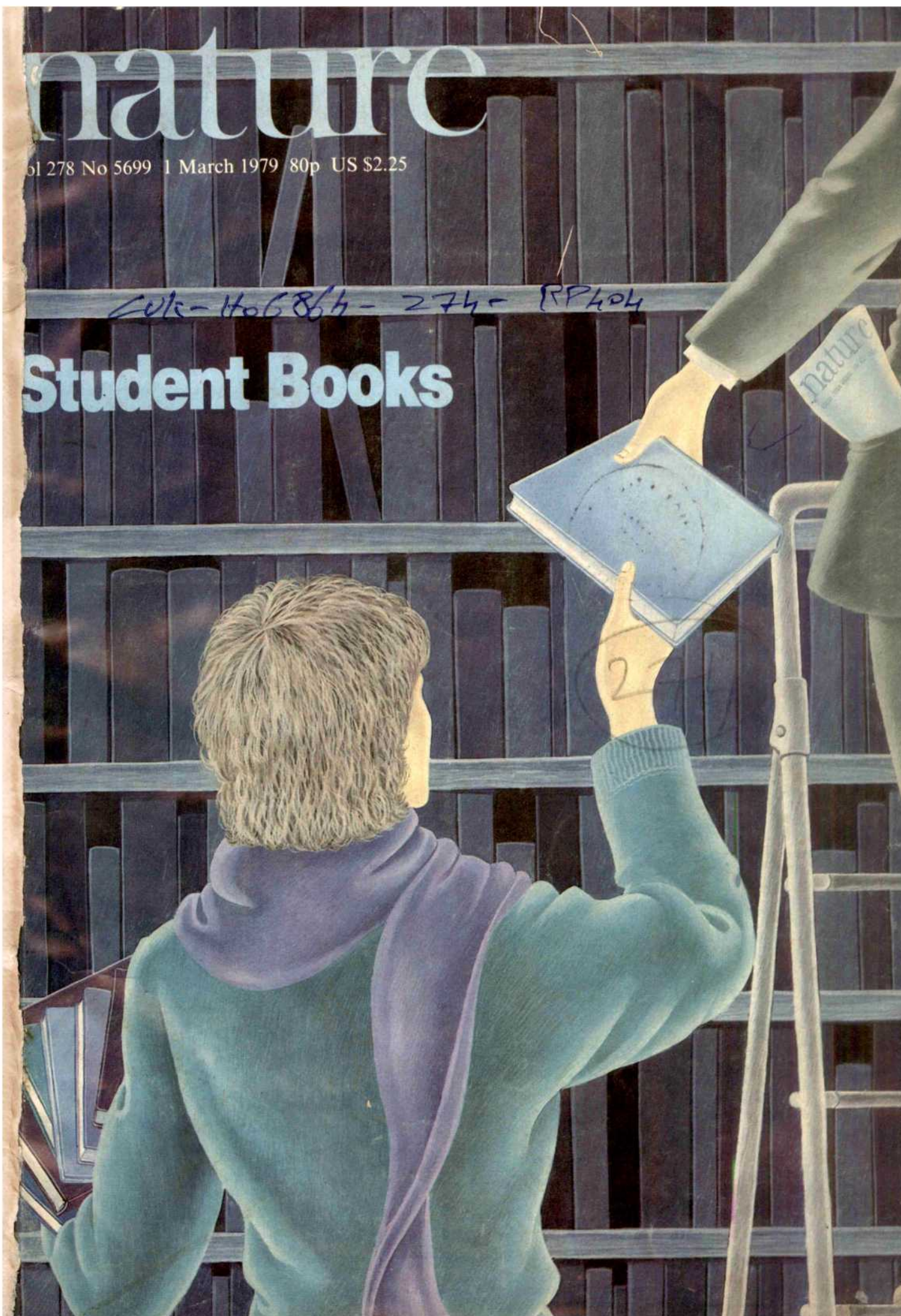


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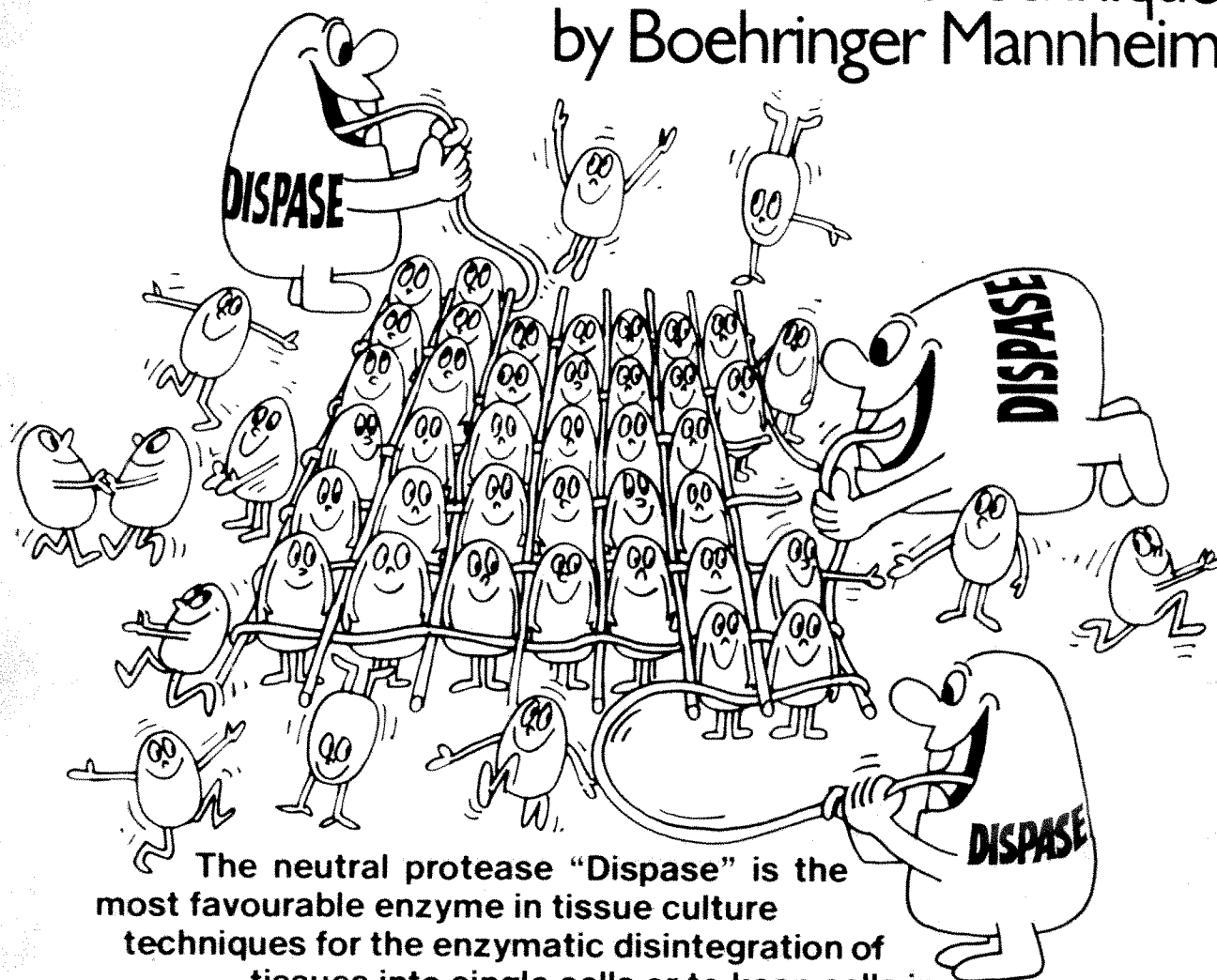
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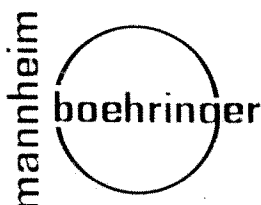
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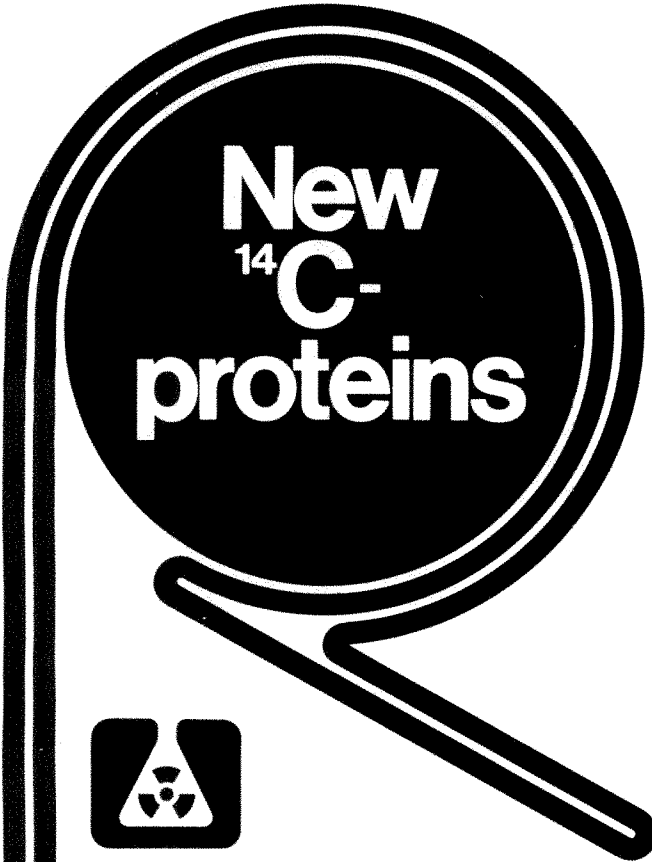
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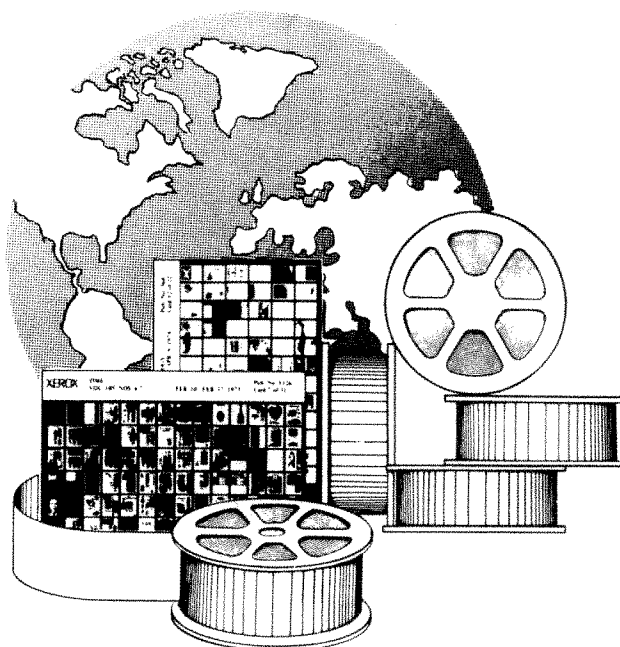
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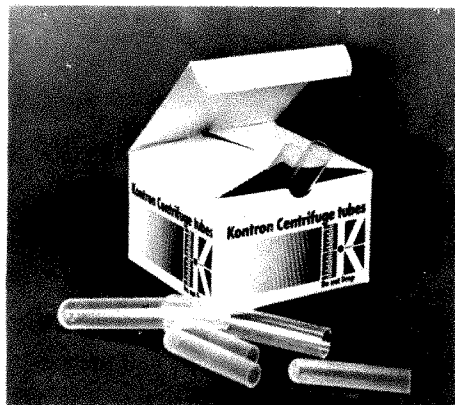
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1 March 1979

Science to grow again?

WHAT is the minimum rate at which expenditure by research councils should grow in real terms in the next few years to sustain the vigour of the scientific community in Britain? A difficult question, and in some ways a meaningless one. One single aggregated figure for growth would have to cover a wide range of activities from research of great timeliness through to research that might just as well be terminated right now; it would also have to make allowance for discoveries yet to be made but which will call for the commitment of new resources in years ahead. The Advisory Board for the Research Councils (ABRC), however, make a guess at a figure in their just-issued report for the years 1976–78 (HMSO; 70p): 4%. The report gives a valuable survey of past fluctuations in financial support, from the remarkable days of 13% real growth in 1966, through the period of at-best no growth, at-worst cuts of a few per cent in the mid-1970s to the slightly calmer waters of about 1% annual growth promised for the next three years.

What lessons have been learnt from this period? That salaries drift upwards in real terms by up to one per cent each year simply because of increased seniority; that the cost of equipment drifts up also, especially now that much which was bought during the growth period of the 1960s is obsolescent—excepting that computers are still getting cheaper; that without a little financial elbow-room all money gets locked into continuing projects, buildings and staff, and new initiatives never get the start they need; that if too much growth is pumped into the system too quickly, the money injected either cannot be spent and is returned to the Treasury, or is spent hastily and not necessarily wisely. From all these considerations, the figure of 4% looks realistic. Whether it will sell in Cabinet in inflationary times, or with a possible change of government is another matter.

But whilst research done through research councils has been subject to severe restraints these past few years, and whilst the other arm of the 'dual-support system'—financial assistance for universities through the University Grants Committee (UGC)—has, if anything, experienced even greater difficulties in making ends meet, defence research and development has shown relative resilience. The ABRC reports valuably bring together the nation's R & D expenditure in one table. In

1969/70 total government R & D cost £604 million; it now (1978/9) costs £1,735 million. The proportion of this sum which has been spent on defence R & D has risen from 40% in 1969/70 to 50.5% in the current financial year.

It might be argued that during a period in which the government pared down on major civil commitments such as its contribution to the European Launcher Development Organisation, and research on Concorde, it would be natural for defence R & D to seem to grow in significance. One way to test this would be to see whether money for research councils and for the research side of University Grants Committee (UGC) support (which can only be an estimate) has kept pace with defence R & D funding. The calculation is somewhat complicated by the redistribution of funds during the Rothschild re-organisation, so we do it for the Science Research Council only, this council not having had to transfer any of its funds over the past several years to customer departments. The ratio of defence R & D expenditure to SRC plus UGC research funds was 2.55 in 1969/70, dipped as low as 2.39 in 1974/5, but now stands at 2.93. The conclusion is inevitably that defence R & D has not in recent years experienced quite the privations that much of civil science has had to undergo.

Is it right that half of all government expenditure on R & D should go to defence? No-one (as far as we know) sits in Whitehall and preordains percentages that would be appropriate for research in different areas—relative movements are therefore much more likely to reflect changing conditions within individual ministries than any clear-cut policy decision. Nevertheless the remorseless upward trend in importance of the defence R & D figure should not go unremarked. It is next to impossible to tell whether the £875 million spent this year gives value for money, but anecdotal evidence—what little there is—suggests that areas of real excellence in defence R & D establishments are few and far between, and that the general level of competence and drive is not high. Mechanisms for weeding out inferior quality in civil science by peer review are by no means perfect, but at least they exist. Do we know that within a closed organisation, whose employees possess excellent job security, adequate steps are taken to protect the taxpayer's interest? □

Environmentalists fight use of 'untested' pesticide

The EPA has been accused of playing politics over Mississippi's fire ant 'emergency'. Clive Cookson reports

ENVIRONMENTALISTS are fighting tenaciously to prevent the use in the southern United States of an untested and allegedly carcinogenic pesticide. They say the Environmental Protection Agency approved the pesticide for political reasons and against the advice of its own scientists.

Twice last year the EPA agreed to requests by the state of Mississippi to use Ferriamicide, a new organochlorine insecticide, as an emergency weapon against fire ants. Each time the Environmental Defense Fund persuaded a federal court to delay the EPA approval. Last month the EPA announced a third time that it would permit Mississippi to apply Ferriamicide to a limited area this spring. But within a fortnight the EDF persuaded the agency to delay the approval again—this time without court action—by drawing its attention to some Canadian research results.

Ferriamicide, developed by Mississippi in a state-owned chemical plant, is a combination of Mirex*, imported from Brazil, with an amine and ferrous chloride to speed up its photodecomposition in the environment. But, according to a recent report by Dr David Villeneuve, head of the Environmental contaminants section of Health and Welfare Canada (HWC), one of Ferriamicide's main decomposition products, is photo-Mirex, which is even more toxic than Mirex, the active ingredient of Ferriamicide. Pure Mirex was the chief insecticide used against fire ants until the EPA banned it in 1976 after studies indicated that it causes cancer, birth defects, and other toxic effects in mammals.

EDF general counsel William Butler says the Canadian study was widely known before the EPA's most recent approval of Ferriamicide—Dr Villeneuve presented his work at several scientific meetings—but the EPA was apparently not aware of it.

According to the EDF, the agency's whole attitude to Ferriamicide has been influenced by the desire of senior EPA administrators to appease the powerful southern politicians who have been pressing very hard for its approval. Fire ants were accidentally introduced from South America in the 1920s and have spread remorselessly across the South, biting humans and animals and impeding agriculture with their mounds. Controlling the ants has become a big political issue in the nine southern states now infested.

Almost every southern senator and

congressman, as well as state and local politicians, joined the campaign to win EPA approval for Ferriamicide. A public relations drive led by Jim Buck Ross, Mississippi's Agriculture Commissioner, persuaded 20,000 people from the state to write to the agency in support of Ferriamicide.

EPA files, which the Environmental Defense Fund obtained during last year's litigation, show that the agency was highly sensitive to this political pressure, though EPA officials insist that the decisions to approve Ferriamicide were not influenced by political considerations. Barbara Blum, EPA deputy administrator wrote on one internal note. "If it's a political decision we want to make the most of it." She was apparently referring to the opportunity to win more favourable treatment for the EPA and for the Administration's pesticides legislation in Congress—the Senate and House committees which oversee the agency and fix its budget have many prominent members from the South.

None of the scientific branches of the EPA pesticides registration division supported their senior administrators' decisions on Ferriamicide. Some, including the chemistry and fish and wildlife branches, actively opposed them. Nevertheless, Dr Blum ruled that the fire ant "emergency" justified Mississippi's use of its unlicensed and virtually untested pesticide, though she did impose more stringent safeguards than the state proposed. (The eight other infested states lodged similar requests to apply Ferriamicide over a total of 17 million acres, but the EPA has not yet ruled on their cases.)

Dr Blum accepted Mississippi's argument that there is no effective alternative treatment for large, heavily infested areas, although one or two US chemical firms are reported to be developing new ant-killers which may be available soon. The environment-

alists argue, with the support of some agricultural scientists, that Mirex itself has not halted the spread of fire ants; the area infested has increased from 30 million acres in 1962, when Mirex was first used, to 190 million acres in the late 1970s.

According to William Butler of the EDF, the only result of Mississippi's highly visible war on the ants—involving helicopters and converted B17 aircraft—has been political. Voters can see and hear that the state and federal governments are doing something about the insects which are making their lives miserable. Mr Butler likes to compare the psychology behind the war—and its practical results—with the US attempts to bomb the Vietnamese into submission.

The uncomfortable position of the EPA—under fire from the environmentalists on one side and from the politicians and farmers on the other—became more embarrassing when the head of the US Government's other major regulatory agency for toxic chemicals, the Occupational Safety and Health Administration, criticised the latest EPA authorisation of Ferriamicide in Mississippi. What Eula Bingham, Assistant Secretary of Labour responsible for safety and health, objected to was a condition imposed by the EPA that no women of child-bearing age should handle Ferriamicide. OSHA officials are said to fear that this sex bias may be used or quoted as a precedent by chemical firms wishing to exclude women from their work forces.

They also doubt whether the ban on women handling the pesticide makes any sense. Research has indicated that Mirex damages the testes of male rats and may therefore cause fathers to pass on birth defects to their children. Logically, then, only the elderly and sterile should be allowed to use it.

**Chemically Mirex is dodecachlorooctahydro - 1,3,4-methano-2H - cyclobuta-(CD) pentalene. Photo-Mirex is 8-monohydro-Mirex.*



UK union attacks GMAG bureaucracy

THE Association of University Teachers (AUT) in the UK is worried about the paperwork its members could face if the Genetic Manipulation Advisory Group's (GMAG) plans to include self-cloning experiments within its remit (see below) are adopted. Three AUT representatives expressed their concern to members of parliament last week when they gave evidence to the House of Commons Select Committee on Science and Technology which is currently examining genetic engineering.

Dr David Sherratt of Sussex University, one of the AUT representatives, explained to *Nature* after the meeting that most of the concern centres on the interpretation of part of the document published in *Nature* last year (9 November, page 104) on proposed new guidelines for the UK. The document states that laboratories intending to do self-cloning experiments should provide "a block notification" of the experiments to be done over a year, an undertaking to work in category 1* and a retrospective "detailed log of all experiments carried out".

The latter point causes Dr Sherratt most concern. "In our laboratory", he says "12-15 of us are using these techniques (cloning *E. coli* genes into *E. coli* organisms) every day." Providing GMAG with a detailed log of all such experiments (up to 1,000 in a year) could make the amount of bureaucracy the laboratory has to deal with "absolutely enormous", he claims. As yet, however, no-one knows whether a single sentence on each experiment will satisfy GMAG or whether it will require short papers. "It all depends on what they want", says Dr Sherratt.

Under the Williams guidelines, which still operate, his laboratory, together with most others, does not at the moment notify GMAG of self-cloning

experiments. Scientists have generally interpreted GMAG's definition of genetic manipulation as excluding self-cloning. During 1978, however, the Medical Research Council (MRC) indicated that self-cloning experiments should be considered by GMAG. It suddenly started to make the award of some grants for work involving self-cloning experiments conditional on those experiments being notified to GMAG. GMAG went along with this. Prior to 1978, it would seem that the MRC and GMAG had agreed with scientists that these experiments did not count as genetic manipulation.

The trouble is that GMAG's definition of genetic manipulation is ambiguous with regard to self-cloning, so it could be argued that both the MRC's interpretation and that of the scientists are correct. Even GMAG admits that it is ambiguous. Its remit is defined as: "the formation of new combinations of heritable material by the insertion of nucleic acid molecules produced, by whatever means, outside the cell, into any virus, bacterial plasmid or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation". The chief difficulty, says Dr Sherratt, arises in the meaning assigned to "they" in the phrase "in which they do not naturally occur". If it refers to the "new combinations", then all self-cloning experiments are included in the guidelines. If, on the other hand, it refers to the "nucleic acid molecules", as most scientists have assumed it does, then all self-cloning experiments are exempt from regulation. This is the point that GMAG has recently been debating.

Judy Redfearn

GMAG wants self-cloning notification

IN a controversial decision last week, Britain's Genetic Manipulation Advisory Group (GMAG) has opted to include self-cloning in its definition of "genetic manipulation", but to slacken its notification procedures for such experiments. However GMAG has not yet issued a formal statement of its new views, and will not do so until after 16 March, when it meets to decide a final form of words.

That meeting will require decisions on three crucial issues: first, what containment conditions will be required for self-cloning (and related experiments); second, what experiments to include in this slackened category; and third, what notification procedures to demand.

At present, GMAG feels that "good microbiological practice" will be sufficient, somewhat more relaxed than the present Category 1; that three or four systems could be included, such as *E. coli* in *E. coli*, and experiments likely to be undertaken in teaching; and that the notification should be more of a formality than a chore. But precisely where the group stands on these matters is not decided.

For comparison the revised guidelines of the US National Institutes of Health clearly exempt from any control all self-cloning experiments, experiments involving donor-host pairs that naturally exchange DNA, and some cloning experiments with viral DNA in viral vectors.

Robert Walgate



Britain snowed up, January 1979

World climate conference turns to the weather

IT is the short term variability of climate—to most people, year-to-year variation in 'the weather'—that matters most, not the longer term 'climatic' variation with which some (perhaps especially some of the environmentalists) are more deeply concerned. This is a first conclusion to be drawn from the "Conference of Experts on Climate and Mankind" convened recently in Geneva by the World Meteorological Organisation.

Certainly, the effects of short-term variability are serious enough. It was one such phenomenon, the Sahel drought of 1968-73, which in fact brought the need for some sort of climate conference to the fore.

Figures given by Robert Kates of Clark University indicated that between them floods, tropical cyclones and drought cost the world a good \$30 billion each year, claiming the lives of some 250,000 people, 95% of them citizens of the Third World. While the absolute costs of these disasters are many times greater in developed than in underdeveloped countries, in terms of GNP the situation is reversed: "Climatic hazard impacts poor countries 20-30 times the rate of rich countries," Kates suggests.

While the need for research on short-term variability was recognised as the immediate research priority, there was inevitably a great deal of discussion at the meeting of the possibility (rather than the probability) of medium term changes. This brought up the debate between the "ice age" prophets, and those who see the danger of a global warming as the more immediate threat.

By the end of the meeting, both could perhaps agree that their points had been met; there seems to be a general consensus that the Earth is at the start of a potential cooling period of perhaps 10,000 to 20,000 years, possibly not an ice age in the sense of those recorded in geological time, but still a major, long-term change.

At the same time, there was no doubt of the feeling of the meeting as a whole, regarding the possible combined effects of CO₂ emissions and those of other pollutants such as nitrous oxide and many chlorine compounds, including the already notorious chlorofluoromethanes. How little is known about the real effects of all these substances is evident from a note in the report of the working group concerned, that "it is possible that some processes could lead to a cooling" (rather than a warming as with CO₂) "of the atmosphere".

Despite this uncertainty, Ralph d'Arge of the University of Wyoming offered estimates of the economic costs of climatic change. Giving sets of figures for a drop of 1°C, he calculated that losses in rice production could only be offset by an investment of \$19 billion—and rice is the staple crop of the majority of the world's poorest people.

Yet perhaps even more significant, as indicating the knife-edge of climate on which many national economies are balanced, are his figures for softwood

production in USSR: a drop of 1°C would require an offsetting investment of \$28 billion, whereas only a half degree rise would give a \$13 billion bonus.

Where urban wages are concerned, this differential is even more marked: for the United States alone, d'Arge's figures jump from a loss of \$73 billion to a rise of \$31 billion. So it is not surprising that another conclusion of the conference was that planners, in the industrialised countries as well as those of the Third World, should take climatologists far more into their confidence than they do at present.

Not irrelevant in this connection was a response made to B. J. Mason, Director of Britain's Meteorological Office, when he remarked that variability such as the hot dry summer of 1976 need not be so disastrous: whereas certain British crops did badly, others so improved that the total loss was slight. It was the sole Iranian at the meeting who pointed out that perhaps this was because many crops grown in Britain are at the limit of their natural range, and need a warm summer to be really profitable.

Fascinating data on medium term climatic variation was put forward in the Chinese paper, based on studies of fluctuations over much of the last 5,000 years. Mean temperatures are believed to have varied over a range of two to three degrees—as great as that foreseen under a prolonged "greenhouse

effect" today. At the same time, Chinese scientists studying more recent records believe they have identified a number of short-term cycles of from two or three to 11, 22, 36 and 80 years for various parameters in different parts of their vast country with, it is admitted, an extraordinarily wide range of normal variation.

Inevitably, a good deal of attention was paid to the use of computers as the main tools in forecasting, especially perhaps over longer ranges of time, although it was agreed that there is an urgent need for better models to work to. Moreover, although no one was so crude as to point this out, an industrialised country such as Britain may well spend as much on a single giant computer as many, if not most, Third World countries have for their entire annual budget in meteorological services.

This point, at least, was reflected in the final Declaration of the Conference, with a call for assistance to these countries through training and the transfer of appropriate technologies without which they cannot participate fully in the proposed World Climate Programme. This programme, a bare outline of which was appended to the Conference Declaration, will be discussed by the WMO Congress when it meets from 30 April for four weeks, with a view to reaching decisions about content and funding.

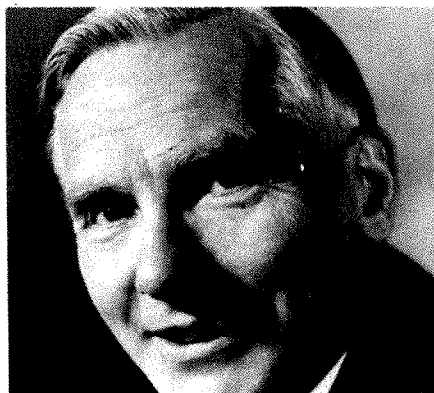
Peter Collins

Call for world (non-nuclear) energy organisation

THE potential dangers of the rapid growth of nuclear energy on an international scale are so great that there is an urgent need to establish a global body to co-ordinate and stimulate the development of non-nuclear sources of energy, according to Dr Joseph Rotblat, emeritus professor of physics at the University of London, and a leading member of the Pugwash organisation.

Speaking at a meeting in London last week organised by the environment division of the Institute of Biology, Professor Rotblat said that although a global energy body already existed in the International Atomic Energy Authority, it existed to promote one form of energy—nuclear. No similar body existed to encourage other energy forms. "It is high time that the balance was restored", he said.

A large expansion of nuclear power would greatly increase the chance of nuclear war, said Professor Rotblat, since almost every nation would have access to plutonium, and would also have the technology necessary to make nuclear weapons. "In the long run nuclear energy is not compatible with the survival of civilisation", he said.



Fortunately the recent slowdown in global demands for energy meant that the expansion of nuclear energy was likely to be much slower than earlier predicted. However the risks of proliferation were still increasing, particularly since several countries had made the export of nuclear energy an important part of their economy, while the demand in third world countries had been stimulated by the "promotional activities" of the International Atomic Energy Agency.

"The most positive thing we could do to reduce the risk of nuclear war would be to encourage countries to opt for non-nuclear sources of energy. A global organisation devoted to this end would be important to give the same aura of respectability to non-nuclear energy sources as is at present given to nuclear energy", Professor Rotblat said.

Professor F. W. Spiers of the University of Leeds told the meeting that measuring background levels of radiation provided an important yardstick for studying the health effects of society's nuclear activities. Whether background radiation was a source of cancer was an open question, he said. "Although we would not anticipate it to have much effect when there are so many other causes of disease".

Levels of background radiation also provided a lower limit for the 'doubling dose' for different cancers, since if this dose was estimated at less than the background level, it would imply that the background radiation was responsible for more than the observed incidence of cancer.

David Dickson

Soviet space effort is strictly for the cash, not the glory

ACCORDING to TASS, the flight programme of Soyuz-32—launched on 25 February—includes docking with the Salyut-6 orbiting station and checking it for a possible further mission aboard the station. This suggests that the Soviet planners are anxious to get their last kopek's-worth out of Salyut-6, which has already been in orbit for almost 18 months.

Although the finances of the Soviet space programme are never published, current Party doctrine stresses that all branches of science should direct their research towards the practical needs of the national economy. The economic pay-off from the Soviet space programme was therefore the keynote of a press conference in Moscow last month given by chief Training Officer Vladimir A. Shatalov and cosmonaut Vladimir Kovalenkov.

According to Shatalov, "the greatest economic impact, the greatest benefit so far can be derived from research in near-Earth space and in the operation of orbital stations". This, he said, would remain the basis of the Soviet space programme for the next few years, because such questions "really do bring considerable, obvious benefits even now".

Illustrating such benefits, Kovalenkov mentioned observations from orbit of dust storms and meteorological and oceanological processes. When observations of the oceans began, he and his fellow-cosmonauts were not told what they were supposed to be observing. They had to work out by trial and error such practical matters as the best porthole to use in relation to the Sun's position. After some time, he said, it was possible to understand "to some extent" the ocean currents, and hence to identify resources of phyto- and zooplankton—to the ultimate benefit of the Soviet fishing fleet.

Observations from orbit of ocean vortices, Kovalenkov added, had led to an oceanological expedition to the Atlantic last summer. From subsequent reports, it appears that this expedition established the existence of isolated closed spiral currents, with diameter up to 200 miles. However these are predominantly in the Bermuda Triangle area, and the unbiased observer should remember that the Soviets, being good dialectical materialists, are at pains to find a physical explanation of the supposed Bermuda Triangle disappearances.

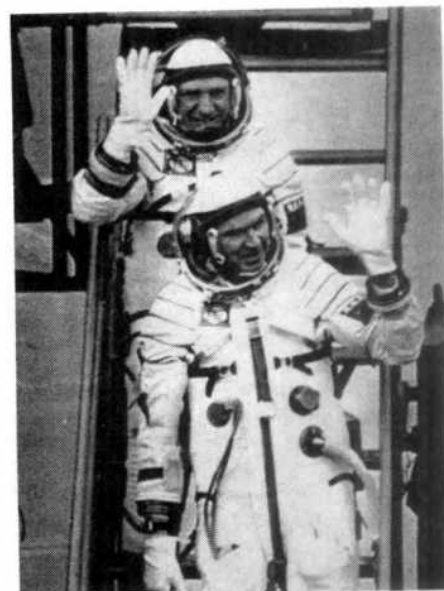
The observation of such processes from space will be a major task of the first Soviet unmanned oceanographic

satellite, Kosmos-1076, launched on 12 February. Soviet space planners have always stressed the great potential of unmanned craft and probes. The US Apollo moonflights, for example, were presented in the Soviet media as a waste of funds. The *Luna* spacecrafts and *lunokhod* rovers, it was stressed, could do all that a manned Soviet mission might do, at half the cost. Kovalenkov's remarks would suggest, however, that without the recent long-term manned Salyut missions, the programme of the unmanned Kosmos-1074 might well have been a somewhat hit-and-miss affair.

Earthquakes are another Soviet concern. A team at the State Nature Centre are using photographs from the Salyut missions to predict earthquakes in the Soviet seismic zones. A spokesman from the centre explained recently on Moscow radio that the seismic zones were first observed visually by Yuri Gagarin. Since some 15% of the terrain of the USSR is seismically active, while some 30% of the population live in these areas, earthquake prediction from satellite observations, he said, could produce "tremendous" savings in construction costs. Preliminary work along these lines was begun by the Salyut-6 crews last year.

Even the exotic triple Cd-Hg-Te semiconductor crystals produced about Salyut-6 in the joint Soviet-Polish "Syrena" experiment have their practical use. They have been handed over to the Polish Institute of Mining Safety, to be used in producing infrared-sensitive pyrometers.

Salyut, it would therefore seem, is paying its way. Indeed, Shatalov himself admitted as much in a recent interview with the Bulgarian daily *Otechestven Front*. Much of his state-



Soyuz-32 send off: Cosmonauts Lyakhov (top) and Ryumin before Sunday's launch.

ment was, naturally, devoted to the training of the Bulgarian cosmonaut candidates, in the Interkosmos programme, but in referring to the work of the Salyut crews "including the international ones" carrying out extremely valuable observations for the national economy, he implied that, in his opinion, from now on the programme would be operating essentially on a break-even basis.

Looking to the more distant future, Mr Shatalov noted that, with bigger spacecraft, there would be a stricter division of crews into scientific and operational personnel. This would cut considerably the training demands on the individual members of the crew who would now need to qualify only as scientists or, alternatively, as spaceship operatives. Ultimately, he said, a whole "Aeroflot-type" back-up organisation would have to be set up as well, to handle the terrestrial processing of the flights.

Vera Rich

Space shuttle may be six months late

A STUDY group of the US National Academy of Sciences predicts that engine problems mean the space shuttle's first flight, officially planned by NASA for November this year, is now unlikely to take place until April 1980.

In a report prepared at the request of the Senate committee on science, space and technology, the group says it is particularly concerned that the engine that will actually fly in the space shuttle will not be the same configuration as that currently being tested, and that, in the circumstances,

plans to perform flight certification tests on the engine, which will be used among other things to help launch the European Space Agency's Spacelab, are premature.

"The only rational option, for safety's sake, is to reschedule the formal flight certification until the configuration is fixed," Professor Eugene E. Covert, professor of aeronautics and astronautics at the Massachusetts Institute of Technology and chairman of the National Research Council group which prepared the report, said last week. □

Supreme Court to hear benzene exposure case: The US Supreme Court has agreed to review an appeal court ruling which struck down the Occupational Safety and Health Administration's strict new limit for benzene exposure in the workplace. The Fifth Circuit Court of Appeals in New Orleans—whose reputation is pro-industry and anti-environmentalists—decided that the limit was invalid because OSHA did not provide a reasonable cost-benefit analysis for it. The industrial groups which challenged the limit estimated that it would cost them \$500 million to comply with the new standard and claimed there was no evidence that lives would be saved by it. The Government argued unsuccessfully that current scientific knowledge was insufficient for OSHA to predict accurately the number of lives saved by a given reduction in exposure to harmful chemicals. If the Supreme Court upholds the decision, the basis for government regulatory activities could be seriously undermined.

Rothschild's Dimpleby lecture on risk is attacked:

"In view of the highly biased nature of Lord Rothschild's lecture we were shocked to learn that the Atomic Energy Authority is distributing copies of (the lecture) through its liaison office in Washington and we call upon you to stop its distribution forthwith" said the UK office of Friends of the Earth in a letter to UKAEA chairman Sir John Hill last week. Czech Conroy, Energy Campaigner of FOE, first raised the protest on 23 February at a lecture given by the UKAEA's health and safety adviser, Professor F. R. Farmer, to students at the Royal College of Art. Conroy pointed out that Rothschild's talk was based in part on "the severely discredited" Inhaber report prepared for the Atomic Energy Board of Canada on the risks of energy production and that Rothschild's talk had been widely criticised in the scientific press. Conroy also protested that in spite of these criticisms the Authority had published the lecture without comment in its monthly magazine, ATOM. The editor of ATOM, who happened to be in the audience, offered to publish a critique of the talk and Friends of the Earth requests in its letter that this be agreed formally. The UKAEA is not yet prepared to comment on the request.



Lord Rothschild

According to the Politics of Health Group of the British Society for Social Responsibility in Science, drug companies are the most profitable industrial enterprises. In the UK for 1975, rates and returns were estimated at 21.4% (Industrial chemicals were second at 16.2%). In 1972, Boots and Beechams showed 54% and 41% returns respectively on capital employed.

(*Insult or injury?* by Charles Medawar. *Social Audit*, 9 Poland St, London W1V 3DG; £1.50.)

German President draws attention to jobs problem: The President of West Germany, Walter Scheel, addressed the Wissenschaftsrat ("Science Council") that advises government on higher education policy on 25 January about the employment problems of young scientists. He hinted that the Heisenberg Programme was not a complete solution. This programme was recently devised by the Deutsches Forschungsgemeinschaft to keep at university those who had won their "habilitation" (right in principle to a university post) but had no job. Only some 70 people had been awarded Heisenberg fellowships so far. "Industry as well ought to be at least aware of this problem", Scheel said. "Perhaps there will be an opportunity to increase exchanges between industry and the universities." However, he added: "We cannot easily refute the claim that not enough basic research is being done in the Federal Republic." And casting a glance at the well-established principle of academic freedom in German universities, he said: "It is the right and duty of the state to insist upon some coordination in basic research". However this would not reduce the freedom and dignity of research. (*Wirtschaft, Wissenschaft, Politik* 5 February 1979.)

Porton Down transfer causes redundancies: The transfer of the Microbiological Research Establishment from the control of the Ministry of Defence to the Public Health Service Laboratory Service Board is expected to reduce the staff by 25% from 300 to 223. Although called "natural wastage", the redundancies include at least 10 senior principal scientific officers within five years of retirement. Most of the job losses are being contested by the Transport and General Workers Union which represents the laboratory assistants involved in the transfer. The Ministry of Defence says that no jobs will be lost through the move except "for some who want voluntary redundancy".

Social Audit audits UK food and drug companies: Social Audit Ltd, a London-based non-profit organisation, has issued a stinging critique of British drug company marketing practices in the Third World. Supporting the World Health Organisation's criticism of the selling of "inessential" drugs in Third World markets (8 February), author Charles Medawar further cites the sale of drugs specifically banned in Britain and suppression of information about dosage and side effects as examples of "disgraceful standards of advertising and marketing by UK companies in the developing countries". Medawar emphasises that drug expenditure, typically 20–50% of a country's health budget, can do little to ameliorate health problems caused by poor sanitation and malnutrition. Preventative measures and ultimately "political and economic solutions" are needed, he says. The Association of the British Pharmaceutical Industry calls the report "an unjustified smear". The ABPI says that, given the confused state of research, there are justifiable differences in the requirements set by different governments.

Oil from coal feasibility study launched in UK: The National Coal Board and the Department of Energy of the UK have signed a financial agreement to cover the cost of creating an engineering design and feasibility study for the construction of two coal extraction demonstration plants capable of processing 25 tonnes of coal per day. The two processes which have been developed to the laboratory stage are liquid solvent extraction (petrol, diesel fuel, kerosene) and super critical gas solvent extraction, a new process that will produce the aromatic compounds, benzene, toluene and xylene, as well as chemical feedstocks for plastics, rubbers, artificial fibres and paints. The Government will put £800,000 towards the total cost of £1.2 million. The actual cost of construction and operation of the two plants, to begin in 18 months, is estimated to be £30 million. Sir Derek Ezra, chairman of the National Coal Board said at the signing ceremony: "Looking at the future availability of oil in terms of world reserves, and currently at the situation in Iran, who can say that the need for this new technology may not emerge sooner than we think?"

'Britain alone cannot end poverty, but it may be able to make a contribution to its end'

Adrian Moyes of Oxfam calls for a transformation of our 'charitable' approach to foreign aid and Third World technology

THERE'S a story of a motorist who stops to ask the way to Andover. The person he asks replies: "If I were going to Andover, I wouldn't start from here." Similarly, anyone trying to help the very poor in the Third World wouldn't start from Britain; most of what needs to be done has to be done by people and governments in the Third World. It is an illusion to think that the wonders of British science and technology can, on their own, do much to benefit the very poor. Whether they do so, depends on the political will in each Third World country.

The use of technology by the very poor has social and political costs. If there are fewer poor people, there may be fewer people prepared to work at low wages for land-owners or manufacturers. Small-scale local production may mean a smaller market for big local manufacturers or multinationals. Urban interests may block investments that will boost agriculture, urban bureaucrats may frustrate policies to direct technology to the poor.

A lot of people benefit from the way that Third World societies are variously arranged at present. They are the people who control the means of production; who own the land or the factories or the transport facilities; who have the money to lend or invest—or to buy modern technology products. They want to keep things that way.

They ensure that there are operating theatres for the townspeople, instead of dispensaries for the rural poor; that R & D is directed to sophisticated or luxury goods and large scale industry; that there is social recognition and praise for research workers who follow international science. They give low priority to extension services and small industry programmes. Foreign investment, some of it from Britain, contributes to this. As *Nature* has put it; "It skews decision-making to favour research and development policies—for example those concentrating on capital rather than labour-intensive production techniques—that benefit the upper and intermediate groups in society, but have little impact on the plight of the poorest".

Technology is a necessary condition for development, but it is not a sufficient one. It cannot get round the need

for political reform, for example by increasing yields so high that even small plots of land become viable and land reform would be unnecessary. Technology is not like that; it cannot solve political problems unless there is political will to solve them.

As a result, most Third World science and technology bears little upon the problems of the very poor. And most scientists and technologists go along with that. They tend to enjoy rather low social prestige (unlike, say, lawyers)—so they turn to the world of international science to get their standing. They go for prestige subjects that will (or at least may) lead to international recognition. Traditional technology and the needs of the poor seldom involve such subjects.

On a personal level too the pressures work against the sort of science and technology that might benefit the poor. Scientists and technologists like the comforts and stimulus of the city; they do not want the inconvenience and discomfort (and perhaps health risks) of spending much time in slums, or in poor, often remote and unpleasant, parts of their country.

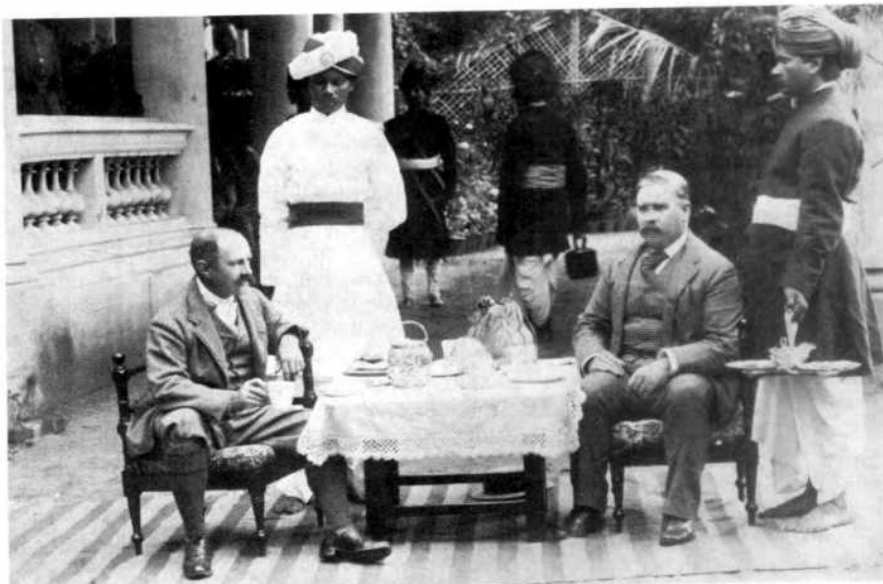
There is not a great deal that outsiders can do about all this. It is not very productive for outside governments to adopt the role of championing the Third World poor against the rich in their own countries.

The difficulties are considerable, but they need not make us despair—Britain alone can't end poverty, but it may be able to make a contribution to its end—it's possible, after all, to get to Andover, even if you start from here.

In the spectrum of technology which runs from the most advanced to the most traditional, Britain's balance of advantage is greatest at the advanced end—so perhaps it is here that Britain can contribute most. Some relatively advanced science and technology can, if social and political conditions allow, benefit the very poor. Examples are education by satellite; the manufacture, storage, and testing of drugs, insecticides, contraceptives; even the equipment needed to devise a better differential for a trishaw; and in the future micro-processors and solar cells. Maybe Britain should concentrate on developing and adapting this type of technology and leave the type of technology that is actually used by the very poor to the Third World countries to handle for themselves.

The drawback to this approach is that left to itself, technology usually favours the rich. It seems to be quite a general rule that if you add to technology to an inegalitarian society (and most Third World—and many other—societies are inegalitarian) the result is increased inequality. This happens because technology is used primarily by those who can afford to take risks—the very poor can't; for them conservatism is life insurance. The miracle seeds of the Green Revolution benefited mainly the farmers who could afford the fertilisers, the irrigation and the pesticides.

The rich are also favoured because new technology tends to drive out old. In doing so it may deprive some people of their livelihood even though at the



Imperial past: is our technology just colonialism in a modern guise?

same time it benefits many of the very poor by providing cheaper and/or more durable products. The mass of consumers benefit from plastic buckets and plastic sandals, but the producers lose work and maybe jobs.

In the 60s Indian metal-workers began making metal dishes and utensils out of aluminium, instead of the "traditional" but increasingly expensive brass and steel. The plates could easily be cut and shaped with simple machines and tools. They were sold in the villages, often by the workers themselves. It cost about £100 to create one job—an ideal application of appropriate technology it might be thought. But the aluminium came from Birla, India's second largest industrial consortium, and so Birla was alerted to the new market. It began making dishes from anodised aluminium. They sold better because they were shiny. That killed the village version.

So if Britain is to implement its policy of More Help for the Poorest,

it cannot merely concentrate on the sort of technology it is best at—and hope the benefits will trickle down. It must take more direct steps to ensure that the very poor are able to benefit from technology (if they want to).

This requires recognition of the need to strengthen and develop traditional technology, rather than using imported technology to downgrade and maybe destroy it. Traditional technology is much stronger than many scientists and technologists (including Third World ones) think. It includes a wide range of skills (ironwork, waste recycling, hygiene, food preservation), a vast amount of local knowledge, and (provided there is little or no risk) a surprising amount of experimentation (often disparagingly referred to as trial-and-error). The way to help the very poor to make greater use of technology is to help them see how much they know already, and to encourage them to adapt that—and supplement it with new technology

from outside.

A good example of this approach comes from Tanzania where a team spent two months in a village helping the villagers to analyse their own storage problems and devise their own methods of improving them. The team based its activities on the belief that the villagers themselves could go a long way towards solving their own problems on the basis of their own skills and resources. They returned to the villagers in a systematised form all the information they got from them, thus giving them confidence that they possessed a concrete technology.

The villagers were not overwhelmed into accepting the team's suggestions: they rejected many of them, including air-tight stores and communal storage, and picked and chose from along their own practices and the team's suggestions so that the end result was something they had designed themselves.

At the end of the two months, 15

NATURE abhors a vacuum. During the 1939-45 war, urban sites devastated by enemy bombs were rapidly covered with vegetation. The number of species of plants and animals which appeared without any help from man was surprising. Within a few years even trees grew up among the rubble, and provided cover for insects and birds, including rarities like the Black Redstart, several pairs of which nested and reared their young within the boundaries of the City of London.

Scientists made some interesting ecological studies of these sites, and city workers interested in natural history derived pleasure looking at them during their lunch breaks. Economic pressures have caused these oases to be destroyed, to be covered with concrete and asphalt, with buildings and car parks. Opportunities for ecological studies or rural refreshment in our cities has thus been greatly diminished.

Fortunately, however, there have been encouraging developments in the opposite direction. In 1977, in connection with the Queen's Silver Jubilee celebrations, a working party was set up to consider the use of neglected areas of land in London for community purposes. The main drive came from Max Nicholson, for many years the Director General of the British Nature Conservancy. He was chairman of the working party's Environmental Committee. This has several interesting projects in hand in various parts of the city, but the one which appeals to me is the William Curtis Ecological Park in Southwark, near the Tower Bridge and just across the Thames from the Tower of London.

Rus in urbe



KENNETH MELLANBY

Until just over a year ago this three acre site was an unattractive lorry park covered with an uneven surface of hardcore, an unpromising area for ecological study. Most of the hardcore and rubble has been removed, to be replaced with soil. This has been carefully landscaped with undulations and valleys, and a good sized pool, surrounded with marshy ground, has been constructed.

Those managing the site had difficult decisions to make—to plant or not to plant. Wartime experience showed that a good cover of plants would quickly appear without human intervention. However, they wished to encourage native species. Unfortunately disturbed sites in London, as well as in remote oceanic islands like Mauritius, or in protected National Parks in Australia, often attract the wrong species. When visiting this Park recently, I saw that the commonest plants developing on

nearby waste ground were the Oxford Ragwort, a native of Italy, first recorded in England in 1794, and rapidly spread when the railways were constructed in the nineteenth century, and Buddleia, only introduced from China in about 1890, and now a rampant weed in Britain.

The Ecological Park has some areas left for spontaneous regeneration, but most of it is being carefully managed. Many native trees and shrubs have been planted, unfortunately not always successfully as the area has a surprisingly low rainfall and artificial watering is difficult. Wild flower seeds unlikely to arrive unaided have been scattered, and young plants are beginning to appear. The pond is already colonised by insects and invertebrates one might not expect to find in such a location. A kestrel nests in the one remaining large tree, all that is left from the rural countryside built over in the last century.

It will be some years before the park resembles in any way the beautiful fields and woods which once existed nearby, but it already provides unique facilities for local children, brought up in a concrete jungle, to get first hand experience of real ecological problems. Perhaps it will also inspire park authorities in other cities to set up more semi-natural areas to complement their tidy lawns and regimented rows of colourful bedding plants. There have indeed already been some such developments, in Glasgow in Scotland in particular. The virtue of the William Curtis Park is that it shows how much can be done with a small and unpromising parcel of wasteland.

new village-designed grain stores were built and in use, several other improved stores were built, even the poorest were using insecticide, the village storage committee had set up a research project to monitor results, the village had agreed to teach 15 other villages to analyse their storage problems, and the committee held a seminar at the university. "It was at this point, recounts the Report on the project, that the committee members really began to see themselves as storage experts"

These Tanzanians had begun not only a process of improving their storage practice but also a process of using their own and outside technology for their own benefit.

Outside technology however cannot usually be applied directly; its principles and techniques may be valid worldwide, but their use varies as much as the people who use them. When technology is transferred from one society to another, the principles and techniques remain the same, but they are re-arranged and combined with a different set of social and economic activities—to make a technology that has been "transformed"

In order to "transform" a technology, people need a range of possibilities from which they can select and test those most relevant to their needs, combine them with elements from their own technology, and work out the new social and economic arrangements needed.

The new technology may mean more time for women, or more work for

women; more money for everyone, or more money for one or two people; less free time in the festival season, too much work in the peak weeding season; community work to empty latrines, community contributions to pay for a maintenance worker. It may mean new institutions at family level, as when a new agricultural technique tends to transfer work from women to their husbands; at village level, as when village meetings have to choose somebody to go for training as a village health worker; or at local government level, as when the technicians are needed to maintain village pumps, cover a whole district and have to be organised from a central office.

Outsiders (such as people in Britain) can develop appropriate technology—techniques or hardware which perform needed tasks. But only the people who are to use the technology can transform it; only they can adapt their ideas (on the role of women for instance, or the importance of festivals) and their social and economic arrangements.

How then can Britain help the very poor to develop their own technology and transform outside technology? The answer lies in a new sort of programme—a programme whose leading edge is in the slums and villages of the Third World where the poorest are. Field staff, many of them local, would seek out, stimulate and finance projects such as the Tanzanian grain-storage one described earlier, helping it to grow into a countryside system, including (if need be) training for extensionists and laboratory and workshop facilities.

The field staff would have access to funds not tied to British salaries or equipment, and the programme would be able to finance non-government bodies in the Third World. There are various ways such a programme could be organised—as a development of one of the Ministry of Overseas Development's existing units, as a new Council for the Use of Technology Overseas (or something), or as a non-government body (perhaps a multilateral one).

A programme of this kind would be quite a change from Britain's present aid for science and technology. The basic premise in Britain is that *they* have the problems, *we* have the solutions. Technology made us rich; we have only to apply it to make them rich. The tradition in Britain is to do things for the poor in the Third World—a kind of charity even when run by the government.

But it is not much help to make people the passive recipients of a technology designed elsewhere; there needs to be a close connection between the development of a technology and the society in which it is to be used. We should be concerned not so much with the appropriateness of a particular piece of technology, as with the appropriateness of the process of technical change. That is what the new programme would be about. □

This article is based on Good servant, bad master by Adrian Moyes, published on 8 March, available from Oxfam Publications, 274 Banbury Road, Oxford, UK, price £1 post free.

Stockholm institute supports Third World scientists

Dr I. M. Nitis, at the Udayana University of Denpasar in Bali, is working on stylosanthes as a companion crop to cassava. He has discovered that the extra green feed produced by the combination of the two crops could support one or two yearling Bali cattle per hectare for a year. His work has been financed by three grants, ranging from \$555 to \$6,600, from the International Foundation for Science (IFS). "The IFS only gives a little money, but it leaves me to work quite independently", he says. "The beauty of the foundation is that it helps young third-world scientists just starting their own research. If their project is successful, a bigger organisation—the UN Food and Agriculture Organisation, for example—will pick them up." Dr Nitis is one of 250 young scientists in 44 developing countries receiving support.

"Our grants have been limited to research in applied biology and agriculture—work which could be carried out in the villages", explained Professor Sven Brohult, founder and President of IFS. "We want to establish self-

Wendy Barnaby reports on the work of the International Foundation for Science

supporting units on the farms and in the villages. It's like the old Chinese experiment of having everyone self-supporting on a small scale so that nobody actually starves. We must avoid contributing to urbanisation and bureaucracy. We leave big investments to the big organisations. Our idea is to encourage indigenous, simple technology."

So far, grants have been given under six categories: aquaculture; animal production, vegetables, oil seeds and fruits; afforestation problems; fermentation and methods for food preparation; and natural products. "We feel the work has been rather successful in these fields", added Professor Brohult, "and are thinking of going into another rural construction. On a very simple level, of course. How to construct a fishpond, for example: you have to have a certain slope around the walls.

And we could help in supplying energy to rural communities. Using simple means to dry fish with solar energy."

To qualify for a grant, a project must be relevant to the needs of a developing country and the researchers must be native to, and carry out their work in, a developing country, where their salaries are paid by the institute that normally employs them. All applications are adjudicated by voluntary scientific advisers.

This sort of volunteer work is gratefully received: IFS's total budget last year was just under \$1 million. The money came as government grants from nine countries: Sweden, Canada, France, the Federal Republic of Germany, Belgium, Norway, the Netherlands, Nigeria and Switzerland.

Although the day-to-day running of IFS is left to its small, Stockholm-based staff, guidance and ideas are provided by about 50 member organisations' academies of science around the world. The most recent to join, to Professor Brohult's great delight, is the Royal Society in London. □

correspondence

GMAG: NIH guidelines are not the way

SIR,—Professor Alan Williamson is well within his rights to complain (1 February, page 346) that there was “no real debate” about the proposed basis of GMAG’s new way of working at the public meeting on 21 December, although it is by no means clear at whom the complaint should be directed. By inference, GMAG is somehow to blame. Professor Williamson could just as well have blamed those among his colleagues who chose to use up what time there was in echoing (and amplifying) what the platform had already acknowledged—that there must be special arrangements for some (but not all) homogenic experiments. He might also blame himself for not having brought up the issues to which he now draws attention.

Of these, perhaps the most important is Professor Williamson’s assertion that the UK could with advantage adopt the NIH guidelines and be done with further introspection. It is therefore worth saying that the NIH guidelines are in several respects defective, at least if one supposes that some of the conjectured hazards of genetic manipulation may (or might be) real. First, their classification of animal virus experiments is irrational, and takes no account of conjectured immunological hazards in particular. Second, the potential importance of expression compared with mere replication has been entirely overlooked in the “broadly based public debate” that Professor Williamson admires. Third, even the new NIH guidelines are not constructed in such a way that what must be presumed to be a growing class of experiments without risk can be progressively identified and excluded from rigorous restraint.

I am one of those who believe that if GMAG does not change its procedures soon, we shall find that events force on us a decision between the NIH guidelines and doing the research elsewhere. It is of course ironical that we should be faced with such a choice when the hazards of genetic manipulation remain conjectural. In my opinion, however, it is disingenuous of Professor Williamson and other would-be genetic manipulators to complain at the constraints which they invited a mere four years ago. Making a bonfire of the regulations now will cause only trouble. Surely it is in the interests of the scientific community that they should be dismantled by rational means.

GMAG’s proposed way of working offers precisely such a possibility, provided that genetic manipulators will play their part in gathering the necessary data. My own belief is that if the proposed system were adopted and willingly accepted, we should find that before the end of 1980 all but a handful of experiments were no more restricted than by the requirements of “good microbiological practice”. Is not that a chance worth taking?

Yours faithfully,

JOHN MADDUX
Nuffield Foundation, London, UK



Genetic manipulation:
new guidelines
for UK

from Nature, 9 November

Risks of recombinant DNA regulations

SIR,—A more appropriate title for your editorial (November 9, page 103) would have been ‘The absence of reason continues’ or ‘Reason ought to prevail’, rather than ‘Now reason can prevail’. Let me explain why I consider the regulations of the recombinant DNA technique and the new GMAG proposal as unreasonable.

● Since the risks of the recombinant DNA technique are principally in the realm of hypothetical scenarios and science fiction, it is totally unreasonable to propose any complex regulations.

● GMAG is proposing to estimate the imaginary risks of various types of experiments, but totally ignores the true risks of regulations themselves. As I have discussed before (*Trends in Biochemical Sciences* 3, NZ43; 1978), the regulations lead to many real dangers to science and society while their benefits are restricted to supporting the livelihood of the bureaucrats who administer the regulatory machinery. It is totally unreasonable to institute regulations without first evaluating the benefit-to-risk ratio of the proposed regulations themselves, and this ratio in the case of recombinant DNA regulations approaches zero.

● As wisely argued by J. D. Watson (*The New Republic* 180, 12; 1979) it is folly to control any human endeavour which may benefit society, just because it is impossible to provide a perfect proof that no risks exist or can be imagined. There is a high probability that such bureaucratic controls will be detrimental to the best interests of society, rather than having any benefits.

The GMAG proposals on the complex numerical estimations of the purely imaginary risks bear a resemblance to the Hans Christian Andersen fable on the ‘Emperor’s New Clothes’ and appear to be a rather pathetic example of where the recombinant DNA folly could lead us. Let’s hope that at the end reason will prevail and all regulations will be converted to a one-sentence statement proposed by D. Stetten, the first chairman of the NIH Recombinant DNA Advisory Committee: “The conditions of containment appropriate for any recombinant DNA experiment are those which are dictated by the most virulent or dangerous organism entering into that experiment”.

Yours faithfully,

W. SZYBALKI
McArdle Laboratory for
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Wisdom in universities

SIR,—Your leader ‘Converting intelligence into wisdom’ (25 January, page 251) draws undeniably sound conclusions about our lamentable shortage of far-sighted generalists. But in my view you err in claiming that education must be ‘immensely wasteful’ to be effective. It is simply not true that most of what we learn at school or college helps illuminate future thinking in an indefinable way. Quite the converse: the insistence on specialist teaching, parrot-fashion learning and the unrelenting adherence to cumbersome orthodoxies in the academic world are the antithesis of what education ought to mean, and drives out of the minds of all but the most stalwart student any aspiration to true independence or heterodox creativity.

Teaching was originally the responsibility of elders who were accepted as sources of wisdom. It is now a source of profitable employment for those who, all too often, lack the wisdom to do anything better. University teachers are rarely taught to teach. University students who do well in their examinations often look back to realise that they did so in spite of how they were taught, rather than because of it; and few people will argue with the claim that most of what they were taught was distinguished by its irrelevance to conditions in the real world. I have recently been looking into some of the attempts made by universities to get together with industry, for example, in an attempt to make available the wisdom that we like to imagine lurks in the academic fold. In many instances the level of common literacy (to cite just that) is as low in the university-sponsored material as it is in the most backward corner of narrow-minded industry. The inefficiency of universities with regard to staff appointment procedures, administration and communication is remarkable—but is entirely in accord with what I have come to expect.

This is not to say that all university departments are incompetently run. But the very fact that *any* are, undermines what faith society ought to have in the seats of learning. Until there is a thorough reappraisal of what education should mean there is little chance of injecting excitement and liveliness into the academic world. There will be few generalists as long as there is no market for them; and until the unthinkable occurs, and the reappraisal you call for in your leader begins, the opportunities for real progress and revolutionary new insights into man’s predicament will remain unwanted, unfashionable, and frankly embarrassing.

It is, I fear, impossible to upset an apple-cart that is so large, and so weighed down with passengers who are enjoying the apples. It’s fun trying, though.

Yours faithfully,

BRIAN J. FORD
Science Unit, Cardiff, UK.

news and views

Artificial reaction centres to mimic photosynthesis

from Roderick K. Clayton

In photosynthetic tissues, tens or hundreds of molecules of chlorophyll and other pigments cooperate in absorbing light energy that is delivered to a single photochemical machine. The concept of a photosynthetic unit, a photochemical reaction centre together with its share of light harvesting or 'antenna' pigments, was advanced by R. Emerson and W. Arnold in 1932 (*J. gen. Physiol.* 16, 191) to explain the yield of photosynthetic oxygen evolution by algae exposed to brief, intense flashes of light. It was C. B. van Niel who proposed, on the basis of comparative biochemical studies of bacterial and green plant photosyntheses (see *Adv. Enzymol.* 1, 263; 1941), that the photochemistry is oxido-reductive: light energy, absorbed by chlorophyll, drives a separation of oxidising and reducing entities. We now know that this photochemical oxido-reduction is the transfer of an electron from chlorophyll (or bacteriochlorophyll, in the case of the photosynthetic bacteria) to an acceptor, often a quinone. Photochemical reaction centres are pigment-protein complexes in which some of the chlorophyll is specialised to react in this way. The reaction centres are embedded in membranes that also carry the antenna pigments and the components needed for electron transport, proton translocation, and ATP formation. Quanta absorbed by the antenna pigments migrate to the reaction centres and initiate photochemistry. The products, oxidised chlorophyll and reduced acceptor, serve as starting points for the secondary events that provide ATP and reduced pyridine nucleotides, which in turn sustain growth.

The first direct sign of photochemistry in reaction centres was the reversible light-induced bleaching of an absorption band near the long wave absorption maximum of chlorophyll or bacteriochlorophyll. This was reported by L. N. M. Duysens using photosynthetic bacteria (bleaching of 'P890'; thesis, State University, Utrecht, The

Netherlands, 1952), and by B. Kok using green plant tissues ('P700'; *Biochim. biophys. Acta* 48, 527; 1961). The reversible bleaching was a small fraction of the total absorbance, most of which was due to antenna chlorophyll. Reaction centres of photosynthetic bacteria were first isolated as spectrophotometric entities by selective degradation of the antenna bacteriochlorophyll and consequent exposure of the absorption spectrum of the reaction centres (Clayton *Biochim. biophys. Acta* 75, 312; 1963; Loach *et al. Photochem. Photobiol.* 2, 443; 1963). They were first isolated as chemical entities through dissolution of the membranes by means of detergents and subsequent fractionation (Reed & Clayton *Biochem. biophys. Res. Commun.* 30, 471; 1968). Reaction centres have not yet been isolated from green plant tissues for either of the two photosystems. However, the detailed chemical and physical analyses of purified bacterial reaction centres (see *The Photosynthetic Bacteria* (ed. Clayton & Sistrom) Plenum, New York, 1979) have provided insight into the composition and functioning of the reaction centres of photosystem 1 and photosystem 2 of green plants (see *Brookhaven-Symp. Biol.* 28, 1976).

Reaction centres isolated from photosynthetic bacterium *Rhodospseudomonas sphaeroides* are pigment-protein complexes in which four molecules of bacteriochlorophyll, two of bacteriopheophytin (pheophytins) are chlorophylls in which the central magnesium atom has been replaced by two protons), one molecule of ubiquinone and one iron atom are bound non-covalently to a protein composed of three polypeptides, total molecular weight 10^4 . In the first photochemical step following excitation, an electron is displaced from two of the four bacteriochlorophylls, acting cooperatively as electron donor, to one of the two bacteriopheophytins. The electron moves on to ubiquinone in less than a nanosecond. The quantum efficiency of this process is close to 100%. The products, dimeric bacteriochlorophyll cation ($B_2^{+ \cdot}$) and anionic ubisemiquinone ($Q^{\cdot -}$), are stable against a back-reaction for about

1/10 in the absence of external electron donors or acceptors. In the living cell these products interact with secondary electron carriers in less than a millisecond.

The elucidation of photochemical mechanisms in reaction centres has been aided by studies of the properties of chlorophylls and pheophytins *in vitro*, and our new knowledge of how reaction centres behave has in turn guided attempts to construct artificial reaction centres that mimic the natural ones in terms of reactions, rates and efficiency. One motive for this is to invent a photovoltaic cell, useful for solar energy conversion, patterned after the natural system. On page 54 of this issue of *Nature* M. J. Pellin *et al.* describe for the first time an artificial system that resembles, in its composition and photochemical properties, a truncated version of the natural photosynthetic reaction centre. In the model system the analogue of bacteriochlorophyll is pyrochlorophyll *a*, chosen for its greater stability against irreversible photo-oxidation. A dimer of pyrochlorophyll *a* is linked to an alcohol derivative of pheophytin *a*, the latter acting as a hydrogen bonding nucleophile. Measurements of flash-induced absorbance changes indicate that electrons are transferred with high quantum efficiency from dimeric pyrochlorophyll to pheophytin in this molecular complex. The photoproduct is formed in less than 6 ps and returns to the neutral ground state in 10-20 ns. This is exactly the behaviour of natural reaction centres from which the ubiquinone has been removed: electron transfer from dimeric bacteriochlorophyll to pheophytin in less than 6 ps, followed by relaxation to neutral ground state bacteriochlorophyll and bacteriopheophytin in 10-20 ns. The success of this experiment suggests that the rapid and efficient operation of photosynthetic reaction centres does not depend to an exquisite degree on the angular and translational coordinates of the components. The artificial system shows promise that more stable variants of the natural system might be developed for solar energy conversion. □

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An expanding Earth?

from W. B. Harland

THE concept of an expanding Earth belongs to this century because until the discovery of radioactivity and its application to geoscience there was no knowledge of an internal source of heat that would prevent the Earth from shrinking. Indeed, until about 1950 most geotectonic debate was between the hypothesis of thermal contraction with a reduction of radius of less than 0.01 mm yr^{-1} and a convection hypothesis for continental drift that assumed a relatively constant radius. The primary features to be accommodated were the orogenic belts with a debatable degree of convergence. So for continental drift it had to be assumed that the oceans must be expanding to maintain a steady-state Earth. The concept of convection with spreading oceans was probably first developed by Osmund Fisher (between 1881 and 1891) but not until 1933 was the concept of an Earth expanding so as to open all the oceans enunciated by Hilgenberg. This was not then taken too seriously as no convincing mechanism was proposed to account for so large a change so suddenly in the later part of Earth history. Indeed it was Carey's detailed and accurate reconstructions of continental drift especially from 1958 onwards that perhaps more than anything else, demonstrated the validity of tectonic evidence to settle the debate active since Wegener's successive formulations (between 1912 and 1928). Strangely, however, Carey's reconstructions required more ocean spreading than could be swallowed up in orogeny and so he wanted continental drift to be largely explained by an expanding Earth.

This recurring question was considered at a recent meeting in London*.

At this meeting S. Warren Carey (University of Tasmania) argued, for example, that the great circle approximately encompassing the Pacific Ocean had expanded substantially with little evidence of contraction along its length. Again, most Earth models postulate a large Tethyan Ocean that successively reduced in size through Palaeozoic and especially Mesozoic time to be finally eliminated; but Carey argued that the Tethys is an artefact formulated only to avoid an expanding Earth and that it had no reality; the same would apply to other postulated earlier oceans. He concluded that the Earth has expanded exponentially, this expansion is recognisable only in the last 200 Ma and the rate has increased to that of the present day $5\text{--}6 \text{ cm yr}^{-1}$. Carey also argued

that the main orogenic belts had begun with equatorial dispositions and that major sinistral shear took place along those lineaments. Such transcurrent juxtaposed features whose dissimilarity had instead suggested to many an oceanic gap.

In the intervening years, from magnetic striping, the course of ocean spreading has been delineated with considerable precision so that latterly the problem was to estimate how far this generation of new crust was compensated by the, much more difficult to estimate, subduction in orogenic belts. H. G. Owen (British Museum (Natural History)) has plotted the striping in a series of detailed maps that also require a more modest, but nevertheless significant, expansion of the Earth during the past 200 Ma at least. Owen's expansion is less than Carey's because he recognises the evidence of subduction zones. It might be added that most plate tectonic reconstructions seem to get by with a steady size Earth but they generally do not plot ocean floor spreading details. In this respect Owen's work is so thorough that it cannot be ignored.

To complete the series one might add a commonly held third hypothesis that the Earth is only slowly expanding (about 0.05 cm yr^{-1} as suggested by Egged, in 1969 and Holmes in 1965) so as not to affect ordinary plate tectonic reconstructions. Such could easily be accounted for by thermal expansion from radioactivity.

Tests of these hypotheses were discussed in general and in fundamental terms by S. K. Runcorn (University of Newcastle) and with rigorous quantitative and geometrical constraints by A. D. Stewart (University of Reading).

The palaeomagnetic test compares the palaeolatitudinal spacing on a continent with that on the present Earth. This assumes an inextensible continent, and a continuing geocentric magnetic dipole and would be capable of settling the question if only the errors were not too large. Results would seem to rule out any extreme expansion but might allow up to 5% over the past 200 Ma.

Earth's moment of inertia would change with increasing radius and there would be a marked slowing with expansion. Estimates of the number of days in a year have been made of around 400 days at about 400 Ma. This would not permit the recent rapid expansion hypothesis; but the critical observations on corals on which these figures are based have yet to be repeated more generally.

The principal mechanism suggested for expansion over and above what secular thermal expansion would effect is that of Dirac's decline of the gravitational constant with time. But that would hardly fit the sudden expansion of radius from 0.6 cm yr^{-1} at 500 Ma and 0.7 cm yr^{-1} at 250 Ma (respectively about 11 and 5% of Earth history and nearer 1% of the age of the Universe). An interesting test of such extreme expansion is provided from extraterrestrial bodies. The smooth lunar maria, formed at about 3.8 Ga, are preserved with no subsequent distortion as would be the case if a cosmic process were to account for a differential expansion of the Earth's crust. An extensile feature on Mars would suggest only a small expansion.

Stewart distinguished planetary interior expansion (perhaps due to decline in G) rupturing the outer shell, from general expansion of the Universe. The latter could not directly produce differential motions such as continental drift but only a change of Earth's radius of 0.5 mm yr^{-1} . A test of palaeogravity change from mineral PT systems does not seem to distinguish expansion models up to 0.5 cm yr^{-1} .

His main contribution was a rigorous quantitative study of the geometrical constraints on the interior expansion hypothesis. On a constant diameter Earth, spreading varies from zero at the poles of relative rotation between two plates, to a maximum 90° away. But on a purely expanding Earth, spreading rates should be essentially constant. It is thus possible to get a measure of the expansion rate by measuring spreading pole-spreading equatorial distance. The result is less than 0.4 cm yr^{-1} over the past 5 Ma. This would eliminate Carey's rapid expansion but not Owen's which is 0.5 cm yr^{-1} average over the past 200 Ma.

Several estimates of subduction rates were used to test the balance of crustal gain and loss. This is simply a return in more sophisticated fashion to the old problem of orogenic shortening. Here there is space only to conclude that the subduction required for a steady state Earth seems to be approximately ($\pm 50\%$) consistent with several methods applied to individual zones, and so at least half of all oceanic crust formed must have been subducted which would rule out the extreme but not Owen's expansion.

Finally Stewart's assessment of the standard palaeomagnetic test from McElhinny's data concludes a rate of expansion of radius only 0.11 cm yr^{-1} which would rule out both Carey's models. This is what most scientists

*A meeting of the Joint Association for Geophysics with the Geological Society of London was held in London on 17 January.

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have generally concluded though one speaker questioned whether the data-set used to obtain this figure was properly chosen. But the challenge of an expanding Earth will remain until either the tests have been more rigorously repeated or the critical features of Carey's and Owen's models are incorporated in a steady-state model with or without minimal expansion.

Mechanism of platelet-collagen interaction

from J. L. Gordon

THE rapid adhesion of platelets (or of their non-mammalian counterpart, the thrombocytes) to the vascular wall when the endothelial lining is breached has been a subject of interest since Wharton-Jones showed in 1851 that thrombocytes accumulated at points of local damage in blood vessels of the frog's foot. It eventually became clear that the substance that platelets 'recognised' was a component of connective tissue, and of the various components tested only fibrillar collagen induced platelet aggregation *in vitro*. The importance of platelet-collagen interaction in haemostasis and thrombosis was sufficient justification for many research groups to study this phenomenon but its speed and specificity also intrigued some of those with an interest in cell adhesion *per se*, who joined the search for a collagen 'receptor' on platelets and for the determinants on collagen that platelets recognised.

When Jamieson *et al.* (*Nature new Biol.* 234, 5; 1971) advanced the hypothesis that a glycosyl transferase on the platelet surface mediated adhesion by forming an enzyme-substrate complex with incomplete disaccharide groups on the collagen molecule this provided a great stimulus for research on platelet-collagen interaction. Eventually, the results of further experiments (see for example Menashi *et al.* *Nature* 264, 670; 1976) made this hypothesis untenable, despite the impressive rear-guard action mounted by its proponents (Jamieson *et al.* *Thrombos. Diath. haemorrh.* 33, 668; 1975). An important factor in this debate was the discovery that an ordered, fibrillar, quaternary structure was required for collagen to stimulate platelets (for a review see Jaffe in *Platelets in Biology and Patho-*

logy (ed. Gordon) 261, Elsevier, 1976). Thus, in experiments using monomeric collagen (that is, individual molecules composed of three polypeptide chains as a triple helix) where some platelet response to stimulation (for example aggregation or secretion) was measured, collagen polymerisation was a prerequisite for activity. Hence, it was sometimes erroneously concluded that inhibitors were blocking platelet-collagen interaction when in fact they were interfering with collagen fibrillogenesis. This was also exemplified by studies with collagen subtypes which show varying rates of fibril formation: for example, type III is a much more potent stimulant than type I when they are mixed with platelets in monomeric form (Hugues *et al.* *Thrombos. Res.* 9, 223; 1976) but the potency difference is minimal if preformed fibrils are tested (Barnes *et al.* *Biochem. J.* 160, 647; 1976).

Even when it was accepted that collagen must be in fibrillar form to stimulate platelets the question still remained whether monomeric collagen (or even fragments of individual molecules) could bind to platelets without inducing aggregation or secretion. Much intellectual and experimental effort was expended on this but the results obtained were often conflicting and the discussions generated more heat than light. One reason for this was that collagen molecules and fragments readily self-associate either when free in solution or during attachment to a matrix such as sepharose beads; hence, varying amounts of pseudofibrils may be present. Platelet-collagen binding experiments are also open to the criticism that weak interactions probably do not survive the separation procedures. The main conclusion to emerge from such studies was that platelets may bind to submolecular fragments of mammalian collagen but, if so, the binding was much weaker than to fibrils. These findings were consistent with the hypothesis advanced by Lüscher *et al.* (*Ser. Haematol.* VI, 3, 382; 1973) to explain platelet stimulation by polymers. This introduced the concept of platelets interacting weakly with monomers or fragments through binding sites of low specificity and affinity; when multiple, simultaneous interactions were possible with several sites in optimal alignment (that is, in an ordered polymer with regular repeating sequences) binding was much stronger and a cellular response was induced. Polymerisation alone is not enough—platelets are not stimulated by gelatin (polymerised, but denatured, collagen). It appears that the native fibril formed *in vivo* by collagen type I or type III exhibits an

optimal configuration: platelet aggregation can be induced by $< 1 \mu\text{g ml}^{-1}$ of type I collagen in suspension. In contrast, suspensions of native type IV collagen (which has an amorphous structure) are inactive. However, if collagen types I and IV are dissolved and reassembled as SLS ('segment long spacing') fibrils they are both active platelet stimulants, with an activity threshold around $10 \mu\text{g ml}^{-1}$ (Barnes, Bailey, MacIntyre & Gordon; unpublished work). It therefore seems that the steric arrangement of collagen molecules is much more important than chemical differences between subtypes in regulating interaction with platelets.

The recent paper by Bensusan *et al.* (*Proc. natn. Acad. Sci. U.S.A.* 75, 5864; 1978) sheds some light on platelet-collagen interaction by concentrating on the platelets rather than the collagen. They induced platelet-collagen adhesion by mixing a suspension of type I native collagen fibrils with well-washed platelets, disrupted the adherent platelets by sonication, and determined what platelet proteins remained adherent. The most important platelet membrane component attached to the collagen was fibronectin, a glycoprotein of $\sim 2 \times 10^5$ molecular weight that is a major component of many cell membranes. Also present were actin, myosin and tropomyosin, which suggested that fibronectin on the platelet membrane was involved in binding to collagen and was also connected to the platelet contractile proteins. The presence of fibronectin was confirmed immunologically, and further evidence for its functional significance was obtained by demonstrating that collagen pretreated with fibronectin did not stimulate platelets, whereas anti-fibronectin antibodies induced platelet secretion. Other points remain to be clarified: for example, plasma fibronectin binds to triple-helical or denatured collagen (Dessau *et al.* *Biochem. J.* 169, 55; 1978) whereas the isolated platelet protein apparently binds to collagen fibres but not to gelatin—that is, shows the same conformational specificity as for platelet-collagen interaction. Assuming that the findings and interpretations of Bensusan *et al.* are confirmed, however, more emphasis in future considerations of platelet-collagen interaction should be given to the mechanisms involved in fibronectin-collagen interaction. This has great significance in many cell adhesion phenomena apart from those involving platelets (Yamada & Olden *Nature* 275, 179; 1978). One quibble with Bensusan's paper, terminological rather than technical, concerns the use of the term 'receptor': as usually employed

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(for example in the context of cholergergic, aminergic and other sites) this implies a degree of specificity and affinity which is at variance with the characteristics of fibronectin in mediating interaction with collagen. This is, however, a small criticism of a paper that invites a major reassessment of attitudes in this field of research. □

Proposed optical Stern–Gerlach experiment

from Peter Knight

THE atomic beam work of Otto Stern and his colleagues in Hamburg was published in a series of papers bearing the subtitle 'Investigations with the Molecular Beam Method' (*Untersuchungen zur Molekularstrahlmethode*). The thirtieth and last, in 1933 by R. Frisch, reported the observation of the deflection of an atomic beam by the momentum carried by photons (*Z. Physik*, 86, 42; 1933); it closes with the remark: "It would certainly be possible to do more careful experiments. Unfortunately, for reasons beyond our control, the experiments had to be stopped prematurely". The phenomenon was revived by the work of Ashkin and his coworkers (most recently in *Phys. Rev. Lett.* 41, 1361; 1978) who demonstrated a large enhancement of the radiation pressure effect using laser radiation. Since then photon deflection has been discussed for atomic trapping and cooling, for spectroscopy and for laser isotope separation (although the latter now seems an outsider in the separation stakes).

A recent paper by R. J. Cook presents a new and quite simple analysis of atomic motion in a resonant laser electromagnetic field (*Phys. Rev. Lett.* 41, 1788; 1978). He shows how an atomic beam can be split by traversing an amplitude gradient of the resonant radiation in a fashion reminiscent of the old Stern–Gerlach demonstration of space quantisation. This splitting had been discussed earlier by Kazantsev (*Sov. Phys. JETP* 39, 784; 1974) and aspects of the subject reviewed by Stenholm (*Phys. Rept.* 43, 151; 1978). Photon deflection is produced by excitation and emission recoils, and by a force caused by the interaction of the induced dipole moment with the amplitude gradient of the radiation field. Cook's paper is concerned with the latter and his results are valid only if spontaneous emission can be neglected during the interaction time.

For an atom driven by a laser such that only two energy levels (with wave func-

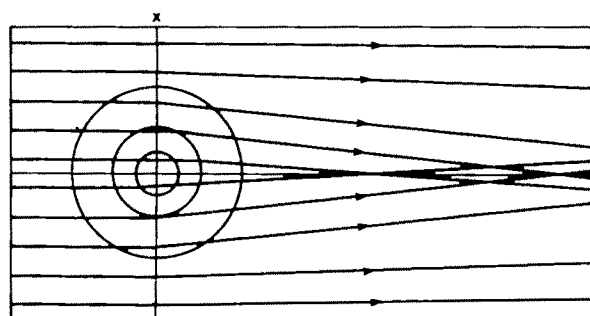


Fig. 1 Focusing of atomic trajectories associated with the wavefunction u_- (Cook *op. cit.*)

tions c_1 and c_2) are relevant, the analysis is particularly simple. The Schrödinger equation for the time-dependent wavefunction for the atom interacting with an incident field leads directly to two uncoupled equations of motion for the symmetric ($u_- = 2^{-1}(c_1 + c_2)$) and anti-symmetric superposition ($u_+ = 2^{-1}(c_1 - c_2)$) of states. These two combinations represent special 'eigen-waves' for the atomic trajectories which propagate independently. An atom initially in its ground state is equally likely to be in one or the other of these superpositions. The 'eigen-waves' obey similar Schrödinger equations except that the potential energy for the u_+ is of opposite sign to that for u_- . The forces acting on atoms in the two waves are thus in opposite directions so that an amplitude gradient will split a collimated ground-state atomic beam into two components: an optical analogue of the Stern–Gerlach experiment. A well-collimated beam of atoms intersecting a Gaussian laser beam at right angles will see the optical beam as a cylindrical lens which focuses u_- waves but defocuses u_+ waves. (Fig. 1 shows the focused trajectory.)

Cook estimates that a 1 W laser beam focused to a 50 μm beam waist (with waist intensity about 10^4 W cm^{-2}) will act as a lens of focal length approximately 1 cm for an atom of slow thermal speed and typical mass and transition dipole moment. An atomic beam centrally traversing such a cylindrical 'radiation lens' would have a cross-section flux at the focus as shown in Fig. 2 where the u_+ wave has been deflected out of the

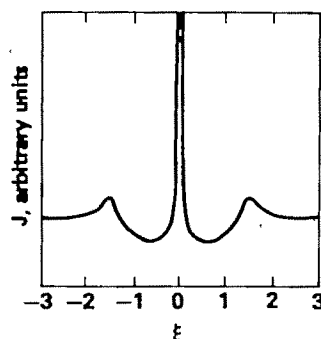


Fig. 2 Total atomic flux J at the focus of the 'radiation lens' for a centrally positioned beam of atoms, in units of laser beam radii (Cook *op. cit.*).

centre and the u_- wave focused.

The lens effect is an interesting demonstration of the inversion of our usual ideas. A lens is normally made of a medium (consisting of polarisable dipole oscillators) shaped so that a collimated light beam is focused by the density gradient of polarisability. Here we have a collimated atomic beam focused (or defocused) by polarisability effects in a light beam shaped to give an electric field gradient. Experimental investigation of these ideas does not seem too difficult and is presumably under way. □

Complexities of cultural evidence in the Lower and Middle Pleistocene

from John A. J. Gowlett

For many years the study of human evolution became, in the words of Irving Hallowell, 'more and more restricted to biological problems dealt with by physical anthropologists'. The cultural evidence, which is essential to a study of the distinctiveness of man, and which is mainly the province of the archaeologist, was scarcely considered as an aspect of biological evolution. The splitting of the study of early man into separate disciplines of archaeology, physical anthropology, and to some extent evolutionary biology, has its roots in the 19th century, and although much has been achieved in the way of interdisciplinary studies since Hallowell was writing, the central importance of the cultural evidence is still sometimes overlooked.

Archaeological work has however built up a record of behavioural complexity of a cultural nature going back at least 2 million years. Man can therefore investigate the evolution of his present cognitive capacity through the permanence of the cultural record. Considering the clear evidence of humanity that is available, it is surprising first that philosophers have made

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so little adjustment to this long time-scale, and second that animals other than man are so often used inappropriately as models for human evolution.

Most of the earliest archaeological evidence comes in the form of stone implements, and although these convey much more information when found in context than in isolation, their study is still an important part of Palaeolithic archaeology: they do, after all, fall clearly within the domain of culture, indicating not only the regular transmission of ideas as learned behaviour, but also the 'imposition of arbitrary form on the environment'. In this sense, Holloway has suggested the potential of the artefacts as a behavioural or sociopsychological record, whereas archaeologists have often been more concerned with classification and typology. Even within the Lower Pleistocene period, which as now defined lasts from ~1.8 down to 0.7 Myr ago, stone-working exhibits design regularities, indicating consistent linkages of ideas (and thus allowing typological description: Fig. 1)^{3,4}. In archaeological terminology, the artefacts which a group of prehistoric people 'manufactured in one area over some span of time' form an 'Industry', but in practice the major taxonomic entities of the Lower Palaeolithic are 'Industrial Complexes' or 'Traditions', defined as groupings of Industries 'considered to represent parts of the same whole'. The vagueness of this definition is of limited importance for descriptive purposes as in the Lower Pleistocene only two industrial traditions of stone-working are generally recognised: the Oldowan, known almost entirely from Africa, and the Acheulean, which continues through the Middle Pleistocene, becoming widespread in the Old World.

The Oldowan, which has an approximate age range of 1.8–1.6 Myr at Olduvai Gorge in Tanzania^{5,6}, is characterised by the regular practice of simple flaking, and the production of a range of tool forms, often based on cobbles. Among these, the discoids and choppers show bifacial working: the production of a resolved edge through flaking from both sides, a complex process involving both feed-forward and feedback. Industries from East Turkana⁷ and the Omo⁸, aged about 2 million years, can be ascribed to the Oldowan, but show some differences which may be accounted for partly by differences of raw material. A variant defined as the 'Developed Oldowan' by Mary Leakey succeeds the Oldowan at Olduvai, having a range of ~1.6–1.2 Myr, and reappearing according to her in higher beds dated to ~0.8 Myr. The Developed Oldowan has a slightly wider range of tool forms, with more em-

Typology after M.D. Leakey (1971)	Oldowan	Developed Oldowan A&B	Acheulean	Acheulean typology (E. Africa) after M.R. Kleindienst (1962)
Bifaces a, b, c, d e, Cleaver f, g, Pick		B		Hand-axe
				Cleaver
				Pick
'Proto-biface'		A		Knife
Discoid				Diak
Chopper, a-e				Chopper
Polyhedron				Spheroid-Polyhedron
Sub-spheroid				
Spheroid				
Scraper (heavy-duty)				Core scraper
				Scraper (heavy-duty)
Scraper (light-duty)				Flake scraper (light-duty)
Awl; Burin; Outil Écaillé				Small flake tools
Laterally trimmed flakes				Trimmed pieces

Fig. 1 A comparison of commonly used Oldowan and Acheulean typologies, which stresses continuity as well as the addition of new forms. Apart from the bifaces, the two typologies differ mainly in detail of description, designated sub-categories being indicated by lower case letters. Correlations of light-duty tools are approximate.

phasis on bifacial working, including some 'poorly made and generally small bifaces' at Olduvai. The Karari Industry from East Turkana⁹, an industry from Chesowanja in Kenya¹⁰ (both probably aged ~1.5 Myr) and an industry from Gadeb¹¹ in Ethiopia have all been described at least provisionally as variants of the Developed Oldowan, demonstrating the usefulness of this term as a descriptive label.

The Acheulean Tradition continues with most of the Oldowan forms, but introduces a new elaborate conjunction of ideas, in the working of bifaces with a well-defined long axis, made on blanks which are specially struck long

flakes (Fig. 2). The bifacial working clearly was a precondition for this development, but it is not clear whether or not the ideas of a long axis and lateral symmetry preceded the use of long flakes. This point has a relevance to the difficulties which archaeologists find in relating the Developed Oldowan and the Acheulean, which are sometimes contemporary, for the Acheulean is first found within the time range ~1.4–1.3 Myr at Olduvai site EF-HR, and Peninj site RHS, also in Tanzania¹². Few other Lower Pleistocene Acheulean sites are known—among these are the Olduvai Bed IV sites, almost certainly the large site

complex at Kilombe, Kenya¹³, and perhaps Kariandusi—although in the Middle Pleistocene Acheulean sites are very common. The Developed Oldowan and the Acheulean have been interpreted at Olduvai as separate cultural traditions, with the suggestion that different hominid groups were involved as makers. Such ideas have, however, been offered only as possibilities, and with very careful qualification^{3,6}, but the existence of two or more hominid species at least in the earlier Pleistocene now seems incontrovertible. In Olduvai Bed I a robust Australopithecine (*A. boisei*) and *Homo habilis* were both found in association with Oldowan artefacts. From both Olduvai Bed II and from East Turkana there is convincing evidence that *A. boisei* and *H. erectus* existed as contemporaries in the period ~1.5–1.3 Myr. At Chesowanja, finds made in 1969 of *A. boisei* were close to recent excavations where artefacts have been found *in situ*¹⁴.

At present neither the palaeontological nor archaeological evidence seems likely to show whether in fact more than one hominid species made stone tools, but Holloway's work on cranial endocasts of fossil hominids does indicate that the brain organisation of various Plio-Pleistocene hominids, including the Australopithecines, was basically human-like rather than ape-like¹⁵. As other assessments of what is 'advanced' in a hominid are largely subjective, it must be recognised that we have no *a priori* grounds for saying that any of these hominids did not make artefacts.

This issue is an important one in the study of human evolution, and needs to be faced openly, but it is not necessary to demand immediate answers, and indeed the growing emphasis on contextual studies encourages other lines of approach. It may be observed that the current definition of 'Industrial Complex' allows use either in a simple descriptive sense, or in a more specific sense of cultural entity, whereas the artefact record in the Lower and Middle Pleistocene, seen in general, shows much continuity of ideas (Fig. 1) coupled with much variability in detail. Some of this variability, which occurs in size, morphology, and proportional frequencies, can be explained by factors such as (1) short-term stylistic change; (2) activity variants, where a human group might use specialised tool kits for individual tasks¹⁶; (3) raw materials, the form and fracture properties of which might influence the end-product; and (4) random or stochastic variation¹⁷. In contrast, remarkably high levels of standardisation are sometimes achieved at a particular locality, implying strict controls. Such combinations of flexibility and stability may reflect the essential adaptive

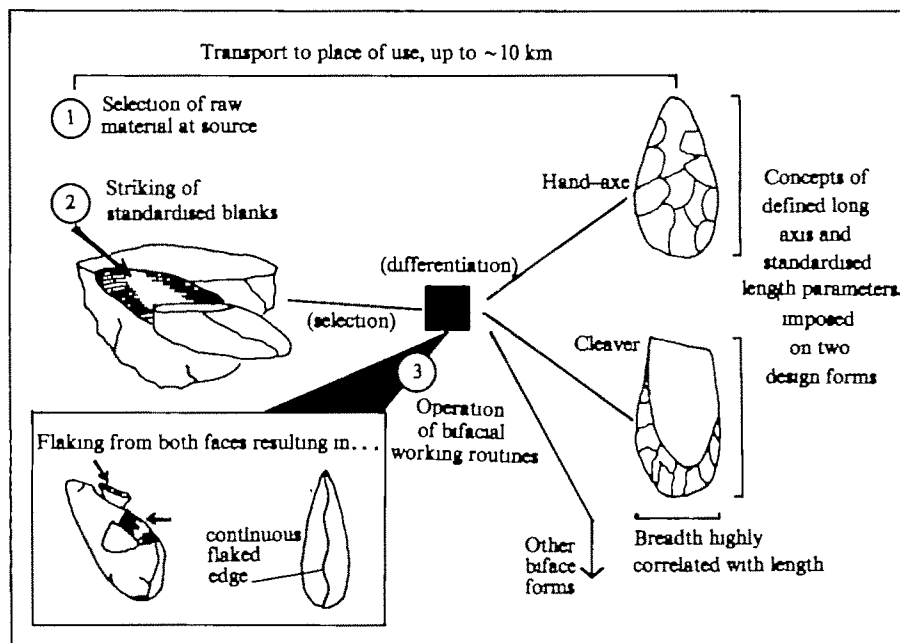


Fig. 2 A simplified diagram of the complex pattern of biface manufacture encountered on Lower and Middle Pleistocene Acheulean sites. Operated as a multi-stage process with geographic separation of stages, it is powerful evidence for organised forward planning, and metrical data provide evidence for remarkable levels of control.

advantages of culturally controlled behaviour. Terms like Oldowan and Acheulean lay stress on selected continuities, and unless used purely descriptively may eventually prove too rigid to embrace the complexities of the archaeological material.

The contextual evidence helps to illustrate this complexity. Even in the Oldowan, artefacts were transported to site, sometimes several kilometres from the source rock, and careful selection of raw material is apparent. In the Acheulean the process is much more complicated and given the distances of transport, and quantities of material, it is interesting for what it can tell us about goal-seeking behaviour, forward planning, habitual and systematic exploitation of an environment, and cognitive mapping of that environment. A great deal can thus be gained from the study of sites in relation to the sediments that contain them. In general sampling density is very low because of the great timespans, and coupled with the high sample variability presents 'in methodological terms... a tough situation'¹⁸. Isaac has treated the background to this in papers which make an important contribution to theory¹⁹. Certain exceptional experimental circumstances, with for example high densities of sites in continuous sediments (such as Olorgesailie or Kilombe), or with sediments extensive over many square kilometres (such as at East Turkana)¹⁹ allow tests which may give some firm answers about the nature of variability in controlled spans of space and time.

In spite of limitations imposed through poor time calibration within sequences, and biases from non-random patterns of exposure, such experiments can work on the density and spatial distribution of occupation traces, and when linked with studies of the transport of raw materials, suggests a whole range of research possibilities touching on factors of interest such as group size, territoriality, and localised or seasonal adaptations.

To further such work, much more archaeological fieldwork is needed, as the corpus of 'routine' finds is very small by the standards of other areas of archaeology, and as only through such work will exceptional finds of unexpected nature crop up from time to time.

It is, unfortunately, too easy for a dogma to arise about future research objectives and methods of working. In the past few years, the exploration of certain extensive areas of sediments, belonging especially to the time-span 3–1 Myr, has dictated the terms of 'palaeoanthropological' research, and led to a particular interdisciplinary balance, which relates mainly to work in the field. Judging from past experience, it cannot be assumed that these approaches will be maintained unaltered in the future. The 'environmental paradigm' though indispensable, forms only one segment in the possible range of approaches: in human evolution, the study of human intellectual development is clearly no less important than that of 'origins', and the potential for cooperation between, for

example, workers in archaeology, hominid neurology, and psychology, is already becoming apparent, although it can hardly happen in the field. To set this subject matter within the context of work in the philosophy of science is also desirable, for the archaeological record has a potential for broadening time-perspectives, and perhaps significant contributions to make towards studies of the phenomenon of consciousness or self-awareness.

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Relativistic nuclear collisions

from A. M. Poskanzer

In the past few years the Bevalac Accelerator at Berkeley has allowed the study of collisions of nuclei at energies which before were accessible only with cosmic rays. The maximum energy attainable, 2 GeV per nucleon, is two orders of magnitude higher than was previously available in the laboratory. At present, beams as heavy as ⁴⁰Ar are in use, but a 2-year improvement project has started which will allow acceleration of beams up to uranium. The construction of similar relativistic heavy ion accelerators has been proposed in West Germany, Russia, and Japan.

The main goal of studies of relativistic nuclear collisions is to make nuclear matter at high density and temperature, with the aim of studying the equation of state of nuclear matter. A sketch of the theoretical binding energy of nuclear matter as a function of density is shown in the figure; the derivative of this curve with respect to density is the equation of state. What is known experimentally about nuclear matter at present is one point on the graph; the equilibrium density of nuclei, and the binding energy of nuclear matter at this density. Also, we know that at this point there is a minimum, which is to say, nuclei are bound. Even the curvature at this

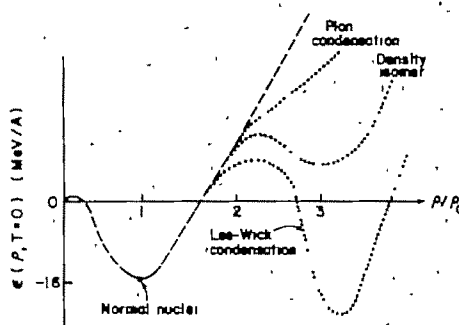


Fig. 1 (From Poskanzer *Comm. Nucl. & Particle Phys.* 7, 41; 1977).

point, that is, the compressibility, is poorly known experimentally. The various curves are some of the speculations about what might happen at high density. At still higher densities it is possible that the nucleons might break up into their constituents to produce quark matter.

It is clear that the first requirement for making nuclear matter at high density and temperature is to collide a high energy heavy projectile at small impact parameter on a heavy target. Goldhaber (*Nature* 275, 114; 1978) estimates that densities of four to ten times normal nuclear density could be achieved in collisions on uranium nuclei with uranium ions of 2 to 8 GeV per nucleon. Thus, in a system containing almost 500 nucleons, one may produce both matter and energy

densities far in excess of any previously attained in a volume of nuclear size.

However, research proceeds in smaller steps for practical reasons and the first results on central collisions were for 250 and 400 MeV per nucleon ²⁰Ne ions on a uranium target (Gosset *et al.* *Phys. Rev. C* 16, 629; 1977). These results, on the energy spectra of protons and light nuclei emitted in the reactions, generated enormous interest, and by now have been fitted by 20 groups of researchers using 12 distinct models. The extreme disparity in the bases of these models is best typified by comparing the fireball model, a so-called thermal model, to the intranuclear cascade model. In the nuclear fireball model one assumes a clean-cut geometry which divides the nucleons into spectators and participants, and then that the participants achieve thermal equilibrium before separating. In the cascade model the two-body collisions of all the constituents are followed. The aim in these model calculations has been to describe the 'background' physics of what goes on, so that one could search for interesting new effects. In the metaphor of the Berkeley Summer Studies, the models are tools for cutting through the weeds to look for the flowers. The amazing thing is that, within factors of two, most of the models were able to fit the data. It was thought that energy and momentum conservation together with a spectator-participant concept, constrains the models so that differentiating between them must await more accurate and extensive data. However, let us examine another possible explanation.

The most recent publication of a calculation is by J. D. Stevenson (*Phys. Rev. Lett.* 41, 1702; 1978). It contains results from one of the five intranuclear cascade calculations which are in progress. Because of the reasonable fit obtained he concludes that "this calculation shows that the radical assumption that a hot nuclear fireball is formed in nucleus-nucleus collisions is not necessary to explain existing experimental results." On the other hand, he also shows that the average emitted nucleon has undergone five to six scatterings and quotes results from gas studies to the effect that this is more than enough to show equilibrium features. Thus, it appears that Stevenson has shown microscopically that there is some justification to the macroscopic thermal models. He has possibly also given another explanation of why most of the model calculations agree: the large degree of thermalisation obscures the initial assumptions of the models. The significance of this point buried in Stevenson's article is not trivial. Although one can follow collective motion microscopically, macroscopic models are enormously simpler, and

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much more easily relate effects to observations. In addition, all the interesting effects we are looking for are macroscopic effects. Classical microscopic calculations, like the one by Stevenson, are useful in testing model assumptions, such as the degree of thermal equilibration, but provide little insight into the collective properties of hundreds of nucleons. We need macroscopic models characterised by a few bulk parameters, such as the compressibility modulus, which are adjusted to experimental data in order to learn new physics. For instance, for compression and re-expansion effects, some macroscopic calculations have just recently pointed out the effects which might be seen experimentally.

The data fitted by Stevenson were taken with a detector of small solid angle so that the experiment looked at only one particle from each event but accumulated data for many events. However, present experiments measure the multiplicity of charged particles associated with the particle in the main detector, and thus are able to select central collisions on the basis of their high multiplicities. Experiments that measure two particles in each event are in their early stages. These two-particle correlation studies will give a much better indication of the degree of thermalisation, because in a single nucleon-nucleon scattering the two nucleons tend to be correlated at 180° in the centre of mass, while in a thermal model they would be uncorrelated. The future lies in measuring as many of the final particles as possible in each event and thus determining the collective motion of the nucleons emitted. One hopes that one day it will be possible to relate data of this kind back to the equation of state of the compressed nuclear matter. □

Insects need cilia

from Michael Sleight

It is generally agreed that arthropods lack water-propelling ciliary organelles of the 9+2 pattern, except on the sperm of some members of the group. Recent observations by Bradley and Satir (*J. Cell Sci.* **35**, 165; 1979) suggest that insects have found it necessary to develop alternative structures to perform the function of fluid propulsion or at least the transport of uric acid crystals, in the Malpighian tubules. The authors have named these structures axopods, by analogy with the stiff microtubule-supported pseudopodia of the Heliozoa and Radiolaria.

The axopods found in the lumen of

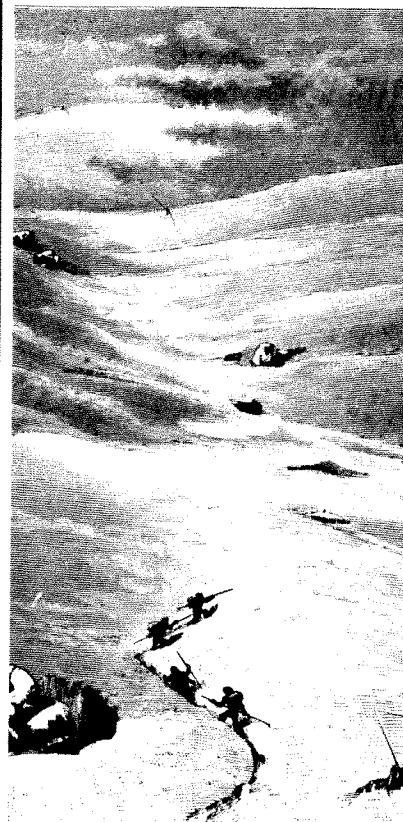
Malpighian tubules of the bug *Rhodnius* are branching projections occurring among, but longer than, the microvilli of the cells lining the lower tubule; they contain between 1 and about 50 microtubules and vary in thickness between about 0.2 and 0.8 μm . The microtubules are separate and regularly spaced, several diameters apart, but without any of the specific patterns or links found in protozoan axopods (Cachon & Cachon *Ann. Biol.* **13**, 523; 1974). Where the insect axopods branch, groups of microtubules separate and pass into each section. At the base of the axopod the microtubules extend deep into the cell to end near an inner cell boundary or in a cluster of mitochondria; no association has been found with a centriole or other microtubule-organising centre. Sections of the axopods reveal groups of longitudinal microfilaments close to the peripheral membrane.

It has not been possible to gain a clear view of the insect axopods in motion, but the movement of uric-acid crystals is consistent with propulsion by the axopods, and no other means of propulsion appear to be present in the experimental situation. The propulsive action is assumed to take the form of active lashing of the axopods. Analogous microtubular organelles other than cilia and flagella do not normally show this form of motion; the straight axopods of *Heliozoa* merely change their length to promote cell movement (Waters *J. Cell Sci.* **3**, 231; 1968) and the haptonemata of certain flagellates, flagellum-like projections that contain a small number of single microtubules, perform coiling movements (Leadbeater *J. mar. biol. Ass. U.K.* **51**, 207; 1971). The appendages of the marine protozoan *Sticholonche* may come nearest to insect axopods, for the numerous, stiff, microtubular exopods of *Sticholonche* are pivoted at the base and are caused to perform rowing motions by the contraction of (non-actin) microfilaments attached to their bases (Cachon, Cachon, Tilney & Tilney, *J. Cell Biol.* **72**, 314; 1977). It seems likely that microvilli of insect malpighian tubules may have been invaded by microtubules; while these provide the stiffness necessary for the propulsion of fluid, the actual bending forces may be provided by microfilaments, as Bradley and Satir suggest. Fluid propulsion of a similar type has been observed in a number of other situations in arthropods; it will be interesting if similar forms of modified microvilli have been adopted elsewhere or whether true cilia do exist on epithelial cells of arthropods. □

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A hundred years ago



OUR readers may remember that early in the year General de Nansouty, the hardy director of the Pic du Midi Meteorological Observatory, was cut off from communication with the world below, the severe weather having so affected the telegraph as to prevent it from acting. Fears were entertained for the General's safety, and M. Albert Tissandier resolved to organise a party for the ascent of the Pic and the succour of the veteran observer. An interesting account of this ascent appears in *La Nature*, to which we are indebted for the accompanying illustrations. The snow-storm having somewhat abated at Bagnères-de-Bigorre on January 9, M. Tissandier resolved to attempt the Pic next day, in company with three of General Nansouty's usual guides.

They set out at 9 A.M. on the 10th, and in spite of the deep snow and fallen avalanches, the ascent was at first not difficult. After equipping themselves for snow work at some huts occupied only in summer, the ascent was begun in earnest. The weather was grey and uncertain, the temperature 0°Cent. , with a thick mist that prevented anything being seen beyond 300 metres. The snow became deeper and deeper as they advanced, and one of the guides went before to show the way, the others following the marks of his footsteps up the steep slope of the mountain side. Sometimes they were buried to the waist, and often they had to rest to recover breath.

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review article

Nerves and genes

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Work with single-gene mutations in a variety of organisms is yielding insights on how genes build nerve cells and specify the neural circuits which underlie behaviour.

It is the business of scientists to explain away the magic in the world. The largest coherent body of magic remaining is the behaviour of men and animals. Behavioural geneticists try to construct plausible explanations for behaviour patterns by studying genetic variants. Historically, they have sought to compare inbred strains, or to cross them and examine the progeny¹. Such comparisons inevitably involve many genetic differences, making the results virtually impossible to interpret. A more recent approach initiated by Delbruck and Benzer has been to generate single-gene mutations which affect behaviour, to analyse the mutants, and to try to infer general principles about how normal behaviour is created. One advantage of such single-gene changes is that now behavioural patterns can be dissected into their component parts. This genetic extension of traditional ethology should have something important to say about how behaviour is organised and regulated, and has been reviewed recently². A more ambitious and potentially more powerful strategy is to examine the effects of single-gene mutations on the ultimate common denominator of behaviour—the physiology of nerve cells and the wiring patterns of neural circuits. This review traces the recent progress of this approach, selecting the work which seems to offer the best prospects for the future.

Single cell physiology

The underlying mechanism for neural activity is ionic. Typically, a metabolically dependent ion pump moves Na^+ out of and K^+ into the neurone, creating a steady potential difference across the membrane. In an action potential, depolarising the cell membrane beyond a threshold voltage opens channels in the membrane specific to each ion, the sodium channel opening and closing more rapidly than the potassium channel. The resulting large change in membrane potential depolarises nearby patches of membrane, allowing the propagation of the impulse through the cell. When the action potential reaches a presynaptic terminal, Ca^{2+} enters through its own channels, causing the release of chemical transmitters which diffuse across the synaptic junction to the next cell, opening still another class of ion channels in the next cell, and thus continuing transmission of the electrochemical signal.

The elucidation of this picture, and the explanation of most of the cell's electrical properties in terms of a few ionic conductance changes, is the major triumph of neurobiology in the last half-century. A substantial problem remaining for neurobiologists is to understand the molecular operation of the various ion channels (presumably proteins) in the neuronal membrane. Two organisms have been found in which the channels can be altered by mutation.

Paramecium

Although unicellular, *Paramecium* behaves as if it were rational. Ordinarily, it propels itself forward through the water by the coordinated beating of cilia distributed over its exterior. However, if it swims into a physical barrier or a noxious chemical, it quickly reverses the direction of its ciliary beat, swims backwards for a time, then sets off in a new direction³. The simultaneous reversal of all the cilia suggests an electrical basis for the behaviour, and experiment bears this out. It is possible to record intracellularly from *Paramecium*, and observe an extraordinary repertoire of ion conductance changes⁴. These can best be understood by following events during an avoidance reaction.

Mechanically stimulating the front of the animal (mimicking collision with a barrier) depolarises the membrane, probably by local influx of sodium ions. This depolarisation spreads throughout the cell by passive current flow and raises the membrane potential above threshold. At this point, a separate, calcium conductance channel is activated, and several regenerative action potentials occur, based on calcium influx. The increased Ca^{2+} concentration inside the cell somehow changes the pattern of ciliary beating⁵, reversing the direction of power strokes so the *Paramecium* backs up. After 1–5 s, the calcium level in the cell decreases (presumably by active ion pumping) and the animal resumes forward swimming.

This fixed action pattern involves relatively few events, and at least two of them are ion conductance changes. Therefore, one can screen for behavioural mutants in *Paramecium* with the hope that some will have altered channels. Using ingenious selection techniques, Kung *et al.* have found many abnormal behaviour patterns⁶. Two are especially dramatic: *pawn* mutants, like chess pawns, cannot back up. This is because their membranes will not produce calcium spikes even when depolarised far above normal threshold. Apparently, *pawn* mutants lack the normal voltage-dependent calcium conductance channels. The *paranoliac* mutant, on the other hand, shows bouts of reverse swimming whether it encounters noxious stimuli or not. Electrophysiology shows that *paranoliac* mutants have normal sensory generator potential and calcium spike initiation, but are blocked in membrane repolarisation after the action potential⁷. Instead of a series of spikes, they show a long, flat peak. This abnormality of *paranoliac* is expressed only in the presence of external sodium⁸. The membrane component affected by *paranoliac* mutations remains unknown. However, radioactive tracer studies indicate that the depolarised membrane in the mutant becomes abnormally conductive to many ions, including calcium, which explains the long periods of ciliary reversal⁹.

So far so good. The visible behaviour of *Paramecium* is closely correlated with the electrical properties of its membrane, and some behavioural mutations affect ion conductances in an apparently straightforward manner. Sophisticated electrophysiological experiments are feasible, such as clamping the membrane voltage quickly to a new level and watching the kinetics of conductance changes. Oertel *et al.*¹⁰ built such a voltage clamp and compared *pawn* mutants (which have no calcium spikes) with normal *paramecia*; thus, they were able to measure the conductance changes specifically attributable to the calcium channels. One could hope to do similar voltage clamp studies with 'leaky' *pawn* mutations which do not completely abolish calcium conductance, compare the kinetics of calcium conductance changes in normal and mutant individuals, and try to gain some insight into the mechanism of voltage-dependent channel activation and inactivation (gating).

Prospects for chemically isolating the channel proteins also look good. The lipid membrane which sheaths each cilium is continuous with the plasma membrane of the cell. When treated with agents such as chloral hydrate, the cilia become stiff and break off from the cells; they can be isolated and membrane fractions purified from them using conventional biochemical methods¹¹. The calcium channels, at least, are concentrated in the ciliary membrane (de-ciliated *paramecia* show no calcium spikes^{12,13}). This raises the possibility of finding altered membrane proteins in *pawn* mutants, then examining the chemical alterations in the mutant channel. Explaining ion channels and gating in chemical terms is a serious problem; *Paramecium* seems a logical place to try.

However, the behaviour of *Paramecium* is much more complicated than the outline above suggests. An increasing variety of unexplained behavioural mutants (spinners, sluggishes, staccatos) have been isolated. Detailed electrophysiology shows a correspondingly bewildering array of ion conductance channels in the cell membrane. It is as if *Paramecium* tried to compact all the functions of a nervous system into one cell.

These eccentricities of *Paramecium* do not detract from its real promise. Voltage-dependent calcium channels are present in the ciliary membrane. It is a matter of time before the channel proteins, apparently specified by the *pawn* genes, are chemically isolated. *Paramecium* is the one system where electrophysiology, membrane chemistry and genetics can all be directly applied to find out what a conductance channel looks like and how it opens and closes.

Drosophila

At first glance the fruit fly seems unpromising for neurophysiological studies: most of its 10^5 neurones are very small ($<5\ \mu\text{m}$); they interact in a tangle of neuropil, and they receive their oxygen supply through tracheoles, making respiration of the preparation a problem. These difficulties limited the success of the earliest studies on adult mutants by Ikeda *et al.*¹⁴⁻¹⁷. They were overcome by the inspired naivete of L. Y. Jan, Y. N. Jan and W. A. Harris, who simply slit third-instar larvae down their backs and pinned them out with the nervous system and ventral muscles exposed. The muscle fibres in two longitudinal cords are wide, flat and easy to record from, and the motor nerves can be cut and stimulated directly with a suction electrode. Using this system, Jan and Jan have worked out the bases of the muscle resting and synaptic reversal potentials, identified L-glutamate as the probable transmitter^{18,19}, and shown that in low calcium solutions neuromuscular transmission in *Drosophila*, like that of the frog²⁰, is quantal, each unit of postsynaptic excitation presumably being caused by the release of transmitter from one synaptic vesicle. Thus, given a mutation affecting synaptic transmission, they could determine if the abnormality was presynaptic (affecting the number of quanta released) or postsynaptic (affecting responsiveness to transmitter and hence the apparent size of quanta). Such analysis also allows the detailed kinetics of transmitter release to be followed.

Screening of previously isolated mutations which affect whole-fly behaviour revealed one in which the larval neuromuscular preparation was also altered. Flies carrying a *shaker* mutation²¹ show spontaneous rhythmic leg movement under anaesthesia. Larval neuromuscular transmission is relatively normal in physiological conditions but becomes obviously different in low calcium solution. Paradoxically, it shows greater synaptic efficacy, with more transmitter released over a longer time course. Jan *et al.*²², applying classical physiological techniques, showed that the abnormality in *shaker* is presynaptic. Conductance to calcium was prolonged up to 1 s after stimulation of the mutant nerve terminal. Apparently, the calcium channels in the mutant open normally but are slow to close, so that calcium continues to enter the presynaptic terminal, causing greatly increased vesicle fusion and transmitter release.

It is then natural to wonder whether the abnormality is in the calcium channels themselves or in the action potential which causes them to open. In fact, the action potential is abnormal in the mutant. Normal synapses can be made to behave like *shaker* mutant synapses by applying tetraethylammonium or 4-aminopyridine, drugs which block potassium conductance. Furthermore, extracellular recording shows electrical activity in the mutant motor nerve which persists after stimulation has ceased (Jan, Jan and Wu, unpublished data). All this indicates that the *shaker* mutation blocks potassium channels in the motor neurone, so the voltage-dependent increase in potassium conductance which repolarises the nerve after an action potential²³ never occurs. Extended depolarisation of the nerve then leads to prolonged calcium conductance and enhanced vesicle release at the terminals.

This experimental success stimulated a direct search in Benzer's laboratory for new physiological mutations, specifically temperature-sensitive paralytics which affect larvae as well as adults. One such mutant, *nap*, was found²⁴. Its name describes both its outward appearance (torpor) and its physiological abnormality (no action potential) at 35 °C. Motor nerves in mutant larvae fail to conduct action potentials at this elevated temperature, whereas those of normal larvae are unaffected. Most probably, the mutant gene specifies voltage-dependent sodium channels, which become blocked at high temperature.

If the above arguments are true, then mutants are available in *Drosophila* which affect the two conductances classically defined by Hodgkin and Huxley: *shaker* blocks g_K while *nap* blocks g_{Na} at high temperature. We are left with the problem of what to do with the mutants. It would be impossible to voltage clamp a mutant motor nerve to measure conductance kinetics directly, although it may be worthwhile to search for larger neurones in the fly which are similarly affected. Prospects for biochemical studies with the mutants also look intimidating. Nevertheless, if one set out to apply genetics to the phenomena underlying the Hodgkin-Huxley model—ion selectivity and voltage-dependent conductance changes—one could not hope for a better start.

A less easily explained physiological mutant, the *bang sensitive* (*bas*) fly, has been found in several laboratories^{25,26}. These mutants pass out for several seconds after mechanical shock, as when their culture vials are rapped on a table. Jan and Jan²⁷ discovered that one of these, *bas*^{MW1}, is specifically enhanced in long-term facilitation at the larval neuromuscular junction. Long-term facilitation, sometimes called post-tetanic potentiation, means increased synaptic efficacy after a train of repetitive stimuli. It is phenomenologically similar to ordinary (short-term) facilitation, except that the amount of pretreatment stimulation required for its onset is greater and it lasts longer. The two processes can now be formally distinguished using mutations: *bas*^{MW1} enhances only long-term facilitation, whereas *shaker* specifically increases short-term facilitation. In *bas*^{MW1}, long-term facilitation requires sodium (but not calcium) influx to the presynaptic terminal during the pretreatment period. The increased synaptic efficacy after pretreatment is due to a prolonged period of calcium conductance in the terminal after each stimulus. How a rise in intracellular sodium might

induce the calcium gates to remain open is not understood. Whether other ion channels are affected is not known.

Normal flies (and other invertebrates²⁸) show long-term facilitation like *bas^{MW1}*, although they require longer and more intense pretreatment stimulation. Also, the physiological properties of *bas^{MW1}* can be mimicked by treating a normal neuromuscular preparation with ouabain, an inhibitor of the sodium-potassium pump. This suggests that the *bas^{MW1}* mutation may simply reduce the efficiency of the pump. Alternatively, it could accentuate the mysterious process by which intracellular sodium leads to facilitation.

Neural circuits

The behaviour of multicellular animals is the result of interactions between nerves. Conceptually at least, the question of how nerves combine to produce behaviour may be broken down into two parts: how nerve circuits work, and how these circuits come to be appropriately wired during development. In reality, however, the study of circuitry inevitably leads to questions of neural development and specificity. This is because mutations affecting wiring usually begin to act during development. As a result, genetic studies of nerve interactions which have proceeded beyond the level of drawing the circuit diagrams—no mean task in itself—have either evolved into studies of development or abandoned the 'genetic lesion' technique for the laser-beam scalpel, or both.

Mapping *per se* is an attempt to define which cells are connected to which, and to characterise the anatomical interactions as strong or weak, electrical or chemical, inhibitory or excitatory, and so on. Direct physiological confirmation of these descriptions has rarely been possible, however, for reasons encapsulated in Gould's corollary of Quinn's law. Quinn's law states that the convenience of genetics multiplied by the quality of the behavioural repertoire is a constant for all animals. Hence, bacteria have superb genetics and marginal behaviour, while undergraduates have very interesting behaviour but cumbersome genetics. Gould's corollary states that the quality of the genetics multiplied by the ease with which single nerve cells can be identified and penetrated is also a constant.

However, mapping of isogenic organisms has produced one very encouraging general result: the overall pattern of wiring—which nerve cells are connected to which—is found to be remarkably constant from one animal to the next, whereas the details of the wiring—the specific number and anatomical location of the connections—are quite variable. As the same genes produce the same behaviour against a background of synaptic and dendritic variability, the hyper-fine structure of neural wiring can reasonably (and fortunately) be ignored²⁹.

Nematode nervous system

The most ambitious attack on the genetic control of neural wiring is being made in Brenner's laboratory with the nematode *C. elegans*. This millimetre-long creature has 'good' genetics³⁰, compensatingly simple taxis behaviour³¹⁻³³, and a seductively finite 258 nerve cells. Intuitively, it seems possible to map all the nerves and look for general principles of circuitry. Goldschmidt had the same idea 70 years ago, and mapped the large nematode *Ascaris* using serial sections and a light microscope³⁴. Brenner *et al.* have mapped most of *C. elegans* using an electron microscope, 20,000 serial sections per nematode, a computer, and much persistence^{35,36}. In nearly all respects, *C. elegans* is found to be a miniature *Ascaris*.

Conceptually, the nematode nervous system is divisible into four parts: the head, which contains virtually all the sensory apparatus; the pharynx, which mediates ingestion; the nerve ring or 'brain' in the neck; and everything further back. The muscle system can be classified along the same lines: the head muscles, which aim the head; the pharynx muscles, which manipulate and pump food; the neck muscles, which help aim the head and aid in swimming; and the rest of the body, which swims.

The 50 or so nerves in the head have been mapped and guesses have been made about their functions. Specific chemotaxis and tactile mutants have been created which display variable anatomical aberrations at least roughly corresponding to these guesses³⁵. However, the incomplete penetrance and pleiotropic nature of these sensory mutations is frustrating.

There are about 20 nerves in the pharynx—sensory cells, interneurons, motor neurones, and multi-functional cells which seem to act as both mechanoreceptors and motor neurones. These have been sorted into a hypothetical but plausible circuit with typical ethological releaser and fixed-action pattern units³⁷. The present impossibility of doing electrophysiology on this animal and the lethal nature of defects in a feeding circuit make analysis difficult.

The 200 or so nerves which interact in the nerve ring create a maze so complex that little can be said about them except that the sensory nerves from the head as well as the head and neck muscles go into it, and the interneurons which control the ventral cord come out of it³⁸.

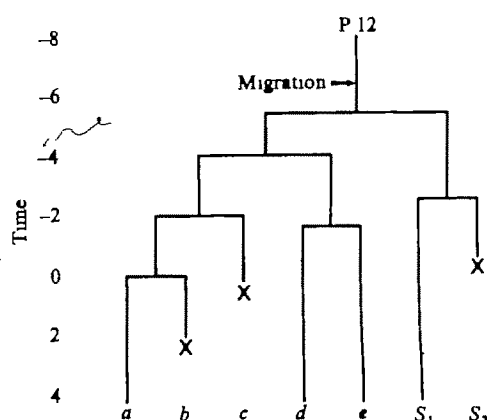


Fig. 1 Schematic representation of post-embryonic development of cells in a portion of the nematode ventral nerve cord, showing patterned divisions from a single precursor cell P12. Cells a-e exemplify five anatomical classes of neurones present in the mature animal; S₁, S₂ are somatic cells; X indicates cell death.

The rest of the nematode, although long ignored, has turned out to be by far the most rewarding to study. The nerve ring sends out a ventral cord which runs the entire length of the animal and contains about 60 motor neurones. Muscles in the ventral half of the body send processes to the ventral cord. The dorsal cord, which services the muscles in the dorsal half, is discontinuous, being made of pieces sent up from the ventral cord³⁹.

A closer look reveals that this swimming motor system consists of 12 almost identical wiring groups in tandem, each with five classes of cell. Developmental studies^{40,41} indicate that these groups arise from 12 precursor cells which divide several times each (Fig. 1). The pattern of division and specialisation is intriguing. After a division, the posterior daughter cell is almost always more like the parent than the anterior cell, and is also more likely to be programmed to die. Relative position may be the crucial cue for nematode differentiation. Although the different groups retain different classes of cells, the same pattern of division is always followed (Fig. 1). For example, although in all female *C. elegans* cell c dies, it is always necessary to make cell c so that its sister can go on to divide into cells a and b. More remarkably, in two of the groups, cell b is also necessarily made and killed so as to produce a cell a (Fig. 1).

Again, the wiring diagram leads immediately to speculation about how the circuit works. Cells a and b look like antagonists, one (who knows which) excitatory, the other inhibitory. Cell d might be responsible for the deep ventral bend which seems

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necessary to reverse direction. Cell *e* is perhaps part of a reciprocal inhibition system used to coordinate movement. If these guesses are correct, then *C. elegans* swimming, like the flight of many insects, is myogenic, confirming an earlier and equally elegant behavioural analysis⁴².

C. elegans, quite unexpectedly, has been found to be a very useful animal for developmental studies. It is possible in principle to establish the lineages of all its cells, and this has already been done for 180 of its 550 embryonic cells⁴³. Post-embryonically, new neurones are formed (Fig. 1) and some old ones undergo remodelling⁴⁴. In larvae, class *d* cells innervate only the ventral muscles, whereas in adults they innervate only dorsal muscles. A study with mutants⁴⁴ sheds some light on the general rules of neural development in *C. elegans*. One mutant lacks all the late-developing motor neurones. These are the only cells which innervate the class *d* nerves. Nevertheless, the *d* cells rewire themselves as usual even in the absence both of normal input from the neurones which drive it and of others which take over its connections to the ventral muscles. In *C. elegans*, at least, no synaptic input is necessary to make, break and remake normal synaptic output connections. Unfortunately, the reverse is true of other animals, especially mammals. In any case, despite a limited behavioural repertoire and the impracticability of physiological confirmation, nematodes are providing fascinating information about neural development.

At this stage the same questions might be answered by laser-beam lesions rather than mutations, but the ultimate power of mutant analysis is clear. For example, a mutation has recently been found which rearranges the pedigree of developing nerve cord cells, and another interferes with the cell divisions shown in Fig. 1, producing multinucleate neurones with strikingly novel morphology (H. R. Horvitz, personal communication).

Songs and sensory circuits of insects

Most of the *Drosophila* nervous system is quite complex, and neural analysis of behaviour patterns is slowly progressing from the outside of the animal in. For example, courting males sing a species-specific 'love song' by extending and vibrating their wings towards females⁴⁵. The circuits underlying this behaviour can be dissected with gynandromorphs—animals which are part male and part female. Flies with male heads and female bodies will extend their wings to females as if in courtship, so apparently the 'will' to begin the song is located in the brain⁴⁶⁻⁴⁸. However, the fly goes on to produce a proper *Drosophila* song only if there is some male tissue in the thorax as well; the actual pattern-generating circuit is probably located in the thoracic ganglion. This parallels the situation in crickets, where males with specific brain lesions are normally incapable of song production, although a headless cricket body will produce a complete song if the thoracic ganglion is stimulated⁴⁹. In both species, the decision to sing and the actual song pattern are mediated by separate neural structures. Other experiments with hybrid crickets⁵⁰ suggest that the male circuit which generates the courtship song pattern is specified by the same genes as the female circuit which recognises the song (they may even be the same circuit).

Crickets have also been used to study neural development. One single-gene mutation causes the loss of the mechanoreceptive filiform hairs on the cricket cerci⁵¹. As a result, the mutant shows no sensitivity to touch on the cerci. The sensory nerves from the missing hairs are completely normal, but the large interneurone to which they project is 'withered'. Apparently, normal stimulation is unnecessary for the maintenance of receptor cells, but is crucial for the integrator cell. This has striking parallels in mammalian visual development. In cats, for example, failure to see out of one eye during a critical period leaves the receptor unaffected, but prevents the normal development of the visual cortex, causing irreversible blindness in one eye⁵². We would like to believe that there is a general principle of neural organisation here somewhere, and the dis-

covery that cricket cerci have a critical period makes that possibility more likely⁵³. If one cercus is waxed over for a time early in development so that the hairs cannot be stimulated, the animal becomes deaf on that side. Removal of the wax has no effect.

Mouse mutants

Mouse brains, although small and unintelligent, are organised like ours and thus allow investigation by conventional neuroanatomical, histological and physiological techniques. Moreover, large areas of the mammalian brain, such as the cerebellum and visual striate cortex, are composed of similar circuit elements repeated over the surface of the brain^{54,55}; one can therefore study a small patch of neural tissue with the expectation that the wiring in the rest of the structure follows the same rules. Finally, there is the hope that functional insights gained with any mammalian brains may be applied to ourselves, and that genetically derived pathologies in mice may have clinical analogues in man. However, the system has one great disadvantage: it is currently too expensive deliberately to induce mutations in mice and screen the progeny for a desired phenotype. This means relying on inbred stocks which have been retained because they acted very abnormally—reeling, trembling, twirling, staggering, and so on. Most neurological mouse mutants chiefly affect the cerebellum, whose anatomy is conveniently simple and repetitive, with only five types of cell, and whose circuitry and synaptic interactions are well understood⁵⁴. Unfortunately, the mutants isolated so far are drastic^{54,57}. Two, *weaver* and *staggerer*, cause massive degeneration of granule cells (the most numerous neurones, which receive the major input from the rest of the brain). Two more, *nervous* and *pcd*, lead to loss of the Purkinje cells (the largest neurones, which provide the only output from the cerebellum).

Genetic lesions such as these are useless for understanding the subtleties of neural development or finding out how the normal cerebellum functions to control posture, coordination and balance. They do, however, raise interesting questions about neurogenesis. For example, the mutation *pcd*, in addition to rapid degeneration of cerebellar Purkinje cells, also produces slow degeneration of mitral cells in the olfactory bulb and photoreceptors and induces structural abnormalities in spermatozoa, leaving other cells in the animal unaffected^{57,58}. Are the four affected cell types under similar metabolic stress, which is in some way aggravated by the mutation? It is difficult to see what they have in common. Do the neurones occupy analogous positions in an unrecognised developmental hierarchy, and thus become dependent on the same controlling element? Evidence from other mutations would be needed to consider such an idea seriously.

One important question is whether the primary lesion is actually in the affected cells. Unlike those of nematodes, vertebrate neurones may die from unrequited synapses, either because they lack proper input or because they fail to establish connections with their target cells. Thus, normal granule cells could degenerate because cryptically abnormal Purkinje cells refuse to interact with them or vice versa. This issue has been elegantly settled for the *pcd* mutation. Mullen⁵⁹ made chimaeric mice⁵⁹ whose cerebella contained mixtures of *pcd* and normal cells. By properly selecting parent strains and using enzyme markers, he was able to ascertain the genotypes of individual cells and it was thus possible to show that all the *pcd* cells had atrophied and all the normal cells survived. Therefore, the defect is cell autonomous: that is, if a Purkinje cell dies it is its own (genetic) fault. This is unsurprising. However, the opposite is true for *reeler*, a mutation which generally affects neuronal migration during development and specifically induces abnormal, twisted morphology in Purkinje cells. In chimaeric normal *reeler* cerebella, Purkinje cells of both genotypes were sometimes normal-looking, sometimes abnormal⁶⁰. In this case the morphology of the cells is specified from outside, by the neural environment.

With the mutant *staggerer*, there is some indication that the major observed effect, granule cell degeneration, is due to a defect in the Purkinje cells which fail to form proper dendritic spines, the normal target for granule cell synapses^{61,62}.

So far, in spite of these elegant analyses, the mouse mutants have taught us little about the development of the cerebellum and nothing about its function. However one is of particular interest; *reeler* affects not only the cerebellum but cerebral cortex as well, altering its development radically. The embryonic cortex forms and develops as a hollow shell of cells around the fluid-filled lumen of the anterior neural tube. Neuronal cell divisions occur only on the inside surface of the shell. In normal development, the earliest neurones to form tend to remain near the lumen to form the deepest layer of the cortex, and cells formed at later stages migrate past all the others to lie in more superficial layers. The position of a neurone in the cortex is thus determined largely by the time of its last cell division, with the youngest cells on the outside. In *reeler*, the developing cortical neurones fail to migrate past each other so that the oldest cells remain in superficial layers with younger cells progressively deeper⁶³.

Caviness⁶⁴ has examined the visual area of this 'inside-out' cortex and found that incoming fibres carrying sensory information from the eyes terminate in the correct layer; that is, instead of penetrating the cortex from below, making a few synapses in the deepest layer, then rising to terminate at an intermediate layer, as in the normal mouse, the same fibres immediately ascend to the most superficial cortical layers, form some synapses, then reverse direction to terminate in intermediate layers. This inversion rule also holds true for different classes of output axons coming from the cortex.

When he examined individual neurones in *reeler* cortex, Caviness found that some looked normal, others were apparently inverted, and still others had axons which abruptly reversed direction after leaving the cell soma. The findings above, taken *in toto*, look very discouraging from the mouse's point of view. Nevertheless, physiological recordings by Dräger⁶⁵ show that most of the cells in the *reeler* striate cortex have normal retinotopic organisation and visual response

characteristics, including binocularity and orientation and direction selectivity. The upside-down *reeler* cortex is functionally indistinguishable from the rightside-up normal one.

No one has studied chimaeric cortices containing both normal and *reeler* cells. It would be interesting to see whether normal organisation is enforced on the mutant neurones and, if not, whether proper visual receptive fields persist with competition between normal and inverted cortical organisation.

It is worth noting that Siamese cats are also neurological mutants. The gene (temperature-dependent albinism) which specifies the pale colour of their body fur also reduces the pigmentation of the cells behind the retinas in their eyes⁶⁶. This pigment deficit apparently induces dramatic miswiring of optic nerve fibres carrying visual information to the brain, causing many to reach the wrong destination. This miswiring leads to a cascade of neurological defects in centres involved with vision—in the thalamus, in primary and secondary visual cortex, and perhaps in even higher areas. Although the original mutant defect is probably non-neural, work with the visual system of these cats (reviewed in refs 67–69) may tell us much about the developmental rules which normally lead to proper arrays of neural connections and orderly maps in the mammalian brain.

Conclusion

At its inception 15 years ago, the neurogenetic approach seemed very promising. Since then it has led to many elegant experiments, much tantalising information, and some insight. In general, however, behavioural geneticists have asked one set of questions and answered another. The central questions of neurobiology—the chemical bases of ion gating and synaptic transmission, the mechanisms involved in specifying a neurone's shape and determining its interaction with other cells, the changes in neurones and circuits which underlie learning—are all untouched by the approach. The field is still promising, in the best and the worst sense. If progress in the next decade equals that of the last, the promise will begin to be fulfilled.

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articles

Very large array observations of solar active regions

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The first very large array (VLA) synthesis maps of solar active regions are presented here. The observational capabilities, and the basic techniques of using the VLA to observe the Sun are also described. Comparisons of VLA maps with magnetograms are also made.

OBSERVATIONS of the Sun with three-element interferometers at centimetre wavelengths and angular resolutions of a few arc seconds have shown that solar active regions contain intense (10^5 – 10^6 K), small-scale (less than $15''$), circularly polarised (above 30%) sources which dominate solar emission at these wavelengths and angular resolutions^{1,2}. The radiation from these sources has been interpreted in terms of gyromagnetic processes involving strong magnetic fields and thermal electrons in the dense coronal regions above sunspots^{2–6}. As long as the active regions are not emitting flares, the intensity, angular size, and degree of circular polarisation of the small-scale sources remain remarkably constant for intervals of at least 12h. This stability makes these solar sources ideal candidates for the aperture synthesis technique which has been developed by

radioastronomers to obtain two-dimensional maps of celestial radio sources with arc second angular resolution⁷. When applied to the centimetre wavelength radiation of the Sun, these techniques may be used to test the gyro-resonance absorption theory of this component of solar emission. Moreover, the synthesis technique can be used to obtain high resolution maps of circular polarisation which delineate the magnetic field structure in the solar corona. These structures can then be compared with optical wavelength Zeeman effect observations of the magnetic field in the underlying photosphere and chromosphere. Because of scintillations in the terrestrial atmosphere, these optical wavelength observations are limited to the angular resolutions which are now available at radio wavelengths using the synthesis technique. The first synthesis maps of solar active regions at centimetre wavelengths were obtained using the 12 25-m paraboloids (20 interferometer pairs) of the Westerbork synthesis radio telescope (WSRT)^{8,9}. In this case, a synthesised beam of $6'' \times 22''$ at a wavelength of 6 cm was used to show that solar active regions contain small-scale sources (with peak brightness temperatures as large as 10^6 K and circular polarisations of $\sim 50\%$) surrounded by a lower intensity $\sim 10^5$ K halo.

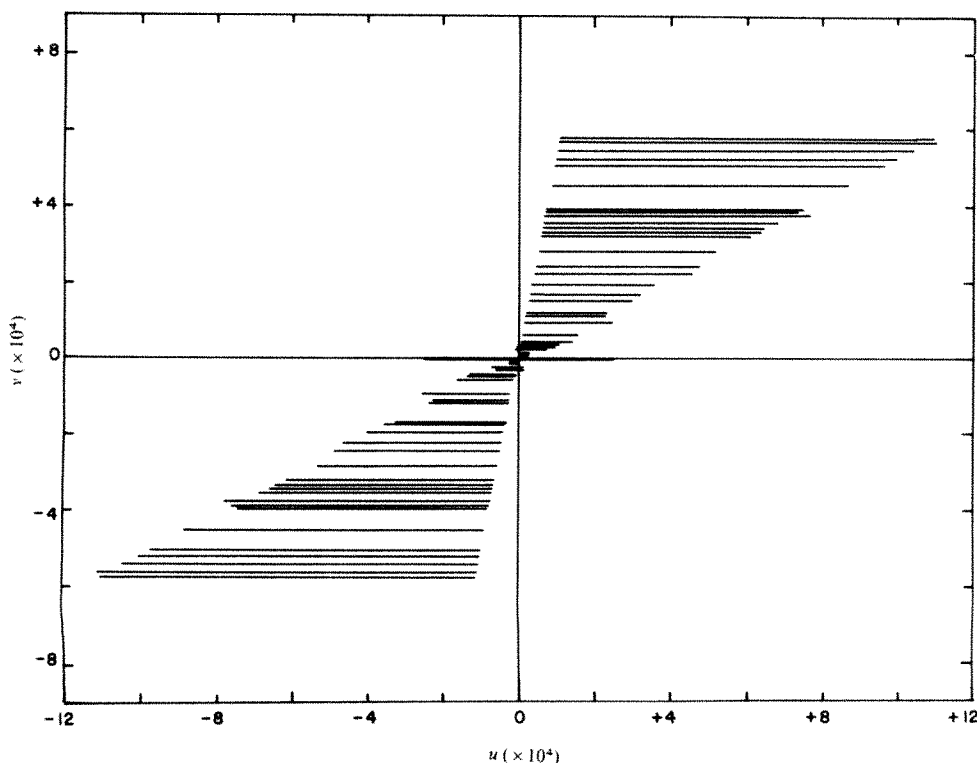


Fig. 1 The u - v tracks obtained while observing the Sun at 6 cm wavelength with 45 interferometer pairs of the VLA when the Sun is at a declination of 0° . Because the longer baselines were not used in our maps, we have omitted here the 10 tracks from the 10 interferometer pairs connecting to the furthestmost antenna located 10.473 km from the array centre. The dark line at $v = 0$ denotes the tracks obtained while observing the Sun at 6 cm wavelength with the 20 interferometer pairs of the WSRT when the Sun is at a declination of 0° .

Although circularly polarised emission was observed from both sunspots and plage peaks, the more intense emission came from sunspots whose magnetic polarity coincided with the direction of circular polarisation. We describe here synthesis maps of solar active regions obtained using 12 25-m paraboloids (65 interferometer pairs) of the VLA now under construction near Socorro, New Mexico by the National Radio Astronomy Observatory¹⁰. In this case a synthesised beam of $1'' \times 3''$ is obtained at a wavelength of 6 cm.

Construction and operation of the VLA

Twenty-seven antennas are being constructed, tested and placed along the three arms of the WYE configuration of the VLA. The position angles of the north, south-east, and south-west arms are, respectively, 355° , 115° and 236° ; whereas the geodetic coordinates of the array centre are $34^\circ 04' 43.497''$ N (latitude) and $107^\circ 37' 03.819''$ W (longitude). With this configuration and location the most complete spatial frequency coverage is obtained when the Sun is at its larger positive declinations. The

permanent antenna stations are placed along each arm at distances ranging between 0.045 and 21.0 km from the array centre, thereby providing effective angular resolutions ranging between $0.57''$ and $4.44''$ at a signal wavelength of 6 cm for the interferometer pairs along each arm (each combination of two antennae makes one interferometer pair). Similar angular resolutions are available for the interferometer pairs consisting of antennae placed on different arms. The individual antennae have 25 m diameter main reflectors with a feed system¹¹ designed to receive signal wavelengths of 1.3, 2, 6 or 21 cm with respective beamwidths of 1.9, 2.9, 8.6 and $30''$ and respective aperture efficiencies of 46, 54, 65 and 50%. About 1 min is required to rotate the antenna feeds to sample signals at different wavelengths. Two independent systems simultaneously accept two oppositely polarised signals at the chosen wavelength, and these signals are converted into intermediate frequency (IF) signals with bandwidths of 1.5 MHz, 12 MHz or 50 MHz. The two IF signals received at each antenna are sent to a central location where they are multiplied together to give the correlated flux for each oppositely polarised signal for every interferometer pair. Each correlated signal is time averaged to give its amplitude and phase every 10 s.

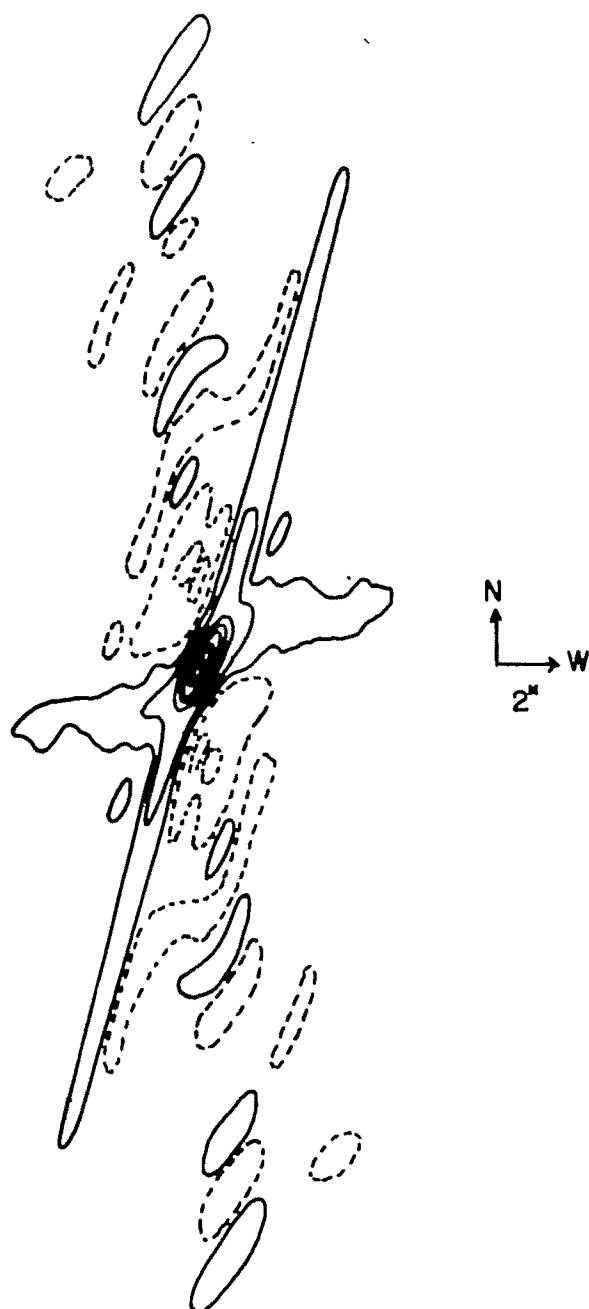


Fig. 2 The dirty VLA beam pattern at a signal wavelength of 6 cm constructed from observations during the daylight hours on 1 April 1978.

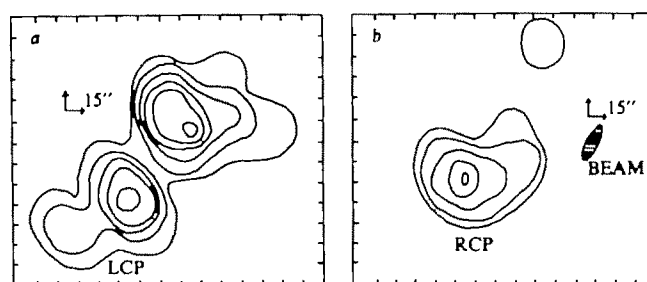


Fig. 3 VLA synthesis maps of solar active region 1046 obtained with one days observation on 30 March 1978. North is up, west is to the right. *a*, The left; and *b*, the right hand circularly polarized signals; $\lambda = 6$ cm.

The present digital system of the VLA is designed to handle up to 12 antennae with two IF signals each, but more sophisticated electronics designed to handle 27 antennae with four IF channels each are being installed. For the observations reported here we used 12 antennae with three antennae positioned along the south-east arm at 0.080, 0.090 and 0.147 km from the array centre and nine antennae placed along the south-west arm at 0.045, 0.484, 0.710, 0.970, 1.589, 3.188, 5.223, 7.659 and 10.473 km from the array centre. Altogether the average correlated flux from 55 interferometer pairs was sampled every 30 s at a signal wavelength of 6 cm for both the left hand circularly polarised (LCP) and right hand circularly polarised (RCP) signals. When observing the Sun at centimetre wavelengths the system noise is completely dominated by the solar brightness temperature of 10^4 – 10^6 K. The theoretical r.m.s. noise fluctuation in the correlated flux density obtained with each antenna pair is 6.4 Jy ($1 \text{ Jy} = 10^{-23} \text{ erg s}^{-1} \text{ cm}^{-2} \text{ Hz}^{-1} = 10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$) for a system noise temperature of 10^4 K with our chosen bandwidth of 12 MHz and an integration time of 30 s. We later averaged the data over 5 min intervals to obtain a r.m.s. noise of 2 Jy for each interferometer pair.

Active regions 1046 ($24^\circ \text{S } 7^\circ \text{W}$ at 12h UT on 30 March) and 1056 ($12^\circ \text{N } 65^\circ \text{E}$ at 0h UT on 31 March) were observed on 30 March and 1 April 1978 respectively, at a signal wavelength of 6 cm. For each antenna pair the correlator outputs were calibrated by observing 3C 84 or CTA102 for 5 min every 20 min, whereas the solar active region was observed during the remaining 15 min of each 20-min period. The frequent observations of the calibrator lessened the effects of tropospheric refraction variations, and calibrated the instrumental gain, polarisation and phase fluctuations.

Measurements

For each polarisation of each antenna pair the correlated flux, $S_{mm}(\text{Sun})$, and the corrected phase $(\phi_m^* - \phi_n) - (\phi_n^* - \phi_m)$ when observing the Sun were taken to be the amplitude and phase of the visibility function, $V(u, v)$. Because corrections for the motion of the Sun across the sky, its rotation about its axis, and its horizontal parallax were incorporated in the computer program which determined the pointing of each antenna, the changing values of the visibility function could then be used to measure the brightness distribution of the active region $I(x, y)$, through the fundamental Fourier transform relationship

$$V(u, v) = I(x, y) \exp[+2\pi i(ux + vy)] \quad (1)$$

where the visibility function and the brightness distribution are determined separately for the two circular polarisations, u and v are the projections of the baseline vector in the direction of increasing right ascension and declination, respectively; and x and y are, respectively, coordinates in the direction parallel to increasing right ascension and declination. These quantities, defined in radians, are given by

$$\begin{aligned} u &= B_x \sin(h) + B_y \cos(h) \\ v &= -B_x \sin(\delta) \cos(h) \\ &\quad + B_y \sin(\delta) \sin(h) + B_z \cos(\delta) \end{aligned} \quad (2)$$

where B_x , B_y , and B_z are the topocentric coordinates of the baseline vector in units of the observation wavelength, and h and δ respectively denote the hour angle and declination of the active region.

As the Earth rotates, the hour angle of the active region changes and the projected baseline describes an ellipse in the u - v plane (the Fourier plane of the brightness distribution). The extent of the coverage in the u - v plane depends on the Sun's declination, the orientation and length of the baseline vector of each interferometer pair, and the total number of interferometer pairs. During the observations reported here, for example, the Sun was near the vernal equinox when the Sun's declination passes through zero degrees, and the elliptical u - v tracks degenerate into straight lines. Figure 1 shows the u - v tracks when the Sun is observed at zero degrees declination with the VLA and with interferometers which are orientated in a strictly east-west direction such as the Westerbork synthesis radio telescope (WSRT). For the east-west baselines, v becomes zero for a declination of 0° . Later in the summer or winter, when the Sun's declination can respectively reach $+23^\circ$ and -23° , the extent of the v coverage by the WSRT broadens. For the VLA, the straight lines given in Fig. 1 become shortened or lengthened ellipses, respectively, for the southern and northern declinations.

The correlated flux when observing the Sun for each polarisation with each antenna pair (m, n) was determined from the equation

$$\begin{aligned} S_{mm}(\text{Sun}) &= \frac{R_{mm}(\text{Sun})[T_{mm}(\text{Sun})T_{nn}(\text{Sun})]^{1/2}}{R_{mm}(\text{cal})[T_{mm}(\text{cal})T_{nn}(\text{cal})]^{1/2}} S_{mm}(\text{cal}) \\ &\quad \times \exp[i(\phi_m^* - \phi_n) - i(\phi_n^* - \phi_m)] \end{aligned} \quad (3)$$

where R_{mm} denotes the correlation coefficient (fraction of the total power which is correlated) for one polarisation of the antenna pair (m, n).

$$\begin{aligned} R_{mm}(\text{cal}) &= G_m G_n \frac{S_{mm}(\text{cal})}{[T_{mm}(\text{cal})T_{nn}(\text{cal})]^{1/2}} \\ &\quad \times \exp[i(\phi_m - \phi_n)] \end{aligned} \quad (4)$$

where G_m and ϕ_m respectively denote the magnitude and phase of the complex gain of the detection system of antenna, m , the $S_{mm}(\text{cal})$ is the correlated flux when observing the calibration source with one polarisation of the antenna pair (m, n), and $T_{mm}(\text{cal})$ denotes the system noise temperature when observing the calibrator source with one polarisation of antenna, m .

When observing the solar active region the correlation coefficient, $R_{mm}(\text{Sun})$, for one polarisation of the antenna pair (m, n) is given by

$$\begin{aligned} R_{mm}(\text{Sun}) &= G_m^* G_n^* \frac{S_{mm}(\text{Sun})}{[T_{mm}(\text{Sun})T_{nn}(\text{Sun})]^{1/2}} \\ &\quad \times \exp[i(\phi_m^* - \phi_n^*)] \end{aligned} \quad (5)$$

Because the parametric amplifiers were used for both solar and calibration observations, an extra IF attenuation producing a phase difference $(\phi_m^* - \phi_n)$ had to be inserted when observing the Sun. This phase difference was measured by observing the calibration source with each antenna with the attenuator in and out. The levels of the two IF signals being fed into each multiplier from each antenna were kept at a fixed value using an automatic level control loop in which the sampled signal level controls an attenuator in the final IF amplifier. The automatic level control loop therefore automatically compensates for the variation in gain magnitude caused by the introduction of an extra IF attenuator when observing the Sun. This meant that we could assume that the gain magnitudes are the same when observing the calibrator and the Sun ($G_m = G_m^*$). The system noise temperature, $T_{mm}(\text{cal})$, when observing the calibrator was measured for each antenna using a switched noise source whose temperature of $\sim 3\text{K}$ was accurately calibrated for each antenna by observing the Moon. The system noise temperature, $T_{mm}(\text{Sun})$, when observing the active region was measured for each antenna by determining the difference in the total power detected when observing the active region and a quiet Sun region whose temperature was assumed to be 10^4K . Even at these high temperatures the front-end amplifiers were in their linear, unsaturated regions. Subsequent observations of more intense active regions with antenna temperatures $\geq 10^5\text{K}$ did, however, require the removal of the parametric amplifiers to avoid saturation. In this case, the gain and phase could be calibrated by observing 3C273 with the amplifiers out, but it also had to be observed with the amplifiers replaced in order to measure the system noise temperatures, $T_{mm}(\text{cal})$.

The correlated flux of the calibration source, $S_{mm}(\text{cal})$, was taken to be 49 and 34 Jy, respectively, when observing 3C84 and CTA102. The gain of each circularly polarised channel was calibrated by adjusting the digitised output of the correlators to give the same values of $R_{mm}(\text{cal})$ for every baseline pair and both polarisations. This is essentially because the calibrator sources have negligible circular polarisation and they are effectively point sources for the interferometric baselines used. These instrumental adjustments were also applied to the solar data to obtain the corrected amplitude, $S_{mm}(\text{Sun})$, and the corrected phase $(\phi_m^* - \phi_n) - (\phi_n^* - \phi_m)$ for each polarisation of each antenna pair every 30 s of each 15-min period of solar obser-

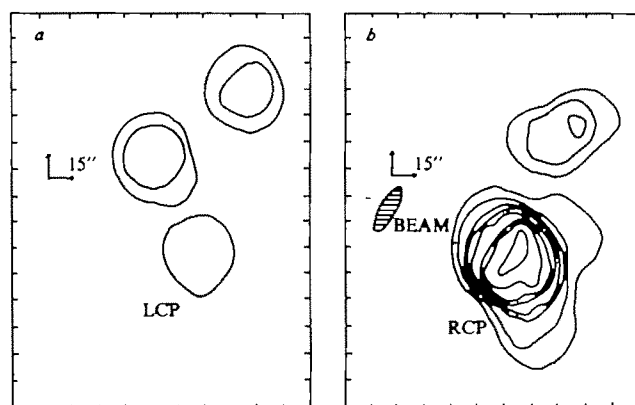


Fig. 4 VLA synthesis maps of solar active region 1056 obtained with one days observation on 1 April 1978. North is up, west is to the right. *a*, The left, and *b*, the right hand circularly polarised signals; $\lambda = 6\text{ cm}$.

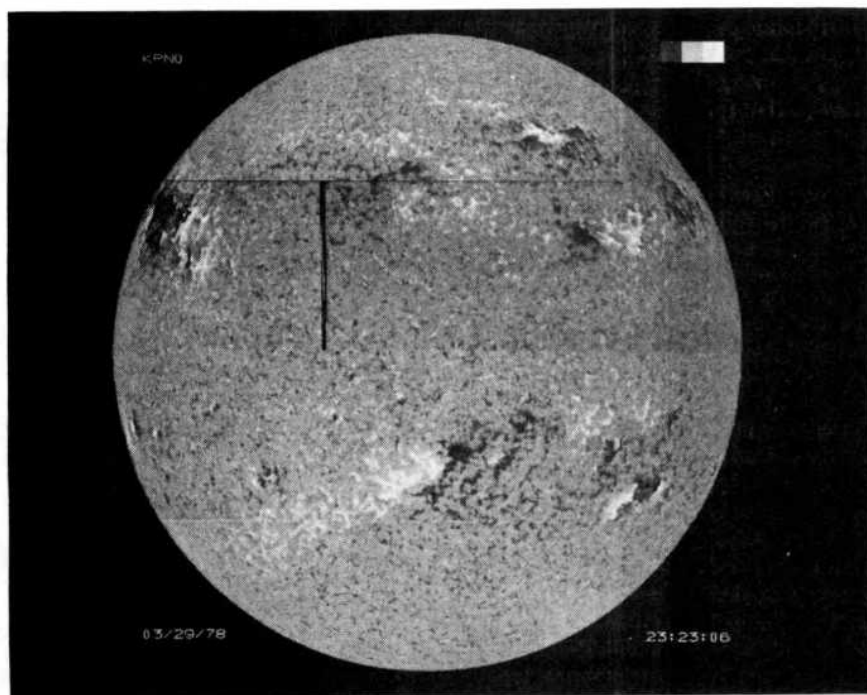


Fig. 5 A Kitt Peak National Observatory magnetogram of the Sun taken on 29 March 1978. Here Zeeman effect observations of the 8,680 Å line of neutral iron have been used to derive magnetic field structure which is limited by scintillation to 2"–3" angular resolution. As in Figs 3 and 4, north is up and west is to the right, and the dark and light regions respectively refer to regions of negative and positive polarity. The angular scale for the magnetogram is determined by the 32' angular diameter of the Sun. The general features of AR 1046 (lower centre) and AR 1056 (upper left) compare well with the VLA maps shown in Figs 3 and 4.

variations. The 30 s values of amplitude and phase were then added together to obtain 5-min vector averages with a phase calibration better than 5° and an amplitude calibration accurate to <10%. These calibrated data were taken to be the amplitude and phase of the source visibility function, and the source intensity distribution was obtained by Fourier transforming the calibrated data using the Tufts University DEC-10 computer.

VLA synthesis maps and comparisons with magnetograms

The procedure we have implemented involves a direct synthesis of a 'dirty' map which is the convolution of the true brightness distribution with the response function of a point source. This response function, or dirty beam is shown in Fig. 2. It has a FWHM of 1"×3", and the highest sidelobe is 14% of the maximum response.

A deconvolution of the dirty map was then performed using the clean procedure in which the effects of the 14% sidelobes of the synthesised beam were reduced¹². Because both active regions observed were resolved on baselines having fringe spacings <15" we have deleted those data having fringe spacings less than this value; thereby minimising the map noise and reducing computer time. The resultant total intensity contour maps are shown in Figs 3 and 4, where the synthesised beam is represented by a shaded ellipse. Here the contour levels represent 0.9, 0.8, 0.7, 0.6, 0.5, ... 0.2 times the maximum response which had the values of 102, 80, 17 and 5 Jy per synthesised beam area (in units of arc s²), respectively, for AR1046 (LCP), AR1046 (RCP), AR1056 (RCP) and AR1056 (LCP). The r.m.s. sensitivity of the VLA system for 12 h of observation of a 10⁴ K Sun is estimated to be 0.06 Jy.

The most striking aspect of the maps shown in Figs 3 and 4 is that the small-scale sources shown in the LCP and RCP maps are not spatially coincident. They suggest the feet of small dipole magnetic fields with positive magnetic polarity corresponding to the regions with strong right circular polarisation. Region 1046, for example, contains two sources ≈30"×30" in size which are ≥80% LCP with one source of similar angular size and ≥60% RCP located between them. Region 1056 contains one intense component about 30"×30" in angular size with ≥70% circular polarisation. In all cases the small-scale sources are surrounded by less intense halo emission ≥1' in angular extent (large angular sizes >1' are undersampled by the VLA system). Assuming a source size of 30"×30" an intensity of 100 Jy per synthesised beam area (7"×20") and a beam efficiency of 80%, we obtain a

source brightness of $\sim 10^{-11}$ erg s⁻¹ cm⁻² Hz⁻¹ rad⁻². Using the Rayleigh-Jeans law with a wavelength of 6 cm and this value of brightness we obtain a brightness temperature ≈10⁶ K. Thus the small-scale sources and the magnetic structures which give rise to their circular polarisation belong to the higher, hotter, coronal regions of the solar atmosphere. Assuming that the radio emission is at the first few harmonics of the gyrofrequency, magnetic field strengths of the order of a few hundred Gauss are inferred².

The magnetic structures obtained with the VLA can be compared with full disk magnetograms taken daily at the Kitt Peak National Observatory (2"–3" angular resolution) and the Mount Wilson Observatory (12" angular resolution). The magnetograms refer to the cooler regions in the medium to high photosphere, whereas the VLA maps refer to the hotter, higher levels of the solar corona. Figure 5 shows a Kitt Peak magnetogram taken on 29 March 1978 (because of inclement weather both Kitt Peak and Mount Wilson could not obtain data on our days of VLA observation on 30 March and 1 April 1978). The general features for the magnetogram of AR 1046 (in the lower centre of Fig. 5) compare well with the magnetic structure seen in the VLA maps (Fig. 3). The dark feature of negative polarity centred about an axis inclined ~45° West of North corresponds to a similar region in the LCP map, whereas the RCP feature corresponds to the more intense white region of positive polarity. The width of ~1', the extent of ~4', and the separation of components (30"–1') are in agreement for the two maps. Similarly, the magnetogram of AR 1056 (in the upper left hand corner of Fig. 5) agrees well with the VLA map (Fig. 4), for both indicate a predominantly positively polarised region (RCP) of a few arc min in extent. Thus we may conclude that the general magnetic structure inferred from magnetograms for the photosphere agrees with that obtained by VLA observations for the coronal regions.

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Optimal wavelength region for communication with extraterrestrial intelligence: $\lambda = 1.5$ mm

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This article shows that the best capability and reliability of communication at astronomical distances can be achieved at millimetre wavelengths. The cost of communication, and the number of directions in which signals are sent and searched for are assumed to be fixed. A characteristic frequency might be 203.385 GHz, corresponding to the splitting of the ground state of the lightest atom—positronium—and coinciding with the centroid of the relic background spectrum.

SINCE the pioneer work of Cocconi and Morrison¹ it has been assumed that, the optimal search range for transmissions from extraterrestrial civilisations is at short decimetre wavelengths² (near the 21-cm line of atomic hydrogen, the most abundant element). This spectral band lies near the intensity minimum of the cosmic radio background, and the 21-cm line itself has a symbolic value that narrows down the range of frequencies to be searched (it is supposed that transmitting frequency is near the line).

The choice of the decimetre wavelengths remains valid even if there is no information available about the locations of transmitting and receiving parties. But some papers^{3,4} point to the advantage of millimetre wavelengths for other strategies of space communication and take into account the scattering of radio waves in interstellar plasma. The aim here is to provide some confirmation of it.

Let us consider the opposite case: the transmitting side knows a fixed number of directions where signals are to be transmitted. This number might be defined, for example, by the number of stars with conditions suitable for the appearance of life and civilisation. The same assumption can be made by the receiving side: they search for signals from a fixed number of the nearest stars of the required type or from the direction towards the galactic centre. This strategy permits the channelling of signals in narrow (and hence very economical) beams. The assumption about the mutual directivity of transmitting and receiving antennae has been used in analysing most of the previous searches for CETI signals (CETI, communication with extraterrestrial intelligence)

Maximum information capacity and reliability

Let us determine the maximum information flow (or its maximum redundancy if the flow is fixed), with a limited amount of expenditure on the construction and the maintenance of the communication system. What wavelength will be optimal in this case? Assume that P_m and $\Delta\nu$ are the mean power and the bandwidth of the transmitter; A_t and A_r are the effective areas of the transmitting and receiving antennae; T is the noise temperature of the receiving system; R is the distance between the receiver and the transmitter; n_t and n_r are the number of directions in which signals are sent and searched for, respectively.

The mean spectral density of the received signal is

$$S_r = (P_m A_r A_t) / (R^2 \lambda^2 n_t n_r)$$

The spectral information capacity of a communication channel taking into account quantum fluctuations according to Lebedev and Levitin⁵ is:

$$C_r = \ln[1 + z_r(1 - e^{-x})] + [z_r + (e^x - 1)^{-1}] \times \ln\{1 + [z_r + (e^x - 1)^{-1}]^{-1} - x(e^x - 1)^{-1}\} \quad (1)$$

where $z_r = S_r / h\nu$ is the spectral density of the number of signal photons and $x = h\nu / kT$ (here ν is the signal frequency h and k are the Planck and Boltzmann constants). Equation (1) does not contain limitations related to the physical nature of the transmitter and the receiver.

For $z_r \ll 1 - e^{-x}$ and $z_r \ll (e^x - 1)^{-1}$, we have $C_r \approx xz_r$, and the total information capacity is:

$$C = \Delta\nu C_r = (P_m A_r A_t) / (kTR^2 \lambda^2 n_t n_r) = (S_r \Delta\nu) / (kT) \quad (2)$$

in agreement with the well-known Shannon formula. It is important to note that for small signals, equation (2) holds true for any frequency and the quantum effects play no role⁵. In this respect, quantum fluctuations have often been treated inadequately in papers on the CETI problem.

Assume that the expenditures of each side, including maintenance and construction of the system, are $\Sigma \propto A^\alpha / \lambda$, $\alpha = 1 - 2$, that is, the cost increases somewhat faster than the antenna area and is inversely proportional to the wavelength, λ . We believe that this dependence is not anthropocentric because it is associated with expenditures proportional to the mass of the structure and the manufacturing accuracy, taking into account real physical effects.

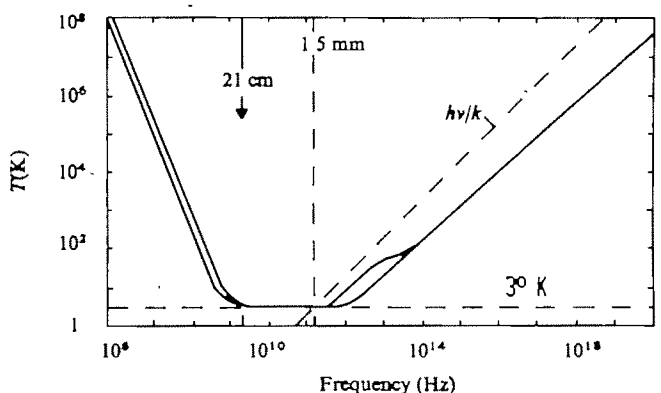


Fig. 1 Frequency dependence of brightness temperature of the cosmic background radiation (solid line). The data in radio, sub-millimetre and infrared ranges are given for the galactic pole (lower curve) and for the galactic plane (upper curve). The vertical broken line is the centroid of the relic background spectrum.

Let us also assume that the expenditures on the transmitter do not depend on the wavelength (the power of laser generators is the same as, if not higher than, radio generators), and are determined only by the amount of energy consumption.

The value T in the limiting case is determined by the brightness temperature of the cosmic background radiation. Figure 1 shows the frequency dependence of the brightness temperature determined by setting the observed sky intensity to the Planck function at each frequency^{2,6,7}. The dashed line $T = h\nu/k$ ($x = 1$) divides observational data in two parts corresponding to classical and quantum fluctuations.

These data show that:

$$T = \begin{cases} \propto \lambda^{-2.6} & \text{for metre and decimetre waves} \\ 3 \text{ K} & \text{for centimetre and millimetre waves} \\ \propto \lambda^{-1} & \text{for submillimetre and shorter waves} \end{cases} \quad (3)$$

With the assumptions discussed above, we obtain:

$$C \propto \begin{cases} \lambda^{-(1.6 \text{ to } 2.6)} & \text{for metre and decimetre waves} \\ \lambda^{-(0 \text{ to } 1)} & \text{for centimetre and millimetre waves} \\ \lambda^{+(0 \text{ to } 1)} & \text{for submillimetre and shorter waves} \end{cases} \quad (4)$$

The millimetre range therefore seems to be most efficient. Some other arguments in favour of this range should be added.

Let us consider some empirical data about the effects of interstellar plasma on the communication system^{8,9}. The effect of pulse broadening due to the dispersive properties of the plasma is $\Delta t_0 = 8.3 \times 10^{15} \times DM \times \Delta\nu \times \nu^{-3}$ s, where DM is the dispersion measure ($\text{cm}^{-3} \text{ pc}$), $\Delta\nu$ and ν are in Hz. Such a distortion of pulses can be partially eliminated by signal processing, depending on how accurately the dispersion measure is known. The dispersive effect is negligible when

$$\Delta\nu < \Delta\nu_0 = 1/\Delta t_0 = 1.1 \times 10^{-8} \times \nu^{3/2} (DM)^{-1/2} \quad (5)$$

Interstellar scintillation is significant for

$$\nu < \nu_c = 8 \times 10^8 (DM)^{0.75} \text{ Hz} \quad (6)$$

In the case of scintillation, the average time when there is no signal within a bandwidth is:

$$\delta t_s = 2.5 \times 10^{-5} \nu (DM)^{-1} \text{ s}$$

where the bandwidth is smaller than

$$\delta\nu_s = \begin{cases} 10^{-26} \nu^4 (DM)^{-2} \text{ Hz, for } DM < 10 \text{ cm}^{-3} \text{ pc} \\ 10^{-24} \nu^4 (DM)^{-4} \text{ Hz, for } DM > 10 \text{ cm}^{-3} \text{ pc} \end{cases} \quad (7)$$

Scattering also results in a longer duration of signal pulses, given by:

$$\Delta t_s = 0.1/(\delta\nu_s) \quad (8)$$

as well as spectral broadening of quasi-monochromatic signals:

$$\Delta\nu_s = 1/(\delta t_s) \quad (9)$$

Note that the value $\Delta\nu_s$, although small, exceeds the effect of the band broadening associated with the uncertainty or the variability of the Doppler shift of a signal frequency $\Delta\nu_D$. The latter effect also limits the bandwidth to $\Delta\nu \geq \Delta\nu_D = \Delta\nu^{1/2}$ (ref. 10). However, future methods of frequency control and generation should provide the accuracy $\Delta\nu_D \approx 10^{-20} \nu t^{-1/2}$ where t is the measurement time in seconds¹¹. The widest band will then be obtained with $t = 1/\Delta\nu_D$, and will be equal to $\Delta\nu_D \approx 10^{-40} \nu^2$, that is, much less than $\Delta\nu_s$. $\Delta\nu_D$ is also negligibly small for frequencies higher than the critical ν_c (the band $\leq \Delta\nu_D$ can hardly be used because of the small information content and difficulties in generation, detection and receiving).

All the effects mentioned here set limits to possible signal bands and pulse durations. Figure 2 gives regions of possible bands for optimal signals ($\Delta\nu = 1/\Delta t$). Pulses from the transmitter will not fade as long as $\Delta\nu > \delta\nu_s$ or $\Delta\nu < \Delta\nu_0$. There is no signal distortion if $\Delta\nu < 10\delta\nu_s$ and $\Delta\nu < \Delta\nu_0$. In the latter case, the distortion can be partly corrected by signal processing.

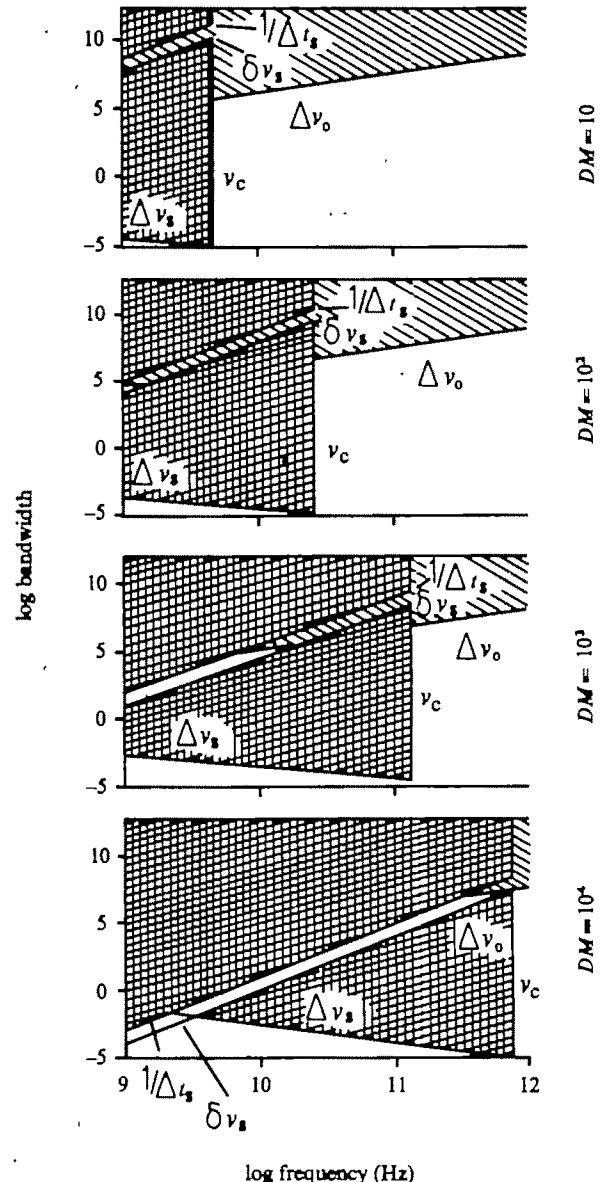


Fig. 2 Limits on the bandwidth and pulse duration ($\Delta\nu = 1/\Delta t$) due to the effects of interstellar plasma. Open regions: no limits. Hatched regions: partial restoration of pulse is possible by processing. Doubly hatched regions: attenuated signals cannot be restored.

Note that all of the plasma effects mentioned above decrease rapidly at higher frequencies indicating the advantage of the millimetre range, especially at large distances. The best region corresponds to $\nu > \nu_c$ and since $DM < 10^3 \text{ cm}^{-3} \text{ pc}$ for most of the Galaxy, the millimetre range satisfies this requirement.

Selection of characteristic frequency

We now discuss several frequencies with outstanding symbolic value in the millimetre range. First of all, this band contains most of the energy of the cosmological relic background radiation, the intensity of which dominates all other radiation in this range. The 'centroid' of the relic background spectrum may be considered as a basic characteristic frequency. Integrating the Planck radiation law as follows:

$$\int_0^{\infty} x^3 (e^x - 1)^{-1} dx = \int_{x_r}^{\infty} x^3 (e^x - 1)^{-1} dx$$

one obtains $x_r = 3.50$, which corresponds to a frequency centroid $\nu_r = 197$ to 220 GHz, or a wavelength $\lambda_r = 1.36$ to 1.52 mm for a relic background temperature from 2.7 to 3.0 K (ref. 12).

By a remarkable coincidence a unique radio spectral line falls in this band. It corresponds to the splitting of the ground state of the lightest atom—positronium (P_0). Positronium forms by recombination of an electron and a positron and is about 1,000 times lighter than a hydrogen atom. Laboratory investigation of positronium provided some of the basic evidence for the validity of quantum electrodynamics. The characteristic feature of positronium is the fact that its properties can be calculated from fundamental physical constants. The lifetime of this atom is very short because of the annihilation process, so a high concentration could hardly be expected in natural cosmic conditions.

The ground state of a positronium atom ($n = 1, l = 0$) is split into singlet $^1S_0$ and triplet $^3S_1$ states (where the positron and electron spins are antiparallel and parallel, respectively). According to most accurate theoretical estimates, this splitting corresponds to a frequency of 203,383 267(86) GHz ($\lambda = 1.47$ mm).

The calculated frequency was confirmed with high accuracy in the experiment by Egan *et al.*¹³ to be 203,384 9(12) GHz.

The annihilation times for the given sublevels are: $\tau(^1S_0) = h/(\pi m_e c^2 \alpha^5) = 1.25 \times 10^{-10}$ s for the two γ quanta annihilation, and $\tau(^3S_1) = 9h/[4(\pi^2 - 9)m_e c^2 \alpha^6] = 1.4 \times 10^{-7}$ s for the 3γ -quanta annihilation (m_e is the electron mass, c is the velocity of light, α is the fine structure constant).

These values may be used as adequate symbols to determine, for instance, the pulse duration of a beacon, and that of the basic information flow. Their reciprocal values (8 GHz and 7 MHz respectively) could denote signal bandwidths. We can estimate the maximum recurrence period for such pulses. If only one pulse is emitted in each direction and if such pulses are sent in a sequence to 10^{11} stars, then the signal recurrence period will be 12.5 s and 3.9 h, respectively.

We shall now estimate the information content of a millimetre channel on the basis of current technology. Given $P_m = 1$ MW, $A_t = A_r = 40$ m² (corresponding to a reflector 10 m in diameter), $T = 200$ K, $\lambda = 1.5$ mm, $R = 100$ pc, $n_t = 10^3$, $n_r = 1$, then from equation (2), C is 2 nit per day [where 1 nit = $1/\ln 2 = 1.44$ bit]. If $P_m = 100$ MW and $A_t = 4,000$ m² (100-m reflector) are assumed for the transmitting side, the same result is obtained for $R = 10$ kpc.

Interestingly, monochromatic radiation near the frequency 203 GHz has already been detected from astronomical objects. Phillips *et al.*¹⁴ observed the radio line from the $3_{13}-2_{20}$ transition of the $H_2^{18}O$ molecule, the laboratory frequency of which (203,407.5 GHz) is strikingly close to the positronium-line frequency. Observations were carried out with an antenna of diameter 11 m and a system temperature of 200 K. The line was discovered in emission from molecular clouds near Ori A and

DR 21. The line intensity corresponds roughly to the expected concentration of $H_2^{18}O$ molecules in these clouds. It should be stressed that with such equipment, it is already possible to search for CETI sources in the millimetre range.

In conclusion, we emphasise that the millimetre region is outstanding among all possible ranges of electromagnetic radiation because it is:

(1) A spectral region of minimum brightness temperature (for lower frequencies, the temperature increases due to synchrotron radiation, for higher frequencies, due to radiation of dust and stars).

(2) A spectral region of minimum absorption and scattering. The most economical strategy of space communications is to align the transmitting and receiving antennae and to send the radiation and the information it carries in a narrow beam.

In this case, the features discussed above for the millimetre range in the present cosmological epoch provide for maximum information capacity and reliability of communication for a given expenditure on construction and organisation.

The most prominent millimetre wavelength, within the bandwidth corresponding to the centroid of the relic background is that of the positronium line at 1.47 mm, which nearly coincides with the $H_2^{18}O$ line. Thus a search for CETI signals would yield simultaneous astronomical results. Alternatively, during normal astronomical observations of the $H_2^{18}O$ line there is a possibility of chance discovery of artificial signals. We once again emphasise the necessity of searching for monochromatic and pulsed signals, bearing in mind that beacons might have a fairly large duty cycle. The use of such signals appreciably reduces the average power required for the transmitter. This fact, along with the known frequency and bandwidth, greatly facilitates the search for extraterrestrial intelligence.

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A novel bacteriophage defence mechanism: the anti-restriction protein

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Bacteriophage T3 and T7 protect their DNA from restriction by producing, as the earliest detectable phage functions, anti-restriction proteins. Although the two phage proteins differ in their chromatographic and antigenic properties, they act by the same mechanism: the anti-restriction proteins inhibit E. coli K12 restriction endonuclease by direct interaction.

A PARTICULARLY vulnerable stage of the bacteriophage life cycle is immediately after infection, when the naked DNA is exposed to host cell nucleases, particularly restriction endonucleases. Some bacterial viruses have developed means of combating the host-controlled restriction system by modifying their DNA by, for example, incorporating unusual bases or by glycosylation. The modified DNA is protected from degradation *in vivo* and *in vitro*. The related *Escherichia coli* phages T3 and T7 apparently possess a different mechanism to overcome

Table 1 Effect of increasing Ado-met concentration on the *EcoK* inhibition by SAMase

-SAMase Initial Ado-met (mM)	+SAMase ATP hydrolysed (% of input)	+SAMase ATP hydrolysed (% of input)	ATPase activity (% of control)	Final Ado-met (mM)
0.5	46.5	6.0	13	0.05
1	61.7	22.7	37	0.1
2	57.7	28.2	49	0.2
5	51.9	31.2	60	1.6
10	41.0	21.0	51	5.6

EcoK and SAMase were purified as described elsewhere¹⁰⁻¹⁴ (SAMase A (refs 10, 11) was used in all experiments; SAMase B (refs 10, 11), however, behaved identically). ATPase was assayed using ³H-ATP (refs 12-14) and resolving ATP and ADP by thin-layer chromatography on polyethylenimine after the incubation. Initial Ado-met concentration was calculated assuming '100% purity' of the dry substance used (Boehringer). ¹⁴C-Ado-met levels were determined after chromatography on cellulose thin layer developed with 64% ethanol²¹. Incubations were for 30 min at 30 °C. The incubation volume of 20 µl contained the components and amount of SAMase described in the legend to Fig. 1 except that the concentration of Ado-met was varied. All components were present from the start of incubation.

restriction: they grow on K12 and B strains even though their DNA is a substrate for the restriction enzymes *in vitro*². Mutants of these phages can be found which are efficiently restricted by the *E. coli* systems. Such mutations map in the first gene of T3 (*S*-adenosyl-methionine hydrolase (SAMase), ref. 3 and N.S. *et al.*, unpublished) and also in the first gene of T7 ('0.3' gene⁴, 'M'-gene⁵). Because the T3 mutations occur in a gene coding for an enzyme that hydrolyses *S*-adenosyl methionine (Ado-met), one of the essential cofactors for the *E. coli* K12 and B (*EcoK* and *EcoB*) restriction enzymes, one could explain the anti-restriction activity as a direct consequence of the hydrolysis of Ado-met. No such explanation can be offered for anti-restriction by T7 because this phage does not code for a SAMase.

T3 SAMase and the T3 anti-restriction activity are independent functions of the same protein

Most T3 mutants which are defective in anti-restriction have lost SAMase in the same mutational event (ref. 3 and N.S. *et al.*, unpublished). One *ts* mutant, however, could not overcome restriction at the non-permissive temperature, although it could still induce SAMase activity (ref. 6 and N.S. *et al.*, unpublished; see also Table 4). The inverse phenomenon has also been described: a mutant (3356) retained the anti-restriction phenotype but had lost the SAMase activity⁷. This can be explained by assuming either that the same peptide chain carried two independent activities or that the two activities were exerted by two proteins encoded in the same DNA segment but read in different reading frames⁸. The latter possibility was ruled out, as out of 20 mutants isolated that were defective in both SAMase and anti-restriction activities, seven were found to be *amber* mutants. The isolation of an *amber* mutant has also been reported from another laboratory³. All these *amber* mutants expressed both anti-restriction and SAMase activities in suppressing hosts. As the *amber* mutation is reading-frame specific⁹, both activities must be expressed by the same polypeptide. Thus, the genetic evidence suggests that SAMase activity is not required for anti-restriction.

SAMase inhibits the restriction system *in vitro*

The effect of purified SAMase^{10,11} on restriction was tested using the *EcoK* system. The restriction reaction proceeds in a series of steps¹²⁻¹⁴, including the activation of *EcoK* by Ado-met, the binding to DNA, the triggering of *EcoK* by the unmodified

DNA site to become an endonuclease, and the ATP-dependent endonucleolytic cleavage. The last reaction involves ATP hydrolysis, and ATPase activity was, therefore, taken as a measure of the overall reaction. This activity was indeed strongly inhibited by SAMase (Tables 1, 2). To elucidate which step in the chain of reactions leading to ATPase activity was inhibited, the steps were examined individually. The possibility that SAMase inhibits by blocking access to the *EcoK* recognition sites on the DNA was unlikely, as we demonstrated by gel filtration chromatography that SAMase did not bind to DNA (not shown). The binding of *EcoK* to the DNA depends strictly on the presence of Ado-met¹²⁻¹⁴. Degradation of Ado-met *in vitro* will, of course, block this initial step and all subsequent steps. Various lines of evidence have, however, shown that Ado-met hydrolysis is not the cause or not the only cause of anti-restriction.

Anti-restriction does not depend on Ado-met hydrolysis and SAMase interacts directly with *EcoK*

If the anti-restriction depended on Ado-met hydrolysis, one would expect to be able to relieve the inhibition simply by increasing the initial concentration of Ado-met. In the experiment shown in Table 1, incubations were made with a constant amount of both *EcoK* and SAMase but different concentrations of Ado-met. The amount of ATP hydrolysed by *EcoK* and the amount of Ado-met remaining were measured. Although the Ado-met concentration varied over a 20-fold range, and its concentration at the end of the experiment varied between close to zero and more than five times that necessary to support a maximum *EcoK* reaction, the amount of inhibition by SAMase was constant at about 50% except at the lowest Ado-met concentration. At this low Ado-met concentration the SAMase

Table 2 Interaction between *EcoK* and SAMase

Complete mixture* preincubated for 10 min, then addition of	ATP hydrolysed (% of input)
—	17.0
150 ng SAMase	0.2
75 ng SAMase	4.0
30 ng SAMase	4.2
15 ng SAMase	11.8

* The incubation volume of 20 µl contained the components described in Fig. 1. All components except SAMase were present from the start of incubation. ATP hydrolysis between 10 and 30 min is shown.

hydrolysed essentially all the Ado-met before the *EcoK* reaction could begin. Above the minimum concentration of Ado-met, the inhibition of *EcoK* apparently required no Ado-met hydrolysis. This result indicates that the inhibition is due to a direct interaction between SAMase and *EcoK*.

Further support for this idea came from a series of experiments with the Ado-met analogue, *S*-adenosyl homocysteine (SAH), which is a potent inhibitor of both *EcoK* and SAMase^{10,11,15}. SAH competes with Ado-met in the first step of the *EcoK* reaction. If SAH was present from the beginning of the incubation, *EcoK* ATPase activity was inhibited (Fig. 1a). On addition of SAMase, *EcoK* was inhibited further, even with high SAH concentrations (Fig. 1a), despite the fact that Ado-met hydrolysis was prevented (Fig. 1c). As expected from the reaction mechanism, SAH was unable to inhibit *EcoK* if it was added late in the reaction at times when free Ado-met was no longer required (Fig. 1b). However, when SAMase was added with SAH late in the reaction, inhibition was observed. The degree of inhibition was constant over all SAH concentrations and thus independent of the amount of Ado-met hydrolysis (Fig. 1b). In fact, it depended solely on the amount of SAMase (Table

2). Changing the proportion of SAMase to *EcoK* caused inhibition of ATPase of between 0 and 100%. The time course (not shown) and reversibility (see below) of the reaction suggest a non-catalytic interaction. Although the proportion of active enzyme in both preparations *EcoK* and SAMase is somewhat uncertain, an approximately stoichiometric interaction is compatible with our data.

The experiment in Fig. 1b showed that SAMase could inhibit *EcoK* after proper binding to DNA. The inhibition of activated *EcoK*-DNA complexes was also measured directly. Such complexes, purified by passage through Biogel, hydrolyse ATP. The hydrolysis was still inhibited by the addition of SAMase (not shown) although Ado-met was no longer required for *EcoK* ATPase and was only present in concentrations (10^{-10} M) far below the K_m of SAMase^{10,11}. These data confirm the conclusions from the genetic results described initially that Ado-met hydrolysis is not required for anti-restriction and that the SAMase protein purified for its affinity to SAH^{10,11} causes anti-restriction by interaction with *EcoK* after the Ado-met-mediated step.

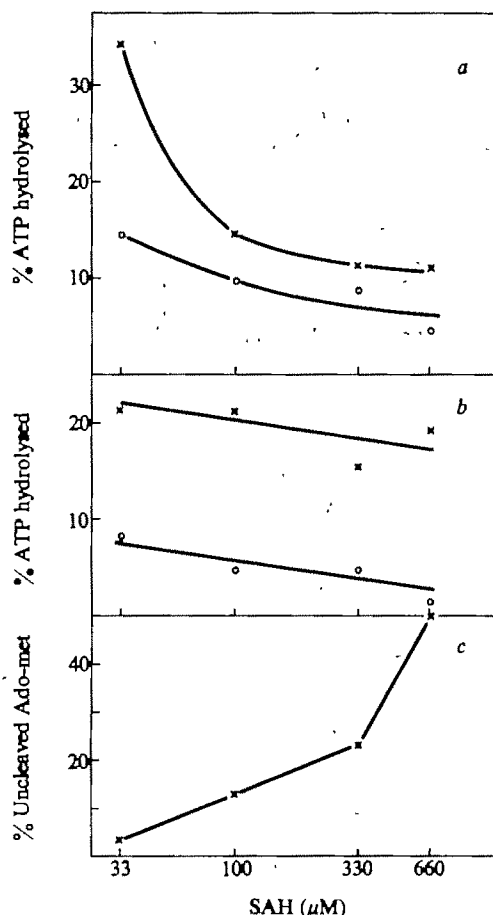


Fig. 1 The influence of SAH on anti-restriction *in vitro*. The reaction mixtures contained in a total volume of 20 μ l (refs 12-14): 2 μ mol HEPES, pH 8.0, 1.4 nmol EDTA, 60 nmol 2-mercaptoethanol, 0.12 μ mol $MgCl_2$, 0.2 μ g unmodified λ (0) DNA, 1.1 nmol purified Ado-met¹²⁻¹⁴ (^{14}C Ado-met at 22 Ci mol⁻¹), 2 nmol ATP (^3H -ATP at 540 Ci mol⁻¹), 100 ng *EcoK* plus 60 ng SAMase where indicated. SAH was added as plotted in the abscissa. ATPase activity was determined as described elsewhere¹²⁻¹⁴. a, Action of SAMase on the overall reaction. All components were present at 0 min. Incubation was for 30 min. x, Minus SAMase; o, plus SAMase. b, Action of SAMase on the activated DNA-Ado-met-*EcoK* complex. ATP, SAH and SAMase were added after 10 min of preincubation of the other components. Further incubation was for 20 min. x, Minus SAMase; o, plus SAMase. c, Ado-met hydrolysis in the incubation mixtures of a. Ado-met remaining at the end of incubation was plotted (in %).

Table 3 Inhibition of SAMase anti-restriction by antibodies

	ATP hydrolysed (% of input)	ATPase activity (%)
Expt 1		
-SAMase (control)	32.9	100
+SAMase at 10 min	8.5	26
+SAMase plus anti-SAMase at 10 min	22.4	68
Expt 2		
-SAMase (control)	40.3	100
+SAMase	12.1	30
+SAMase, + additional Ado-met at 10 min	15.9	39
+SAMase, + anti-SAMase at 5 min	10.4	26
+SAMase, + anti-SAMase at 5 min, + additional Ado-met at 10 min	29.3	73

The assays and incubation mixtures were as in Table 1 and Fig. 1. The anti-SAMase antibodies were prepared as described^{10,11} (anti-SAMase A and anti-SAMase B were equally effective). The amount of antibody added did not interfere by itself with *EcoK* ATPase. SAMase was present from the start of incubation where indicated.

Experiments with antibodies against SAMase directly support the idea of an interaction between the two enzymes, *EcoK* and SAMase. Not only could anti-SAMase added with SAMase to the preincubated *EcoK*-DNA complex totally prevent inhibition (Table 2, expt 1), it could also reactivate *EcoK* after SAMase had been permitted to act (Table 3, expt 2). This clearly shows that the interaction between SAMase and *EcoK* is readily reversible because, if it were not, the antibodies would have precipitated *EcoK* with the SAMase. The reversibility of the interaction was confirmed in gel filtration experiments (not shown): the association of the two enzymes did not survive passage through a Biogel column.

The antibody experiment also provided support for the model of the *EcoK* reaction mechanism¹²⁻¹⁴, which predicts that cleavage of Ado-met after the initial step does not influence the subsequent course of the restriction reaction. The antibodies are somewhat unusual because although they precipitate SAMase, they do not inhibit its enzymatic activity^{10,11}. In the experiments shown in Table 3, both ATP hydrolysis and Ado-met cleavage (not shown) were monitored. As expected, the presence of the antibodies did not prevent the hydrolysis of essentially all the Ado-met, but had a dramatic effect on the inhibition of the activated *EcoK*. Restriction could proceed in the absence of Ado-met.

Properties of purified mutant SAMase enzymes

To investigate whether the anti-restriction reaction observed *in vitro* reflected the physiological anti-restriction mechanism, we isolated mutant proteins of phage with known *in vivo* phenotypes and tested their behaviour *in vitro*. The mutant proteins were purified as cross-reacting material from lysates of cells infected with different SAMase mutant strains, using anti-SAMase immobilised on agarose. The properties of all except one of the isolated mutant proteins were what would be expected from their phenotype *in vivo* (Table 4): the point mutant SAMase 17 eliminated both anti-restriction and SAMase *in vivo*; the isolated protein showed neither activity *in vitro*. T3 3356 could overcome restriction *in vivo* without inducing Ado-met cleaving activity—cross-reacting material from 3356 was SAMase negative but inhibited *EcoK* ATPase. So far, *in vivo* and *in vitro* phenotype correlate well, indicating the physiological role of the anti-restriction reaction *in vitro*. Cross-reacting material was also prepared from a mutant thermolabile for anti-restriction and thermoresistant for SAMase activity (SAMase 15 21). The isolated protein displayed no temperature sensitivity for anti-restriction *in vitro* (Table 4), suggesting that this thermolability occurred only in specific intracellular conditions.

Table 4 Inhibition of *EcoK* ATPase by mutant SAMase proteins

Protein from:	Incubation temperature (°C)	Ado-met hydrolysis	ATP hydrolysed (% of input)	ATPase activity (%)
—	30	—	55.4	100
Non-infected cells	30	—	46.4	84
T3 SAMase 17	30	—	38.8	70
T3 SAMase ts 21	30	+++	16.0	29
T3 3356	30	—	20.1	36
T7	30	—	42.6	77
—	42	—	72.6	100
T3 SAMase ts 21	42	+++	24.0	33

The assays were carried out as in Table 1 and Fig. 1. Mutant protein was obtained as follows: non-infected *E. coli* B_S-1 or B_S-1 at 8 min after infection^{10,11} were disrupted. An S30 was prepared and adsorbed to anti-SAMase IgG attached to Sepharose 4B. Protein was eluted by 6 M LiCl and precipitated by (NH₄)₂SO₄. 10 µg of dialysed protein was added to incubation mixtures. Non-infected cells and T7-infected cells contained no specific cross-reacting band (as judged from SDS gel). IgG was purified from anti-SAMase A antiserum^{10,11} by DEAE-cellulose chromatography²² followed by chromatography on protein A Sepharose²³ and coupled to Sepharose 4B (ref. 22). All infections resulted in <1% surviving cells. T3 SAMase 17 and 21 were isolated for inability to overcome B restriction (ref. 6 and N.S. *et al.*, unpublished). T3 SAMase 17 is a point mutation which induces no SAMase activity. T3 SAMase ts 21 induces SAMase activity *in vivo* at any temperature. T3 3356 (ref. 7) is SAMase negative, anti-restriction positive.

The T7 anti-restriction protein is unrelated to SAMase but exerts a similar action mechanism

We attempted to purify the anti-restriction protein coded by phage T7 using the anti-T3 SAMase affinity column. However, the T7 protein did not bind to the column (Table 4), indicating that it is immunologically not closely related to the T3 SAMase. Extracts from T7-infected cells also contained no cross-reacting material as judged by immunodiffusion^{10,11} and gel overlay techniques (not shown). The T7 anti-restriction protein was, therefore, partially purified using the *EcoK* inhibition assay. Although SAMase did not adsorb to DEAE cellulose^{10,11}, *EcoK*-inhibiting activity from T7-infected cells was only eluted by high salt concentrations (Fig. 2). Such activity was absent when cell extracts from non-infected cells (not shown) or from cells infected with a T7 *M* deletion mutant (Fig. 2) were used. The T7 anti-restriction protein had no affinity to SAH, which is our standard affinity ligand for the purification of SAMase. Although so different, the inactivation of *EcoK* seemed to occur by a similar mechanism, that is, direct interaction. Like SAMase, the T7 anti-restriction protein could inhibit *EcoK* ATPase at a step after the binding to DNA (Fig. 2).

Is anti-restriction the only function of SAMase and of the T7 anti-restriction protein?

Bacteriophage T3 and T7 protect their DNA from restriction by directing the synthesis of anti-restriction proteins. These proteins are the first detectable phage gene products to be made after infection. A consideration of the numerology of these proteins makes it unlikely that either Ado-met hydrolysis (in T3) or anti-restriction (in T3 and T7) are the exclusive roles of these proteins. Each *E. coli* K12 cell is estimated to contain about 10 molecules of *EcoK* (ref. 15); the intracellular Ado-met concentration is of the order of 1 µM and thus each cell contains about 500 molecules of this cofactor¹⁶. Also, the T3 SAMase is among the most abundant of the phage-coded proteins (compare the densities of protein bands in Fig. 1, tracks 5 and 12, in ref. 10) with each cell containing about 10,000 molecules^{10,11}. That is,

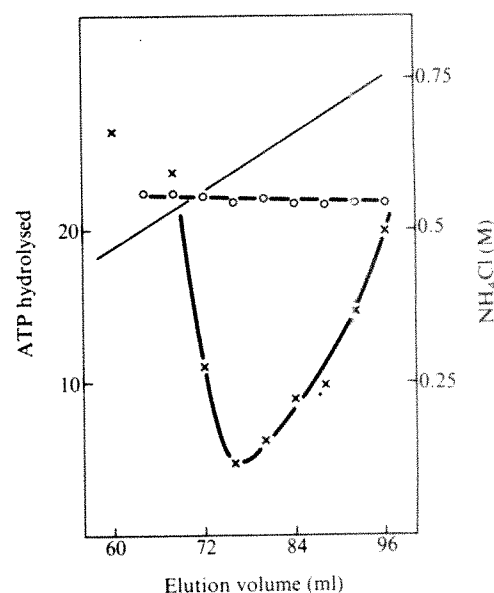


Fig. 2 Purification and action of the T7 anti-restriction protein. *E. coli* B_S-1 were infected with either T7 wild type or T7 D9 at a multiplicity of infection of 10, and collected 8 min later. The cells were disrupted as described elsewhere^{10,11} and 3 ml of an S100 adsorbed to DEAE Sephadex (10 ml bed volume) which had been equilibrated with 10 mM Tris-HCl, pH 7.5, 22 mM NH₄Cl and 1 mM dithiothreitol. Adsorbed protein was eluted using a linear gradient of 22 mM to 1 M NH₄Cl in the same buffer. Aliquots of 0.1 µl of each fraction were added to 20 µl containing activated *EcoK*-Ado-met-DNA complexes plus the components described in Fig. 1. There was no ATPase-inhibiting activity in the non-adsorbed protein. Protein eluted between 0.1 and 0.4 M salt degraded essentially all ATP, ADP and AMP. This activity was similar in both types of extracts; T7 wild type and T7 D9. The graph shows activity which was eluted beyond 0.45 M NH₄Cl. x, T7 wild type; o, D9. T7 D9 carries a deletion of the T7 *M* gene ('0.3 gene', see also ref. 5). Surviving cell count in both infections was 0.05%.

1,000 SAMase molecules per *EcoK* and 20 SAMase molecules per molecule of Ado-met. Similarly, the T7 anti-restriction protein is made in large amounts. We thus have the unusual situation that the enzyme is made in vast excess over its putative substrates. The overproduction suggests an additional role for both anti-restriction proteins. This has been independently suggested by experiments with pleiotropic T7 anti-restriction mutants which at the same time could not induce typical virus-dependent membrane alterations nor exclude super-infecting virus^{5,17}.

The *in vivo* time course of anti-restriction introduces another unanswered question. How does T3 and T7 DNA escape restriction before SAMase and M protein synthesis? It is well known from work with phage λ that restriction is a fast process¹⁸. T3 and T7 infection may involve a delay of restriction possibly related to the membrane alteration which occurs before protein synthesis¹⁷.

The mechanism of anti-restriction described here may serve as a model for future research into other anti-restriction systems. There are indications in the literature to suggest that anti-restriction is a general property of many bacteriophages (refs 19, 20 and S. Iida, W. Arber and T.A.B., unpublished).

During a recent EMBO workshop in Paris, we learned that K. K. Mark and F. W. Studier of Brookhaven National Laboratory had carried out similar experiments and obtained results in agreement with ours. We thank them for communicating their data. According to Mark and Studier (unpublished), purified T7 0.3 protein is very acidic. It inhibits the *EcoB* restriction nuclease but does not affect several type II restriction enzymes; the mechanism seemed to be stoichiometric rather than catalytic.

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letters

Detection of strong H₂O emission from galaxy NGC4945

AFTER the first detection of the 6₁₆-5₂₃ transition of water vapour in an external galaxy by Churchwell *et al.*¹ in M33, other detections were reported by Lépine and dos Santos² in NGC253, and by Huchtmeier *et al.*³ in M33 and IC342. We report here the detection of H₂O maser emission from NGC4945, a bright edge-on spiral galaxy in the Centaurus group. This is intrinsically the most powerful H₂O maser yet detected in any astronomical source. The search for H₂O emission in NGC4945 was motivated by the previous detection of OH and H₂CO absorption by Whiteoak and Gardner⁴ and Gardner and Whiteoak⁵, and also by the similarity of this galaxy to NGC253. The observations were made on 20-22 September 1978, with the 13.7-m Itapetinga radiotelescope equipped with a ruby maser amplifier with an apparent SSB system temperature of about 150 K. The signal was analysed by a 46-channel, 100-kHz (1.35 km s⁻¹) resolution filter bank, and observations were made with the filter bank centred at different frequencies to obtain greater velocity coverage. The 4' beam was pointed towards the nucleus of the galaxy. The overall dimensions of the galaxy are 17' × 3'. The telescope was beam switched at a rate of 100 Hz. The beams were separated by 9.4 arc min and the reference beam was displaced in azimuth, which did not correspond to a fixed direction with respect to the equatorial plane of NGC4945. Antenna temperatures were corrected for atmospheric attenuation and random attenuation using the standard procedure at Itapetinga⁶ and then converted to flux units, the error in the flux scale being ~10%.

The H₂O spectrum of NGC4945 in the velocity range of 630-750 km s⁻¹ (LSR) is presented in Fig. 1. Emission features >5 Jy appear at 668, 674, 698 and 714 km s⁻¹. No other features >3 Jy appeared in the observed velocity range 560-770 km s⁻¹. The feature at 674 km s⁻¹ is probably not resolved and could still be stronger than 12 Jy; its intensity as observed with 100-kHz channels was confirmed by making shifts of the local oscillator. It corresponds to an intrinsic power ~10 times greater than that of the strongest H₂O source in our galaxy, W49, during its maximum period of emission, if NGC4945 is at a

distance of 4 Mpc (ref. 7). The existence of such strong H₂O sources in the Universe raises the possibility of detection of H₂O from galaxies at even larger distances; for instance, an emission similar to that of NGC4945 could have been detected with our receiving equipment if the source were at a distance twice as large.

The velocity range of the H₂O emission coincides precisely with that of the 1,612-MHz OH emission⁴, suggesting that the same region of the galaxy could be responsible for both masers.

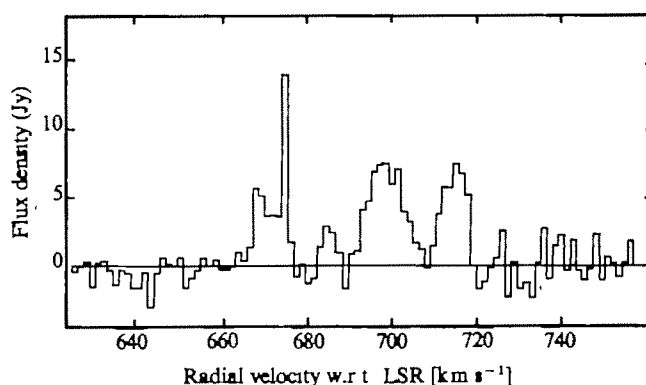


Fig. 1 The H₂O (6₁₆-5₂₃) spectrum of NGC4945. To convert to heliocentric velocities 4.7 km s⁻¹ should be added to LSR velocities.

Gardner and Whiteoak⁴ discuss the possibility of an association of the OH maser with the nuclear region, and point out that the far-infrared radiation from the nucleus is probably the OH pump source. It should be noted, however, that the HI profiles obtained by Whiteoak and Gardner⁸ at different positions along the optical major axis suggest that the velocity range of the H₂O emission may correspond to regions situated to the north-east of the nucleus. A more precise determination of the position of the H₂O masers with respect to the radionucleus (~30" diameter⁹) is planned for a future run of the maser receiver.

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²³⁵U enrichment by pulse gas diffusion

LIGHT-WATER nuclear reactors use uranium fuel enriched in isotope ²³⁵U. One of the enrichment processes is gaseous diffusion. This process is used in large-scale plants in the US and uses the fact that, in the Knudsen diffusion condition, ²³⁵UF₆ diffuses through the porous membrane at slightly higher rate than ²³⁸UF₆. A transient Knudsen diffusion process is proposed here which gives a higher enrichment factor for the lighter species compared with a steady state diffusion process. Because of the higher enrichment, the number of stages required for enriching ²³⁵U to a concentration level suitable for nuclear power reactor fuel is considerably reduced.

The Knudsen diffusion coefficient is reciprocally proportional to the square root of the molecular weight. The difference in molecular weight between ²³⁸UF₆ and ²³⁵UF₆ is 0.86%, and the concentration ratio of ²³⁵UF₆ to ²³⁸UF₆ increases by diffusing through a membrane by 0.429%. Because of the low enrichment factor, many stages are required to concentrate ~0.7% ²³⁵U, in nature, to about 3% as a reactor fuel. A higher enrichment is achieved, however, if the process is operated in the transient condition described here.

Suppose a gas is imposed as a pulse (concentration C_0 for time t_0) on one side of the membrane with small pores in which diffusion is of Knudsen type (effective diffusion coefficient αD_* , membrane thickness L , and porosity a). If the gas concentration on the other side of the membrane is zero, the system is

$$a \frac{\partial C}{\partial t} = \alpha D_* \frac{\partial^2 C}{\partial x^2} \quad (1)$$

at $t = 0$, $C = 0$; at $x = 0$, $C = C_0$, $0 < t < t_0$, $C = 0$, $t > t_0$; at $x = L$, $C = 0$.

The diffusion flux Q at the outlet side ($x = L$) of the membrane is

$$Q = -\alpha D_* \left(\frac{\partial C}{\partial x} \right)_L \quad (2)$$

Substituting the solution to equation (1) into equation (2), we obtain

$$Q = \frac{C_0 D_*}{L} A \quad (3)$$

$$A = \alpha \left\{ 1 + 2 \sum_{n=1}^{\infty} (-1)^n \exp(-(n\pi)^2 \alpha T) \right\} \quad \text{for } t \leq t_0 \quad (4)$$

and

$$A = 2\alpha \sum_{n=1}^{\infty} (-1)^n \{ \exp(-(n\pi)^2 \alpha T) - \exp(-(n\pi)^2 \alpha (T - T_0)) \} \quad \text{for } t \geq t_0 \quad (5)$$

$$\text{where } T = \frac{D_* t}{aL^2} \quad \text{and} \quad T_0 = \frac{D_* t_0}{aL^2}$$

The amount of gas diffused through the membrane for the time from 0 to t_* is

$$S = \int_0^{t_*} Q dt = aLC_0 B \quad (6)$$

$$B = \alpha T_* + 2 \sum_{n=1}^{\infty} \frac{(-1)^n}{(n\pi)^2} \{ 1 - \exp(-(n\pi)^2 \alpha T_*) \} \quad \text{for } t_* \leq t_0 \quad (7)$$

$$B = \alpha T_0 - 2 \sum_{n=1}^{\infty} \frac{(-1)^n}{(n\pi)^2} \{ \exp(-(n\pi)^2 \alpha T_0) - \exp(-(n\pi)^2 \alpha (T_* - T_0)) \} \quad \text{for } t_* \geq t_0 \quad (8)$$

$$\text{where } T_* = \frac{D_* t_*}{aL^2}$$

The total amount of gas diffused through the membrane is, by putting $T_* = \infty$ in equation (8)

$$S_{\infty} = aLC_0 \alpha T_0 \quad (9)$$

The flux of the gas diffused out of the inlet side ($x = 0$) at time t (where $t > t_0$) is

$$Q' = \alpha D_* \left(\frac{\partial C}{\partial x} \right)_0 = \frac{C_0 D_*}{L} 2\alpha \sum_{n=1}^{\infty} \{ \exp(-(n\pi)^2 \alpha (T - T_0)) - \exp(-(n\pi)^2 \alpha T) \} \quad (10)$$

Let us define the effective diffusion coefficient of the heavier species ²³⁸UF₆ by D_* ; $\alpha = 1$. The effective diffusion coefficient of ²³⁵UF₆ is, therefore, αD_* with $\alpha = 1.00429$. In writing concentrations the species ²³⁵UF₆ and ²³⁸UF₆ will be distinguished, respectively, by the superscripts 5* and 8*. The concentrations in the feed are denoted by the subscript 0, and those after the diffusion through a membrane by 1.

The amount of ²³⁵UF₆ diffused through the membrane for the time from 0 to t_* is $S(\alpha = 1.00429)$ and that of ²³⁸UF₆ is $S(\alpha = 1.0)$. The concentration ratio of ²³⁵UF₆ to ²³⁸UF₆ in the collected gas is then

$$\left(\frac{C^{5*}}{C^{8*}} \right)_1 = \frac{S(\alpha = 1.00429)}{S(\alpha = 1.0)} = \frac{C_0^{5*}}{C_0^{8*}} (1 + R) \quad (11)$$

where R is the enrichment factor for the lighter species ²³⁵UF₆.

$$R = \frac{B(\alpha = 1.00429)}{B(\alpha = 1.0)} - 1 \quad (12)$$

Figure 1 shows the enrichment factor R for ²³⁵UF₆ in the gas collected for the time from $T = 0$ to T_* . When T_* is small, the amount of gas decreases, but the enrichment factor becomes very high. If the diffused-through gas is collected for the time from 0 to ∞ , the enrichment factor is $R = 0.429\%$, which is identical with that of the steady diffusion process.

Equation (10) shows that the gas diffused out of the inlet side, at $t > t_0$, also has more of the lighter component than of the heavier, but the enrichment factor for the lighter species is less than that of the steady process.

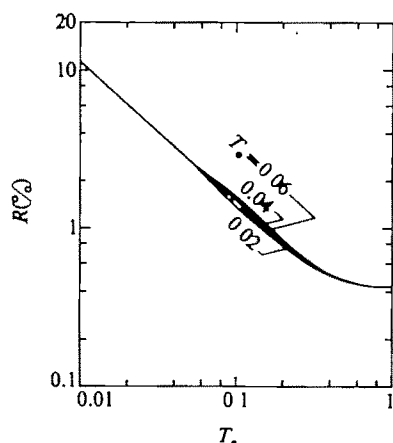


Fig. 1 Enrichment of $^{235}\text{UF}_6$ in gas collected at outlet side of membrane from time $T = 0$ to T_0 .

After the pulse input is turned off, it takes some time for the gas held in the membrane to diffuse out. In case of $T_0 = 0.04$ – 0.06 , equations (5) and (10), respectively, show that no more gas diffuses out of the outlet side and inlet side of the porous membrane after $T = 0.7$. The process is then repeated by imposing the gas mixture on the inlet side of the membrane.

The enriched gas collected from the first enrichment stage is pumped to the second stage for further concentration. If N stages are used having the same operating conditions of pulse period and gas collection time, the concentration ratio of $^{235}\text{UF}_6$ to $^{238}\text{UF}_6$ in the gas obtained from the final stage is

$$\left(\frac{C^{5*}}{C^{8*}}\right)_N = \frac{C_0^{5*}}{C_0^{8*}} (1+R)^N \quad (13)$$

When 0.7% $^{235}\text{UF}_6$ is concentrated to 3%, equation (13) becomes

$$\frac{0.03}{0.97} = \frac{0.007}{0.993} (1+R)^N$$

If $R = 3\%$, the theoretical number of stages is $N = 50$. In steady diffusion process, however, the enrichment factor is only $R = 0.429\%$ and the number of stages becomes as many as $N = 345$.

Vycor type of porous glass may be used as a membrane for the enrichment process. In fact, the measurements made in our laboratory for a gaseous mixture of nitrogen and argon with a 0.12 cm-thick Vycor glass (mean pore radius 45 Å, and porosity 30%) indicated that the lighter species, nitrogen, was considerably enriched in the gas diffused through the Vycor glass at small times, as predicted from the theory.

The effective diffusion coefficient D_e is

$$D_e = \frac{a}{\tau} D_K \quad (14)$$

where D_K is the Knudsen diffusion coefficient, and τ is the tortuosity factor. The Knudsen diffusion coefficient is

$$D_K = \frac{2\bar{v}r}{3} \quad (15)$$

where r is the pore radius. The mean molecular velocity is $\bar{v} = (8R\theta/\pi M)^{1/2}$ where R , θ and M represent, respectively, the gas constant, temperature and molecular weight. According to Satterfield and Sherwood¹, the tortuosity factor for Vycor glass is $\tau = 5.9$.

With the tortuosity value, the effective diffusion coefficient of $^{238}\text{UF}_6$ in the Vycor glass having $a = 0.3$ and $r = 45$ Å is estimated as $D_e = 0.0002 \text{ cm}^2 \text{ s}^{-1}$ at 25 °C.

Suppose the chambers on either side of the Vycor glass plate are, first, evacuated, and then the gaseous mixture of $^{235}\text{UF}_6$ and

$^{238}\text{UF}_6$ is introduced into one chamber as a pulse for 3 s. If the Vycor glass plate is 0.2 cm thick, $T_0 = 0.05$. If the gas diffused through the plate is collected for 0–3 s (or $T_e = 0.05$), $^{235}\text{UF}_6$ is enriched in the gas by 2.7%.

Equations (6) and (7) indicate that the amount of $^{238}\text{UF}_6$ diffused through the plate for the time from 0 to $T_e = 0.05$ is $S = aLC_0B$ with $B = 0.00028$. If the transient diffusion process is repeated in a cycle of $T_{\text{cycle}} = 0.7$, this quantity S is the amount of $^{238}\text{UF}_6$ obtained for the time $T = 0$ – 0.7 .

On the other hand, in steady diffusion condition the amount of $^{238}\text{UF}_6$ to be obtained for the same time $T = 0$ – 0.7 is $S_s = 0.7aLC_0$.

The amount of gas diffused through the porous membrane in the transient condition becomes much smaller than that in the steady condition. This disadvantage is unavoidable in the transient diffusion process, but it will be attractive in that the lighter species is considerably enriched in the gas obtained in a small time.

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Re-evaluation of possible historical relationship between magnetic intensity and climate

HYPOTHESES concerning changes in the internal component of the geomagnetic field and consequent effects on climate^{1–10} are usually based on palaeomagnetic and palaeoclimatic data. Results are often ambiguous due to poor temporal and spatial resolution and difficulties in interpreting the proxy data^{10–12}. Use of historic data partially avoids these problems. Thus, Wollin *et al.*¹³ tentatively conclude “that the trends in intensity from most of the magnetic observatories in the world with records over at least 30 yr correlate negatively with the 10-yr means of air temperature”. Because temperature and magnetic field intensity both have regional characteristics, this conclusion implies that the relationship is a local phenomenon although it will be valid globally. This further suggests that the relationship is causal. Wollin *et al.* presented graphs of mean annual magnetic intensities from 16 magnetic observatories along with 10-yr mean temperatures from nearby meteorological stations; one example is given using a 10-yr running average of winter temperatures and five examples use annual temperatures. Although the graphical data are suggestive of the proposed relationship, the study suffers in three respects. First, data from only 22 of the 43 magnetic observatories listed are presented. Second, the use of 10-yr temperature means yields only three or four data points which may not properly represent the trend of the temperature data. Third, the degree of correlation is not quantitatively evaluated. Here we present statistical evidence that there is no globally valid inverse relationship between geomagnetic intensity and climate for the period covered by observatory records.

We initially selected 98 magnetic observatories¹⁴ which were in reasonable proximity to meteorological stations¹⁵. A record is considered usable if it contains at least 18 yr of data, a maximum number of missing data points equal to 10% of the record length, and no more than one consecutive missing datum. Missing points are linearly interpolated using data from the preceding and succeeding years. Applying these criteria leaves

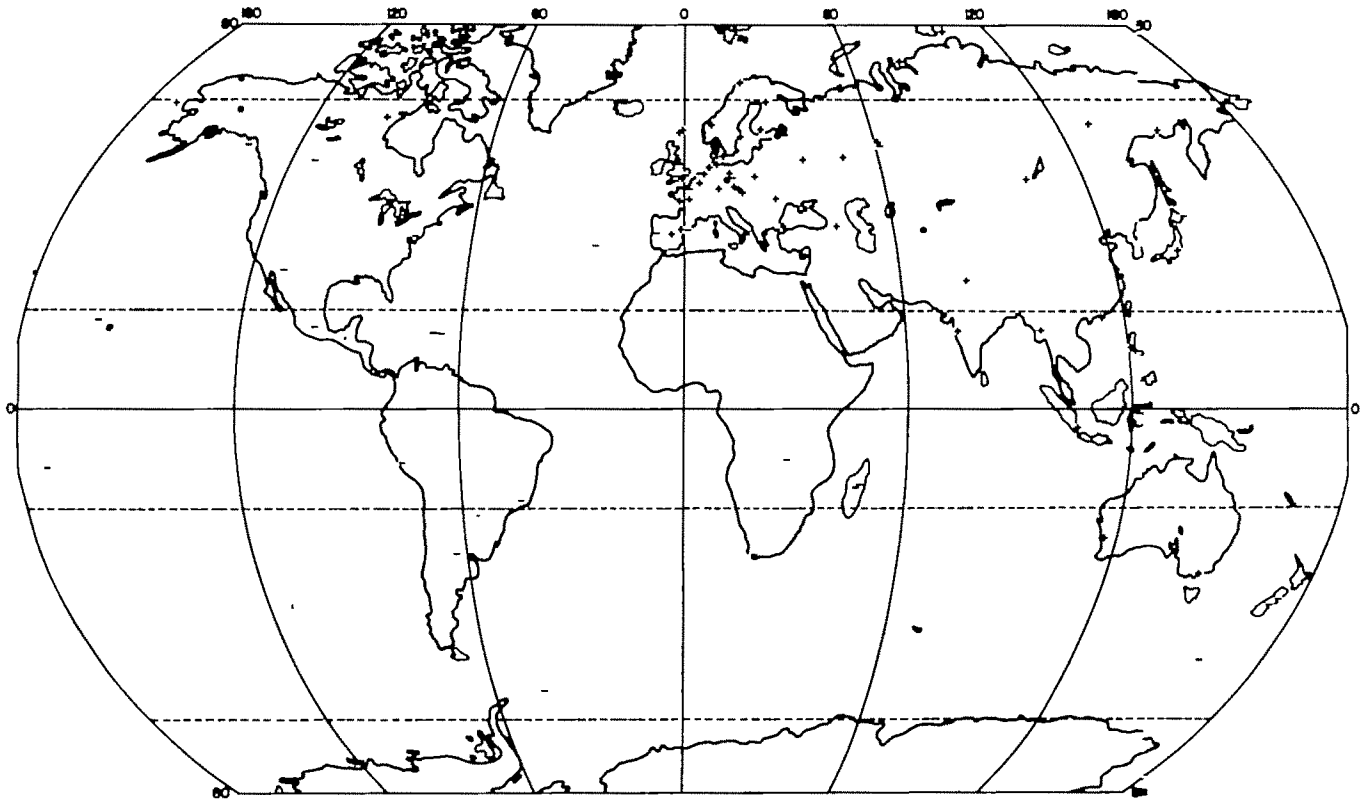


Fig. 1 Geographic pattern of change in the magnetic field strength, r_{TF} , for 88 records (one record used twice with different meteorological stations). +, Indicates field strength linearly increasing with time (0.001 confidence level); -, Indicates decrease with time; 0, indicates no significant trend. These records represent different intervals of time over the past 70 yr.

89 records from 85 stations. Only the relationship between mean annual geomagnetic intensity and temperature is investigated, even though Wollin *et al.* believe that some stations have intensities correlating with winter temperature trends. No stations are excluded from analysis *a priori*, although Wollin *et al.* suggest that the lack of correlation observed for some stations may be due to climatic influences of strong ocean currents.

We investigated the possibility of a linear relationship between temperature and intensity. Cross plots of these two variables show no evidence of a monotonic nonlinear relationship. Correlation coefficients between magnetic intensity and time (r_{IT}), temperature and time (r_{IT}), and temperature and intensity (r_{TI}) were computed for the various records. The geographical distribution of magnetic observatories and the trends for the r_{IT} values are shown in Fig. 1. The histograms of Fig. 2 summarise results from all records used for the three correlation coefficients. Since the magnetic intensity variation has been linear (low frequency), we did not consider the possibility of a frequency dependent relationship. Zero lag was used in computing r_{TF} which seems reasonable considering the linear variation of intensity and our primary concern in trends rather than high frequency changes. The z transformation of Fisher¹⁶ is used to transform the correlation coefficient r by the formula $z = 0.5 \log_e(1 + r/1 - r)$. As r goes from -1 to 1 , z goes from $-\infty$ to $+\infty$. This transformation is advantageous because for any population of correlation coefficients, the distribution of z will be nearly normal, and the standard error of z will have the simple form $S_z = 1/(n-3)^{1/2}$ where n is the number of points for which r is calculated. Figure 3 shows a histogram of the transformed z/S_z values corresponding to r_{TF} . If the r_{TF} values were randomly drawn from a single distribution with a population mean of zero, the z/S_z values would have a standard normal distribution. It is obvious from the outliers that the values do not represent a single population. To quantify the results, we applied several statistical tests¹⁶⁻¹⁸ to the z_{TF} data. Assuming that the

z values are drawn from a single population, a weighted mean and standard error of the mean can be estimated as $\bar{z} = \sum z/S_z^2 / \sum 1/S_z^2$,

$$S_z = \frac{1}{(\sum (n-3))^{1/2}}$$

with the summation taken over the n records. The calculated values are $\bar{z} = -0.89$ and $S_z = 0.019$. A χ^2 test can then be used to see if the z values differ significantly among themselves, the test statistic being $\chi^2_{N-1} = \sum (n-3)(z - \bar{z})^2$. The calculated value is $\chi^2_{88} = 20,140$ which is highly significant, indicating that the z values and hence the correlation coefficients of r_{TF} do not represent a single population. Thus, the $\bar{z}$ and S_z values derived above cannot be considered valid population estimates. A χ^2 test can then be used to test for an overall association between intensity and temperature by use of the statistic $\chi^2_N = \sum (n-3)z^2$. For this test, $\chi^2_{89} = 22,373$ which is also highly significant. The χ^2 statistics are highly influenced by the relatively few outliers observed in Fig. 3. Because of the linear nature of the geomagnetic intensity temporal variation seen in the r_{IT} histogram of Fig. 2, whenever a temperature record is long and linear, the result will generally be a high z_{TF} value. So the χ^2 tests are not sensitive to subtle overall relationships. A sign test should be more indicative in testing the hypothesis as it gives information on the sign of the z values independent of their magnitude. The proposed inverse relation would suggest a preponderance of negatively signed r_{TF} or z_{TF} values. Out of the 89 records, 52 have negative r_{TF} values. Using the normal approximation to the binomial test with continuity correction,

$$P(r^- > 52) \approx P(Z > \frac{51.5 - 44.5}{89/4}) \approx 7\%$$

where Z here means the standard normal distribution. This is borderline statistical significance.

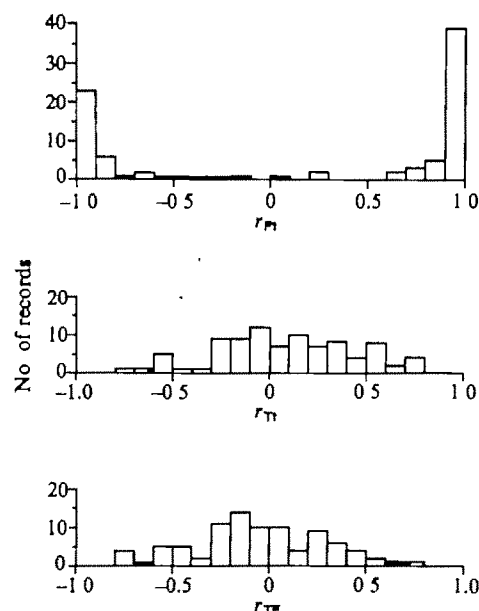


Fig. 2 Correlation coefficients of 89 records from 85 pairs of magnetic and weather observatories. Separate histograms are for the correlation between total magnetic intensity and time (r_{TM}), temperature and time (r_{TI}), and temperature and total intensity (r_{TF}).

Since geomagnetic intensity and climate are both regional parameters showing temporal and spatial coherence regardless of the hypothesis being considered here, data used to analyse the present hypothesis should be well distributed geographically. Otherwise, a spurious regional correlation between intensity and climate might unduly bias the overall results. Of the 89 records, 37 are from European stations although Europe covers only about 2% of the Earth's surface. The European z values were combined into a single datum by taking a weighted average of the individual values, using S_z^2 as weights. Similarly, six other pairs of closely spaced points were combined. The resultant Z_{TF}/S distribution is shown in Fig. 3. The χ^2 tests remain highly significant, but only 26 of the resultant 47 data have $r_{TF} < 0$, and $P(r > 26) \approx 23\%$, which is not statistically significant. Thus, based on the spread in the z_{TF} distribution and the results of the sign test, we conclude that the hypothesis that there is a historic negative correlation between local magnetic intensity and temperature trends is not statistically verified. The European data alone show an overall negative correlation significant at the

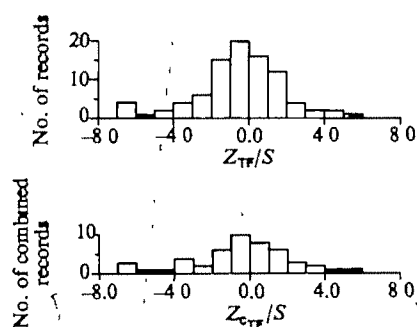


Fig. 3 The Fisher z transformation of the r_{TF} values of Fig. 1 divided by the standard deviations of the z distributions. Transformation of the raw values is given by Z_{TF}/S , while Z_{CTF}/S has combined values from stations in proximity to one another.

90% level, but because of the regional nature of the parameters, it would not be justifiable to conclude a causal relationship between intensity and climate.

Empirical studies of physical relationships are important for indicating appropriate lines of theoretical research. Although no proven theory links climate to geomagnetism, qualitative mechanisms have been postulated^{1,2,7,19}. These generally view the geomagnetic poles as flux tubes along which charged particles can propagate into lower regions of the atmosphere. Changing strength and orientation of the dipole field might then affect the quantity and geographic orientation of the ion corpuscular radiation⁷⁻⁹. However, it would be difficult to conceive of a mechanism that could link all local disturbances of the magnetic field to climatic change.

Because of the highly linear trends in magnetic field strength variation in many parts of the world, there will inevitably be some places where there will be an inverse relationship between the changes in temperature and the magnetic field. Without a viable physical mechanism that could produce such a relationship worldwide, we conclude that the weak, negative correlation between magnetic field strength and temperature in the historical record is a fortuitous result of the linear secular variation of magnetic field intensity and the poor geographic distribution of stations.

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Detection of Ni-Sn phases in temper embrittled Ni-Cr steels

A GENERIC problem in ferritic steels is the reduction in the grain boundary cohesive strength produced by the segregation of trace impurities of Groups IVB and VB of the Periodic Table. Such segregation results in a deterioration in mechanical properties produced, for example, by reversible temper embrittlement¹ and it also promotes enhanced susceptibility to a range of environmental enhanced cracking processes²⁻⁴. It has long been recognised that the embrittlement of steels is enhanced by the

Table 1 Composition of Ni-Cr steels studied

	C	Ni	Cr	Wt% Mn	Si	Mo	Fe
En30A	0.28	4.06	1.20	0.58	0.32	0.11	bal
BE10	0.18	3.33	1.42				bal
	S	Sb	p.p.m. P	Sn	As		
	80	40	130	140	240		
	20	<50	<50	425	<50		

Table 2 Distribution of particles at grain boundaries observed in En30A

Ageing time at 500 °C	Particles at grain boundary (%)		
	Fe ₃ C	M ₇ C ₃	Ni-Sn phase
≤100 h	100	—	—
500 h	96	2	2
5,000 h	80	15	5

presence of certain alloy elements, that is Ni, Cr or Mn, and measurements using Auger electron spectroscopy (AES)⁵⁻⁷ have demonstrated that specific combinations of alloy and impurity elements cosegregate to the boundaries, for example, Ni+Sn, Ni+Sb, Cr+P. These observations can be rationalised using Guttman's phenomenological theory⁸ which attributes the increased driving force for segregation to a positive interaction between alloy and impurity solute atoms. Previous investigations^{1,9-11} have failed to detect the presence of any three dimensional phase using conventional transmission electron microscopy and it is generally considered that the alloy element-trace impurity atoms form two dimensional phases at the boundaries. The only direct evidence for such alloy-impurity interactions and phase formation has been obtained using

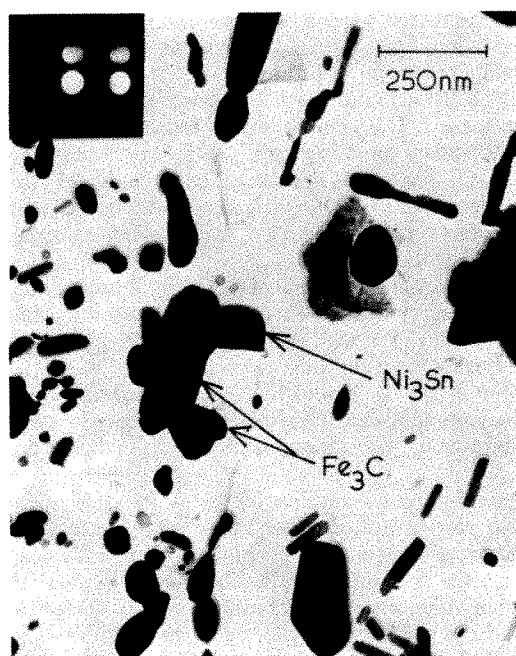


Fig. 1 Micrograph of an extraction replica showing the precipitate distribution in En30A steel after ageing for 5,000 h at 500 °C. A convergent beam microdiffraction pattern of the Ni₃Sn phase is shown in the inset, (211) zone.

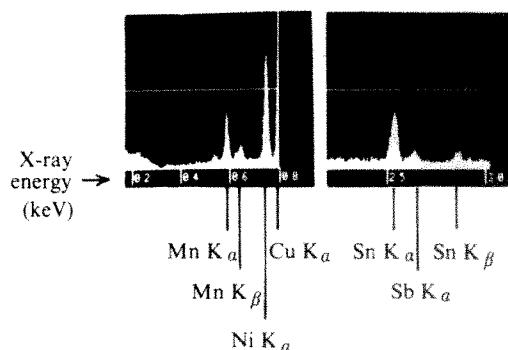


Fig. 2 Energy dispersive X-ray spectrum obtained from the Ni₃Sn phase indicating the presence of Ni, Mn, Sn and Sb.

Mössbauer spectroscopy^{12,13} but it is not possible using this technique to draw any positive conclusions regarding the location of such compounds. Measurements on a pure Fe-3.5 wt% Ni alloy doped with ¹¹⁹Sn¹² showed that Ni atoms associate preferentially with Sn atoms during tempering at 650 °C and the marked change in the spectrum observed on ageing at 500 °C, within the embrittling temperature range, is consistent with the formation of a Ni-Sn compound (Ni₃Sn₂ or Ni₃Sn₄). We report here some initial results from a detailed electron microscopic analysis of the microstructures of a commercial (En30A) Ni-Cr steel and a special laboratory made steel (BE 10) having similar Ni and Cr contents, but doped specifically with tin. The composition of the two steels is presented in Table 1. Both steels were oil quenched from 950 °C, tempered for 1 h at 650 °C and aged at 500 °C for times up to 5,000 h. Carbon extraction replicas, taken from polished surfaces were examined in a Philips EM400 microscope fitted with an energy dispersive X-ray system. Selected individual precipitate particles were analysed using convergent beam microdiffraction and X-ray analysis. Attention was focused on the particles which delineated the lath packet and previous austenite boundaries, that is, high-angle boundaries.

An electron micrograph showing the precipitate distribution in En30A after ageing for 5,000 h at 500 °C is presented in Fig. 1. The results from analysing the precipitates in the quenched and tempered condition and after ageing at 500 °C for times up to 5,000 h are listed in Table 2. For ageing times <100 h at 500 °C only orthorhombic Fe₃C particles, in which 10–20% of the iron is replaced by Cr, were identified. On ageing for 500 h at 500 °C the boundaries contained about 96% Fe₃C and 2% hexagonal M₇C₃ particles containing Fe and Cr in approximately equal amounts. In addition, about 2% of the particles were analysed to contain Ni and Sn and an example is arrowed in Fig. 1. Such particles were invariably located within small clusters of particles and were apparently attached to Fe₃C particles, although frequently M₇C₃ particles were also present in the clusters. The structure was analysed to be cubic with a lattice unit cell size of 0.598 ± 0.004 nm which matches a Ni₃Sn phase previously reported¹⁶ to be stable only above 950 °C. A (211) zone axis diffraction pattern is shown in the inset of Fig. 1. Two sections of the X-ray spectrum from a particle are presented in Fig. 2. It can be seen that the particles contain significant amounts of Sb and Mn in addition to Sn and Ni but no P was detected, although parallel AES studies¹⁴ have shown that P is present at the grain boundaries in significant amounts (~15 %). The copper K_α peak in the spectrum comes from the copper grid on which the replica was mounted. On ageing for 5,000 h at 500 °C the proportion of M₇C₃ and Ni-Sn precipitates increased to 15% and 5% respectively, the balance again being Fe₃C.

A more limited study was made of the high purity laboratory made steel, BE10, doped with 425 p.p.m. Sn (Table 1). An examination of the material aged for 5,000 h at 500 °C showed two significant differences from the ageing behaviour exhibited

by En30A. First, a higher proportion of M_7C_3 was present suggesting that the transformation from Fe_3C is occurring at earlier ageing times. Second, a Ni-Sn phase was again observed but was only found adjoining M_7C_3 particles. Results from microdiffraction and X-ray analysis were consistent with the phase being of the type Ni_3Sn_2 having a hexagonal superlattice structure. Thus, these results provide further support of the Mössbauer results¹² which were also obtained from a special laboratory made steel having a high Sn content.

In the present study, convergent beam microanalysis¹⁵ has been used to characterise individual particles as small as 20 nm diameter in terms of both structure and composition. This technique has enabled us to obtain the first direct evidence for the formation of discrete three dimensional intermetallic compounds from alloy and impurity elements in temper embrittled steels. The results indicate a dependence between steel composition, carbide transformation kinetics and the type of Ni-Sn phase formed and therefore caution is required in predicting the behaviour of commercial steels based on the results obtained from specially prepared casts. The present work demonstrates that careful microstructural characterisation provides an important insight into the processes contributing to grain boundary centred embrittlement phenomena.

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Electron nuclear double resonance signals from three ingredients of coal

ELECTRON spin resonance (ESR) spectra from coals were first reported in 1954¹⁻³. Since then many ESR studies on coals have been conducted, and the signals have been shown to arise from free radicals^{4,5}. Recently Kevan *et al.* studied coals using the electron nuclear double resonance (ENDOR) technique⁶. They detected a single-peak signal at the free proton frequency, thus providing the first direct evidence that the environments of radicals in coals contain hydrogen atoms. We report here on ENDOR signals from three ingredients which were separated out from a coal sample. The ENDOR signal from each ingredient is substantially different from that of the other ingredients. In addition, a proton hyperfine structure (hfs) was experimentally resolved, probably for the first time, for a radical in one ingredient of a coal sample.

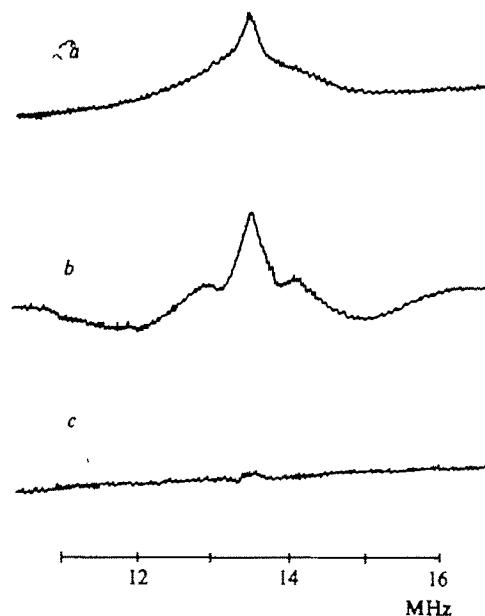


Fig. 1 ENDOR signals from three ingredients of a bituminous coal sample at a relative microwave power of -11 db. The signals were obtained for the out-of-phase mode⁸ at room temperature in air. The RF field was 8.2 G, which was measured by a sideband method¹¹. The free proton frequency was 13.5 MHz. a, Vitrain; b, clarain; c, fusain.

The sample studied was bituminous coal taken from a coal mine in Alabama. This sample showed three distinct layers—bright-glossy, satin-glossy and dull—composed of vitrain, clarain and fusain, respectively⁷. The coal sample was crushed into pieces of average size $0.5 \times 0.5 \times 1.0$ mm. Several pieces of each ingredient were then hand picked, and were put into a Teflon sample tube, which was designed to fit the ESR/ENDOR sample holder.

The ESR absorption from these ingredients showed only a small oxygen effect in intensity⁴. The radical concentration, which was measured using a DPPH probe, was comparable for the three ingredients: $4 \times 10^{19} \text{ g}^{-1}$ for vitrain, $4 \times 10^{19} \text{ g}^{-1}$ for clarain, and $3 \times 10^{19} \text{ g}^{-1}$ for fusain.

The ENDOR technique with an intense radiofrequency (RF) field⁸⁻¹¹ was used. The signal was detected at room temperature in the out-of-phase mode⁸ using an RF field of 8.2 G. Detection of the ENDOR signal was difficult when the RF field was below 3 G. No significant ENDOR signal was detected when the in-phase detection mode was used, even though the applied RF field was higher than 3 G.

The ESR signals from vitrain and clarain samples exhibited a maximum intensity at a relative microwave power of -11 db (-3 db for fusain samples), and the ENDOR signals from the three samples at -11 db are shown in Fig. 1. The signals are quite different for the three different coal ingredients. These spectra indicate that the environment of the unpaired electrons in each ingredient is substantially different from that of the other two.

The chemical compositions of the three ingredients are slightly different from each other⁷: the carbon content is highest and the hydrogen content lowest in fusain, while the content of volatile matter is highest in clarain. Thus it is possible that different ingredients contain different free radical species. Even if these radicals are chemically identical, the environment of the radical would differ from one ingredient to another. Note that the technique used here can effectively detect ENDOR signals from protons in the environment of a free radical¹⁰.

Furthermore, an out-of-phase ENDOR signal, such as that observed in the present case, is highly sensitive to the spin-lattice relaxation time⁸. Thus, it is quite possible that although the

radicals, including their environments, are chemically identical, different ingredients could give quite different ENDOR signals; this difference could originate from the difference in spin-lattice relaxation time, which is due to minor difference in structure deformation of the radical and/or its environment between different ingredients.

For these reasons the different coal ingredients might be expected to give different ENDOR signals. The nature of the difference in ingredients which is responsible for the difference in the ENDOR signals, however, is not yet clear.

The weak signal for fusain (see Fig. 1) can be attributed to an impurity. Examination of the specimen by a magnifier revealed a small bright region which appeared to be vitrain. Another specimen of fusain with a larger bright region showed a substantially stronger ENDOR signal.

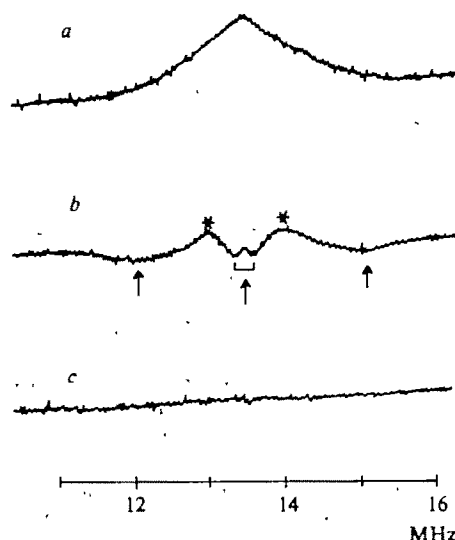


Fig. 2 Signals similar to those in Fig. 1 but observed at a relative microwave power of -20 db. The relative amplifier gain was twice that for the case of Fig. 1. a, Vitrain; b, clarain; c, fusain.

The sharp central peaks of the ENDOR signals from both vitrain and clarain, as seen in Fig. 1, exhibited a line width and line intensity dependence on microwave power which was very similar to the ENDOR signal reported by Kevan *et al.*⁶ The line width of our central peaks (~0.4 MHz) increased appreciably at higher microwave powers. The intensity of these peaks was also very sensitive to microwave power, and at a relative microwave power of -20 db (see Fig. 2), no evidence for this central peak is seen in vitrain and in clarain. In fusain this peak is very weak. This type of single ENDOR peak has been detected previously in irradiated crystals⁶. This peak and the similar one from these coal samples result from RF-field-induced positive phase shifts of the modulated ESR signals. In contrast, ordinary hfs ENDOR signals arise from negative phase shifts. The mechanism for the appearance of these single peaks has not been clarified.

As is seen in the second curve of Fig. 2, the ENDOR spectrum for clarain shows three negative signals (shown by arrows in Fig. 2), arising from hfs splitting. The central signal arises from protons with very small hfs, and the other two from proton couplings with an average hfs splitting of 3 MHz. The latter splitting is probably the first resolved case of hfs in coals.

It is not clear whether the two positive peaks for clarain (starred in Fig. 2) are hfs signals. It is possible that these two peaks appear as a result of a superposition of the signal from a vitrain impurity. In another specimen of clarain, at a microwave power of -20 db the position of the bottom of the central signal (negative in Fig. 2) was higher and in the positive region. This could indicate a higher concentration of a vitrain impurity which, when combined with the central negative signal, could give rise to the two observed positive peaks.

The signal from vitrain was broad and positive (see Fig. 2). Note that vitrain is known to have five modifications⁷. Thus, it is possible that this specimen could be further separated into five or more ingredients.

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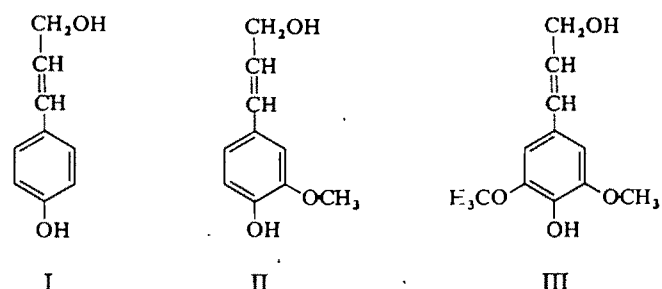
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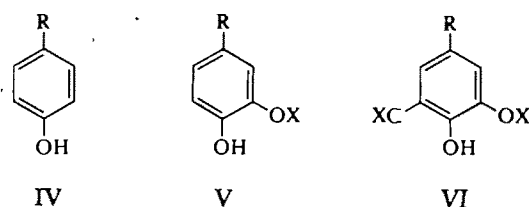
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Lignin-like polymers in coals

ALTHOUGH lignins are major constituents of plants from which coals are derived, their roles in the coalification process and resulting coal structures have not been defined. On the basis of chemical and biological degradations, lignins are considered to be polymers of *p*-coumaryl- (I), coniferyl- (II), and sinapyl- (III) alcohols¹⁻³.



Isolation of coal degradation products related to these three monomers should shed some light on the roles played by lignins during coalification and in the final structure of coals. To date such studies have had only limited success. Many oxidative degradations have also been carried out to break coal down into simpler species; however, isolation and identification of *p*-hydroxyl (IV), vanillyl (V), and syringyl (VI) groups, which are characteristic lignin oxidation products, have not yet been confirmed.



R = -CHO, -COCH₃, or -COOH; X = H or CH₃

Table 1 Summary of CuO–NaOH oxidation of coal*

Wt % of products†	Lignite	Bituminous
Organic acid (benzene–ether extract)	35.3	19.6
Humic acid-like material (methanol extract)	54.3	61.0
Non-oxidised coal	11.0	21.0
Wt % of organic acids‡		
Phenolcarboxylic acid	66.6	54.1
Benzenecarboxylic acid	26.0	36.2
Naphthalenecarboxylic acid	2.0	3.3
Heterocyclic acid	—	1.6
Aliphatic dibasic acid	2.6	1.0
Others§	2.8	3.8
Ratio of phenol/benzene carboxylic acids	2.56	1.49

* Elemental analysis (dry ash free basis): Lignite (Wyoming) C, 66.4; H, 4.7; N, 1.5; S, 1.1; O, 26.3 (by difference). Bituminous (Illinois #2) C, 73.9; H, 5.2; N, 1.4; S, 2.4; O, 17.1 (by difference). Anthracite (Pennsylvania PSOC 85) C, 91.0; H, 3.8; N, 0.7; S, 1.2; O, 3.3 (by difference). Samples were pretreated to remove solvent extractable trapped organic materials before analysis.

† Wt % was obtained from sample on a dry ash free basis.

‡ Wt % was obtained from each benzene–ether extract and the amount of each compound determined from GC.

§ Aromatic aldehyde, keto and unidentified compounds.

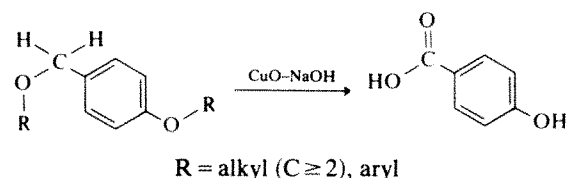
We report here that, using a CuO–NaOH oxidation procedure we were able to isolate phenolic acids which show a relationship between lignins and coals.

Although fulvic and humic acids^{4–6} have been degraded successfully with alkaline solutions to produce lignin-related phenols, this method has not been successful in coals^{7,8}. Reductive degradations of soil⁹ and coal-derived¹⁰ humic acids with sodium amalgam have been reported to produce a variety of phenolic compounds; however, the method used has been criticised because of its poor yields and its being so drastic that resulting chemical structures may have little or no relation to the starting material^{11,12}. In general, commonly used oxidants destroy phenolic rings or give complex products^{13,14}. Some of the oxidants such as nitrobenzene produce reaction byproducts that may interfere with the analysis of the oxidation products^{15–17}. To obtain lignin oxidation products from coals we used the alkaline cupric oxide oxidation method which has been successfully applied to analysis of lignins in plants¹⁵, humic acids^{17,18} and land-derived marine sediments¹⁵. To obtain detailed information of the CuO–NaOH oxidation, control experiments with known phenols, phenyl alcohols, aryl alkyl ethers and alkyl benzenes were also carried out using the procedure employed for the coals.

After removal of trapped organic compounds^{19,20}, 5 g of each coal sample (lignite, bituminous and anthracite, see Table 1) was oxidised with alkaline cupric oxide (52 g of CuSO₄ · 5H₂O, 37 g of NaOH and 185 ml of H₂O) at 200 °C for 8–10 h by the method of Greene *et al.*²¹. After separation of insoluble residue, the alkaline solution was acidified with HCl. The acidic solution was concentrated and extracted repeatedly with benzene–ether (1:3) and finally with methanol. Yields of the oxidation products from lignite and bituminous coals are shown in Table 1. No organic solvent-extractable oxidation product was isolated from anthracite coal. The benzene–ether extracts were derivatised with d₆-dimethylsulphate to yield d₃-methyl labelled derivatives. The derivatives were analysed by gas chromatography–mass spectrometry (GCMS) and high resolution MS using techniques described previously¹⁹. The retention times and mass spectra of the phenolic esters were confirmed with authentic samples. Gas chromatograms of the derivatives are shown in Fig. 1 with numbered peaks identified in Table 2. The methanol extracts from both coals were found to consist essentially of humic acid-like material soluble in alkali but complex and of high molecular weight^{3,22,23}.

As shown in Fig. 1 and Tables 1 and 2 the identification of large amounts of *p*-hydroxy- and 3,4-dihydroxybenzoic acids which are regarded as characteristic lignin oxidation products is very informative. Phenolic di- and tricarboxylic acids, and hydroxynaphthalenecarboxylic acids were also identified which are not obtained from land plants¹⁵ and marine sediments¹⁵ by the same oxidation.

All phenolic acids identified were found as OCD₃-derivatives. The mass spectra showed the complete absence of OCH₃-group. It is confirmed that no isotope exchange of the H in OCH₃ with D occurred during our procedures. Although partial demethylation reaction during the oxidation cannot be excluded from the control experiment, it is probable that the phenolic acids found were derived mainly from the fission of ether-linked aromatic systems as shown in the example below rather than from aryl methoxy units.



Indeed, it is known that very little or no methoxy groups are present in bituminous coals^{23,24}. In the lignite coal aryl methoxy groups are probably not present in significant amounts.

Trihydroxybenzoic acids (syringic group) were not found in the oxidation products. We have found that the authentic syringaldehyde was largely degraded by the oxidation. This may account for the fact that we did not observe them; however, the syringic group may have been degraded during coalification. Authentic mono- and dihydroxybenzenes were found to be stable in the same conditions.

Non-hydroxy substituted benzene-, naphthalene-, pyridine-carboxylic acids and humic acid-like materials found might be

Table 2 Organic acids identified as their d₃-methyl esters by GC–MS and high resolution MS

Peak no.	Compounds
1	Succinic acid
2	Methylsuccinic acid
3	Benzoic acid
4	Methylbenzoic acid
5	<i>p</i> -Hydroxybenzoic acid
6	Hydroxytoluic acid
7	1,2-Benzenedicarboxylic acid
8	1,4-Benzenedicarboxylic acid
9	1,3-Benzenedicarboxylic acid
10	Methylbenzenedicarboxylic acid
11	Dihydroxybenzoic acid
12	Pyridinedicarboxylic acid
13	3,4-Dihydroxybenzoic acid
14	Dimethylbenzenedicarboxylic acid, naphthoic acid (minor)
15	Hydroxybenzenedicarboxylic acid, 4-hydroxy-1,3-benzene-dicarboxylic acid (major)
16	Methyl-hydroxybenzenedicarboxylic acid
17	1,2,4-Benzenetricarboxylic acid
18	1,2,3-Benzenetricarboxylic acid
19	1,3,5-Benzenetricarboxylic acid
20	Methylbenzenetricarboxylic acid
21	Pyridinetricarboxylic acid
22	Naphthalenedicarboxylic acid
23	Hydroxybenzenetricarboxylic acid
24	1,2,4,5-Benzenetetracarboxylic acid
25	1,2,3,4-Benzenetetracarboxylic acid
26	1,2,3,5-Benzenetetracarboxylic acid
27	Methylbenzenetetracarboxylic acid
28	Dihydroxy-biphenyldicarboxylic acid (T)
29	Hydroxynaphthalenedicarboxylic acid
30	Benzenepentacarboxylic acid

(T) indicates that the identification is tentative.

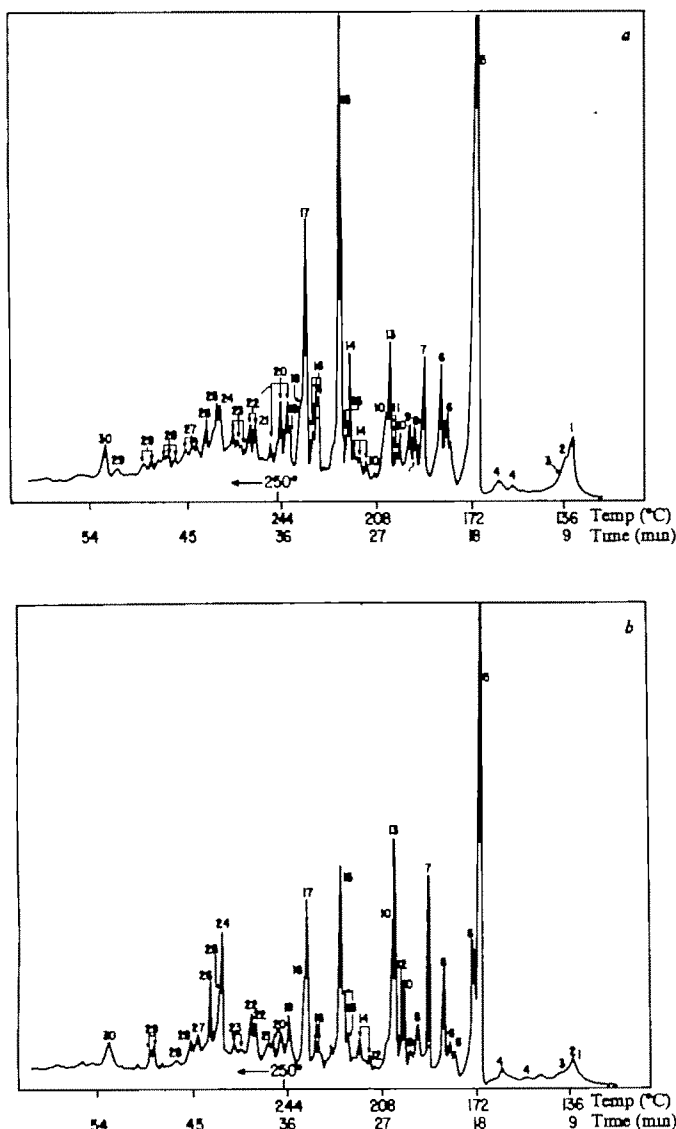


Fig. 1 Gas chromatogram of methyl esters of the organic acid fraction from lignite (a) and from bituminous coal (b). The analysis was carried out on a Perkin-Elmer 3920B gas chromatograph interfaced to a modified Bendix model 12 time-of-flight mass spectrometer with a variable split between a flame ionisation detector and the source of the mass spectrometer. The separation was made on a 15.2 m $\times$ 0.51 mm SCOT column coated with OV17 and temperature programmed from 100–250 °C at 4 °C min⁻¹.

derived from non-lignin or extensively transformed lignin polymers. It is obvious that extensive transformation of lignin-like polymers occurred through several reactions^{22,25} such as demethylation, demethoxylation, dehydrogenation, oxidation, cleavage of ring structures and re-condensation during coalification from lignite to bituminous and anthracite coals. Indeed, we have observed that the CuO–NaOH oxidation of higher rank coals (C, 78–87%) produces very little phenolic acid with an increased yield of non-hydroxy aromatic and heterocyclic acids (data not shown).

Previously²⁰ we characterised aromatic acids trapped in lignite coal, and have found that these acids are qualitatively quite similar to those obtained from the present oxidation of the same pretreated coal. This indicates that the trapped acids were derived mainly from the hydrolytic oxidative degradation of lignin-like polymers during the coalification. Indeed, the later coalification product, the anthracite coal, did not give any trapped acid²⁰ or organic solvent-soluble CuO–NaOH oxidation product. Flaig has also discussed²² oxidative transformation of plant material during humification and coalification.

Our results show that lignin-like polymers are incorporated into the macromolecules of lignite and bituminous coals. It is indicated that the polymers contain more highly cross-linked structures with increased aromaticity compared with the original lignins. This is experimentally shown by the identification of phenolic polycarboxylic acids and hydroxynaphthalene-carboxylic acids which are not found in the CuO–NaOH oxidation products of lignins and plant materials. In addition, plant polyphenols other than lignin could also be precursors for coal formation.

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Fluctuations in SO₂ emission during recent eruptions of Etna

THE eruptive activity of July–August 1977 of Mt Etna consisted of four separate, short eruptive episodes: 16–22 July; 5–6 August; 14 August; 24 August. The rate of SO₂ emission during these eruptions was monitored using a remote-sensing correlation spectrometer. These data suggest that fluctuations in the SO₂ emission rate provide a means for predicting eruptions of Etna.

The July–August 1977 eruptions were confined to the North East Crater vent, while continuous degassing occurred from the central vents. The continuous gas plume was blown downwind immediately after it was emitted while the eruption plumes from the North East Crater rose 500–1,000 m before being blown horizontally. As the plumes moved downwind they were maintained at elevations ranging from 2,000 to 3,000 m above sea level. Monitoring began on 24 July, immediately after the first eruptive episode had ended, and continued until 27 August.

A Barringer Correlation Spectrometer (COSPEC) was used to monitor the SO₂ in the volcanic plume. (The application and design principles have been described by Newcomb and Millán¹.) The spectrometer consists of a Cassegrain telescope, a

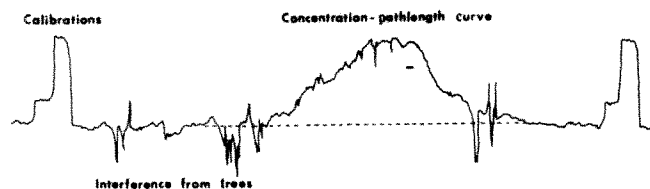


Fig. 1 A typical SO_2 plume cross-section obtained with the COSPEC mounted in an automobile. The instrument is calibrated at both ends of a traverse. The large calibration represents 370 p.p.m. m while the small calibration represents 125 p.p.m. m. The abscissa of the curve is time. A single traverse took ~ 15 min.

grating spectrometer, a correlator disk, and processing electronics section. Incoming radiation is dispersed by the spectrometer. The correlator disk then alternately passes UV spectra of high and low SO_2 absorption. This modulated signal is monitored by photomultiplier tubes which produce a voltage that is processed by the electronics. This processed voltage is proportional to the number of SO_2 molecules in the field of view of the instrument. This is a concentration-pathlength value and has dimensions in parts per million metres (p.p.m. m). The response of the instrument is calibrated by reference cells filled with known concentrations of SO_2 . The instrument also has an automatic gain control to compensate for changes in the radiation source (the daytime sky).

Data reported here were gathered with the COSPEC mounted in a vehicle with a right angle mirror attached to the telescope to allow a vertical field of view. The vehicle was driven under the plume approximately perpendicular to the plume direction. Concentration-pathlength data from the COSPEC are recorded on a stripchart recorder along with odometer readings to provide a distance scale. Instrument calibrations were done before and after each traverse so that instrument drift could be checked. Figure 1 shows the record from a single traverse.

The area under the concentration-pathlength-distance curve represents a SO_2 cross-section of the plume (p.p.m. m^2). This value is corrected for traverses that are not at right angles to the plume direction. A mass flow of SO_2 (tonnes d^{-1}) is calculated by multiplying the p.p.m. m^2 value times the horizontal wind speed of the plume (m d^{-1}) times the density of SO_2 gas (tonnes per p.p.m. m^3).

The traverses were made on the roads shown on Fig. 2. The arrow from the summit shows the average wind direction for the period of this study, while the range of the wind direction

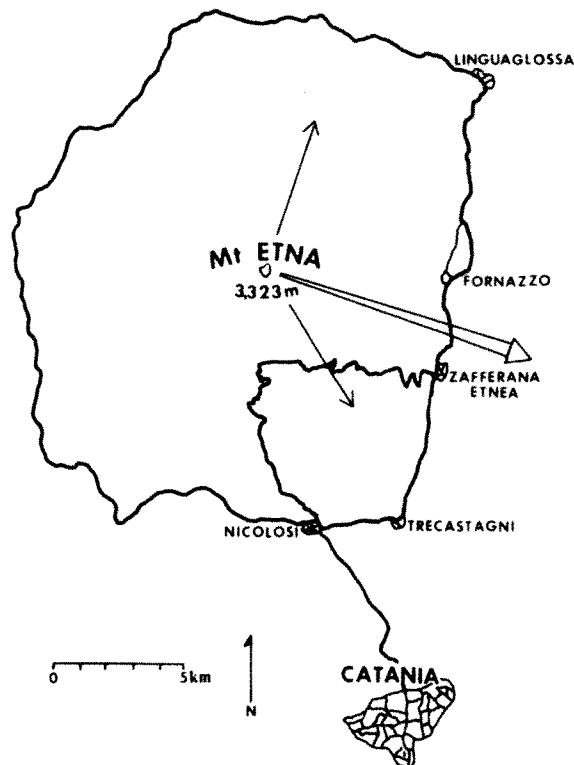


Fig. 2 Map of the roads used to obtain traverse data. The large arrow from the summit represents the mean wind direction while the smaller arrows represent the range of the wind directions.

for the period 16 July–28 August 1977 is shown by the shorter arrows. The roads are all between 7 and 10 km from the summit, and between 1,000 to 2,000 m below the plume as it passed overhead.

Two to eight traverses were made per day. A mass flow value was calculated for each traverse and the values from a given day were averaged to give a mean mass flow value for that day. Cloudy weather (clouds reduce the UV intensity and increase the instrument noise) or equipment failure account for missing days of data.

The activity of Mt Etna during July and August 1977 allowed the measurement of SO_2 output before, during, and after eruptive activity of the volcano. The SO_2 mass flow data are plotted

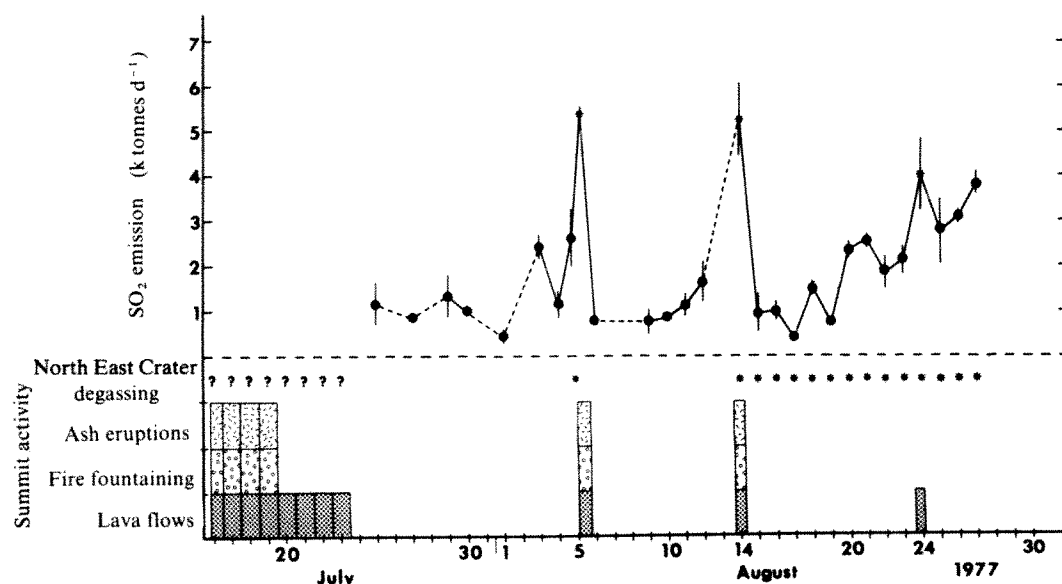


Fig. 3 SO_2 mass flow averages for the period of study. The dotted lines connecting the SO_2 data points are periods with missing days of data. The bars through each data point represent 1 standard deviation of the daily average. The bar graphs represent qualitative estimates of summit activity.

in Fig. 3. The SO₂ fluctuation curve is a plot of SO₂ mass flow (tonnes d⁻¹) versus time (16 July–28 August 1977). The bars through the points equal one standard deviation of the daily data. These should not be interpreted as error bars, but, rather an indication of the natural fluctuation of the volcano for a given day. The observed summit activity categories are meant only as qualitative estimates of the actual summit activity. The dotted lines connect intervals where there were missing days of data.

The data from 25 July to 1 August established a baseline level of ~1,000 tonnes d⁻¹ for SO₂ mass flow during non-eruptive periods. The traverses made on the morning of 5 August showed SO₂ levels up to 3.3 times the 1,000 tonnes d⁻¹ baseline value (1,830–3,330 tonnes d⁻¹). At 14.20 on the same day an eruption began in the North East Crater. Continued monitoring during the eruption showed that the SO₂ levels were above 5,000 tonnes d⁻¹.

By the morning of the 6 August, the activity had ceased and the SO₂ emission dropped to below the 1,000 tonnes d⁻¹ baseline level. The mass flow apparently remained constant until the 10th when a gradual increase began which continued through the 12th. Due to poor weather, the SO₂ levels were not measured on the 13th. At 03.00 on the 14th, the third eruptive phase began with SO₂ levels during the day again above 5,000 tonnes d⁻¹. By the evening of the 14th, the eruption had ceased and SO₂ levels on the 15th returned to the 1,000 tonnes d⁻¹ level.

SO₂ emissions from 15 to 24 August gradually increased to fairly high levels. The 4,000 tonnes d⁻¹ average on the 24th was accompanied by a small lava flow within the North East Crater, but no explosive activity occurred as in the previous eruptions. Monitoring ended on 27 August, with the SO₂ levels still fairly high. However, no further activity was observed until December 1977.

The SO₂ mass flow values reported here are all minimum values. Three factors combine to reduce the measured SO₂ value. These factors are signal attenuation, plume opacity and loss of SO₂ by reaction to SO₄. The COSPEC is a pathlength dependent device. As the distance between the plume and sensor increases, the measured SO₂ values decrease. This has been described by Moffat and Millán² in studies of powerplant plumes. Increasing plume opacity also tends to reduce the measured SO₂ values. This has also been documented in powerplant plume studies³. This factor is important to consider during eruptive activity when there is an increase in pyroclastic material in the plume. A third mechanism for SO₂ signal reduction is the reaction of SO₂ to SO₄, in water droplets in the plume. The COSPEC 'sees' only SO₂ therefore, SO₂ converted to SO₄ is effectively lost. The fact that these values are minimums does not reduce the validity of the SO₂ fluctuation curve since the mass flow averages were all obtained using the same technique, at equal distances from the vent, and at equal vertical distances from the plume.

It is evident that the increases in the SO₂ mass flow from Mt Etna could possibly be used as premonitory signs of impending activity. Two possible types of increases are suggested. First, the sharp increase such as before the 5 August eruption. Second, the gradual increase as exemplified by the periods 9–12 August, and 15–24 August.

The SO₂ mass flow variations correlate well with a model for the movement of magma within the conduit system at Etna proposed by Wadge⁴. He suggests that the eruptive pattern of Etna begins with filling of the conduit system. Activity is initiated by summit eruptions which are followed by flank eruptions. After the flank eruption drains the conduit system, the eruption ends and the cycle begins again. The SO₂ emission levels directly reflect the different levels of the magma.

In theory similar patterns should exist for all volcanoes. As the magma rises, SO₂ gas will be released in greater quantities. One should be able to monitor this increase. The absolute values for mass flow will vary from volcano to volcano, and possibly from eruption to eruption. The time fluctuation between observed

SO₂ increase and eruptive activity could be either compressed or extended.

Previous studies of condensates from high temperature fumaroles adjacent to main volcanic vents^{5–7} has long suggested that the SO₂ volume or S/Cl ratio increases before eruptive activity. The technique of remote sensing by correlation spectrometer provides a simpler and safer method for monitoring the SO₂ emission from the main vents of a volcano.

Several investigators have measured the SO₂ emission from various volcanoes^{8–12}. These data are in the same range as the data collected at Mt Etna, however, much more data are needed from individual volcanoes to determine the range of SO₂ emission and time period fluctuations. Given sufficient data, patterns might become well enough established to allow prediction of eruptions based on SO₂ fluctuations.

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Intra-plate deformation: a quantitative study of the faults activated by the 1978 Thessaloniki earthquakes

THE Thessaloniki region suffered from damaging seismic activity during the summer of 1978. During both major shocks that took place on the 24 May (*M*, 5.5) and 20 June (*M*, 6.5), manifestations of faulting could be observed in the epicentral areas. They showed that the continental crust is stretching out in this region. Measurement of slips on the active faults make it possible to compute the direction of traction (165° N) in the crust. These active faults show close kinematic similarities to previously neotectonically defined faults of Recent Quaternary age (after 400,000 yr) within the same region. Thus, study of Recent neotectonic faults often gives a correct prediction of the direction of possible slipping on faults which could be activated by earthquakes in the same region. We show here that a quantitative study of active and neotectonic faults demonstrates that in this intracontinental situation faulting deformation is satisfactorily explained with a model of continuous deformation.

The epicentral region is made up of a graben (Fig. 1) which demonstrates the presence of recent extensional tectonic activity in this area^{1,2}. An examination of the LANDSAT 1 satellite photographs (Fig. 1) indicates that this angular crank-shaped graben probably results mainly from sinistral wrench movement along NW–SE orientated fault zones. In addition opening occurs along normal faults of E–W to ENE–WSW directions. Similar movements have been observed, SW and SE of the Langadha Lake, on neotectonic faults which have a post Middle Pleistocene age³.

The faults that were formed during both 1978 earthquakes (Fig. 2) confirm this structural setting. Open cracks with an

oblique shear component, having centimetre-sized openings, have been observed in unconsolidated sediments of Upper Miocene-Pliocene and Quaternary ages. These cracks have a shear movement which is essentially dip-slip along planes which strike between 060° N and 090° N (maximum downthrows of 20 cm, see sites 32, 33 and 16 in Fig. 2) or sinistral strike slip (maximum displacements of 10 cm, see site 17) but have an important vertical component along faults striking between 110° N and 130° N. On the other hand, in bed-rock composed of Palaeozoic mica schists (sites 10, 11, 13, 15, 30, 35) the faults contain striations along their shear planes and these have a clear tectonic origin.

These faults and cracks are distributed throughout the epicentral zone and apart from some typical gravitational slide-failures (sites 23 and 26), they are located within Plio-Quaternary fault zones (Fig. 2) which have obviously been reactivated during the 1978 earthquakes.

Numerous cracks observed in unconsolidated sediments appear as small reverse faults. This could have been wrongly interpreted in terms of a compressional mechanism. Slip movement on the active fault plane only takes place where a required overburden pressure is reached (Fig. 3). In the shallow unconsolidated sediments, the motion is made possible by the opening of a curved crack the dip of which can be overturned at the surface. Thus the slip along the normal fault at depth manifests itself on the surface by a crack that has an apparent reverse fault geometry (Fig. 3). All the observed fault planes have a normal faulting component which corresponds to an active extensional tectonic activity.

Along the fault planes affecting the bed-rock, the slip vector corresponds to a frictional striation which can be exactly measured. In unconsolidated sediments of sufficiently rigid quality, the cracks at the surface yield the slip vector for the deeper active fault. This slip vector is equal to the displacement joining two points of contiguous origin being presently at opposite position across the open crack (Fig. 3).

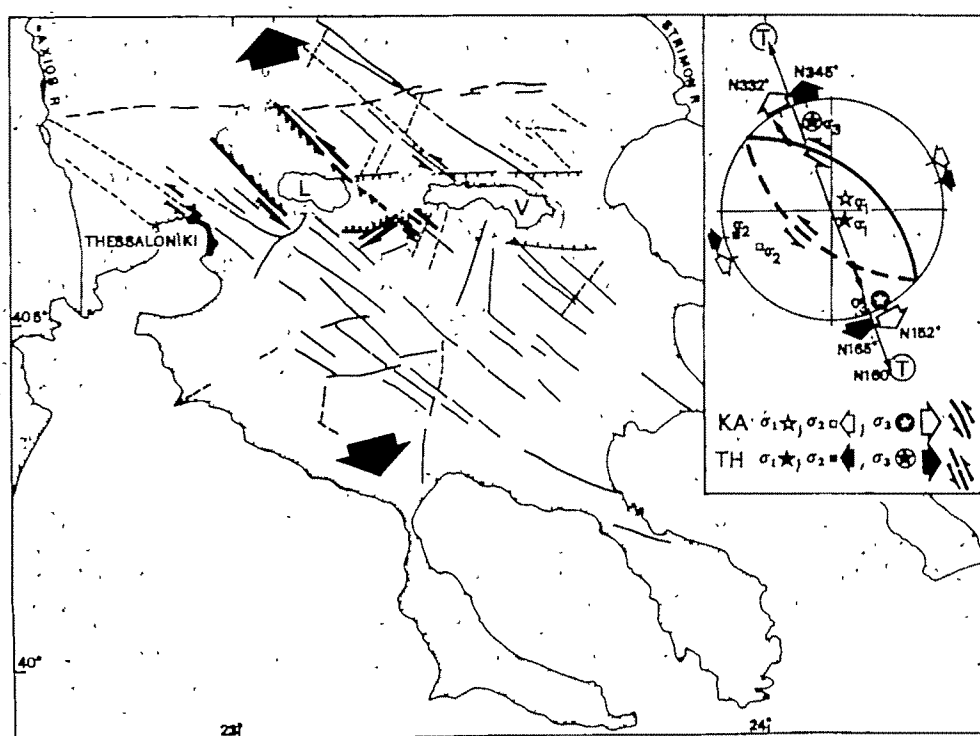
Open cracks could have been wrongly interpreted in terms of gravitational sliding. In such a case, slip-vectors have to be parallel to the steepest slope of the cracks which means that $\theta_w \propto \alpha_{FP}$ (Fig. 4). This linear relationship gives the straight line in Fig. 4. The data, represented by dots, do not agree. The azimuths of the slip vectors lie between 300° and 30° N or

between 140° and 210° N. These two bands are in opposite position on the stereonet; in other words, the cracks cannot have a gravitational origin.

Figure 2 shows that the faults striking between 110° and 130° N have a sinistral-normal behaviour; those between 060° and 090° N are essentially normal and those between 015° and 040° N are also strike-slip but dextral-normal. These faults which are regionally scattered and which have a variable strike direction are a demonstration of the extent of the regional deformation which has taken place.

Regional deformation occurs through finite slip along existing fault planes in a highly fractured medium. In this situation, a model of continuous deformation ought to be tested. The slip $\vec{S}$ on the fault plane has to be parallel to the tangential component $\vec{\tau}$ of the applied stress. The orientation of $\vec{\tau}$ is only function of the orientation of the principal axes and of the ratio $R = \sigma'_1 - \sigma'_3 / \sigma'_2 - \sigma'_3$, where the σ' are deviatoric stresses⁴. Also the knowledge of the slip vectors $\vec{S}$ corresponding to a given family of faults can lead to the determination of the above parameters⁵. The computation searches for the parameter values which minimise the deviation between the predicted striations $\vec{\tau}$ and the observed striations $\vec{S}$ for all the fault planes. Four of the planes would be sufficient to compute a solution. The numerical procedure minimises the function $F = \sum_{k=1}^N (\vec{\sigma}'_k \cdot \vec{u}_k)^2 / |\vec{\sigma}'_k|^2$ where $\vec{\sigma}'$ is the deviatoric stress acting on the fault plane, $\vec{u}$ the vector in the fault plane at right angle with the striation $\vec{S}$, and N the number of observed faults. Such a calculation was performed using only the most accurately measured slip vectors namely 10 bed-rock data from sites 10, 13, 15, 35 and from an additional site close to the sea, as well as three unconsolidated sediment data from sites 16, 22 and 23. This computation shows that the kinematics of active faults is best explained by a horizontal tension σ'_1 of azimuth 165° N. The intermediate stress σ'_2 is compressive, although of much smaller magnitude than σ'_1 and σ'_3 ; its orientation is horizontal with azimuth 76° N. This result gives a deviation between the observed $\vec{S}$ and predicted $\vec{\tau}$ which remains less than 15° . The situation is almost as satisfactory if the computation includes the other eight data from sites 2, 8, 17, 22, 32, 33, also in unconsolidated sediments but of lesser quality. Thus intra-continental tectonics can be satisfactorily analysed in terms of a continuous deformation model. This is because the geological material often contains numerous zones

Fig. 1 Structural map of the epicentral zone: lineaments observed from LANDSAT 1 pictures (thin lines), seismic and neotectonic faults described in this study (thick lines), graben of the Langadha (L) and Volvi (V) lakes (shaded area). Inset, the Wulff stereonet (lower hemisphere) shows the Quaternary fault (KA) to south-west of Langadha Lake and the major seismic fault (TH) with corresponding principal axes. Computed tensional directions are seen to coincide; black, white and T arrows correspond respectively to 1978 faults, Upper Quaternary faults and regional earthquake mechanisms.



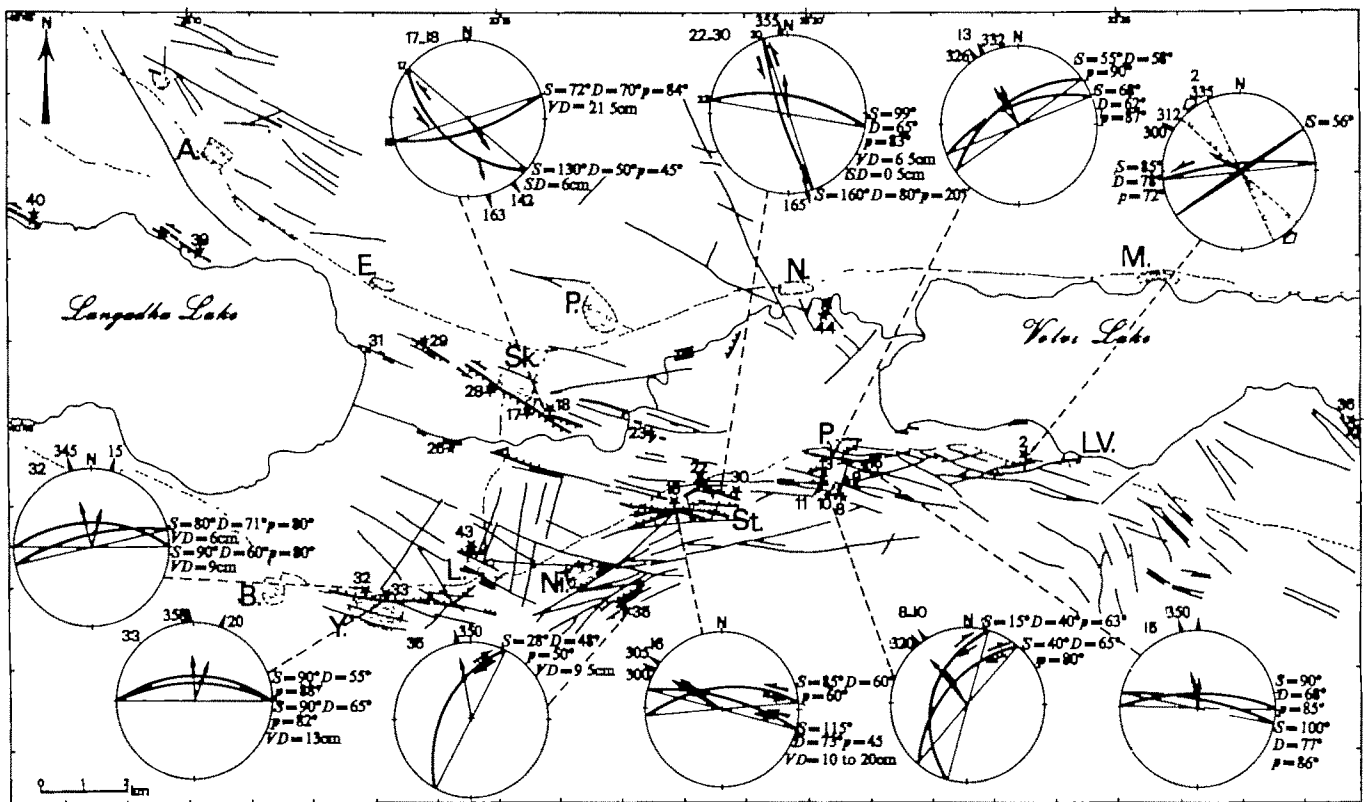


Fig. 2 Detailed map of the epicentral region: lineaments and faults observed on aerial photographs (thin lines), 1978 faults and cracks (thick lines hatched on the side of downthrows). Numbered stars refer to studied sites. Corresponding stereonets illustrate: the strike (S) and the dip (D) of faults, the pitch (p) of the slip-vector (angle between the striation and the horizontal in the fault-plane), the vertical (VD) and horizontal (SD) components of the displacement (D). The arrowhead outside the circles indicates slip-vector orientation while the dot on the trace of the fault plane represents the slip vector, the attached arrow indicating the motion of the upper block. Letters are for the villages of Yerakourou (Y), Nikomedion (N), Stivos (St), Skolario (SK) and Peristeronas (P).

of weakness. A striking feature of our analysis appears in the stereonet in Fig. 1; the tensional direction σ'_1 (thick black arrow) deduced here is compatible with the tension (thin arrow marked T) deduced from regional focal mechanisms^{2,6-8}.

A fault system postdating Middle Pleistocene forms the SW edge of the Langadha Lake graben (Fig. 1). The faults of direction 130° N are normal with sinistral strike-slip³; they have not moved during the 1978 earthquakes. The neotectonic slip-vectors on the various fault planes are compatible with a deviatoric stress tensor very similar to the one obtained for the active faults. In particular, the corresponding tension axis σ'_1 (thick white arrow on the stereonet in Fig. 1) lies close to the tensional orientations described above. Another fault zone (site 35, Fig. 2) was studied before the earthquakes; it was active this time (Fig. 3). The faults are normal with dextral strike slip and the 1978 slip vectors are of similar orientation as those corresponding to earlier motions.

About 500 m to the north of site 35, along the same fault zone, the bed-rock exhibits a mylonite structure in which the fracture planes often contain two different superposed sets of striations. Here the kinematics are quite different from those of recent and active faults; this implies a tension σ'_1 along 120 – 130° N. The faults are probably younger than Mio-Pliocene but older than the Recent. These remarks indicate that only recent neotectonic features can be used to picture the present day active tectonics. It means that, for the region under study the regional stress field has not changed much during the recent period, probably Upper Quaternary, and that the material has not suffered much rotational deformation.

The analysis of ground motions during the last Thessalohiki earthquakes as well as the study of neotectonic data for Upper Quaternary faulting^{3,9} justify the use of models of continuous

deformation to account for intracontinental tectonics. This applies to strains of small magnitudes and hence short geological time periods. The integration over a longer time will give large scale continental deformations which can also be seen in terms of continuous deformation. Such an approach with the use of slip-lines has already been suggested¹⁰. For the Aegean region, the slip corresponds essentially to the dextral strike slip faults of

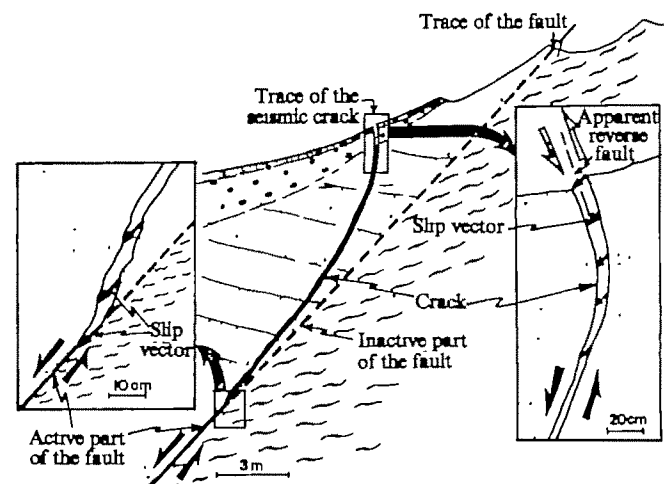


Fig. 3 Field view of the active Nikomedion fault (site 35 on Fig. 2), sketched after visual observation of a subvertical erosion surface. Insets show detailed slip vectors.

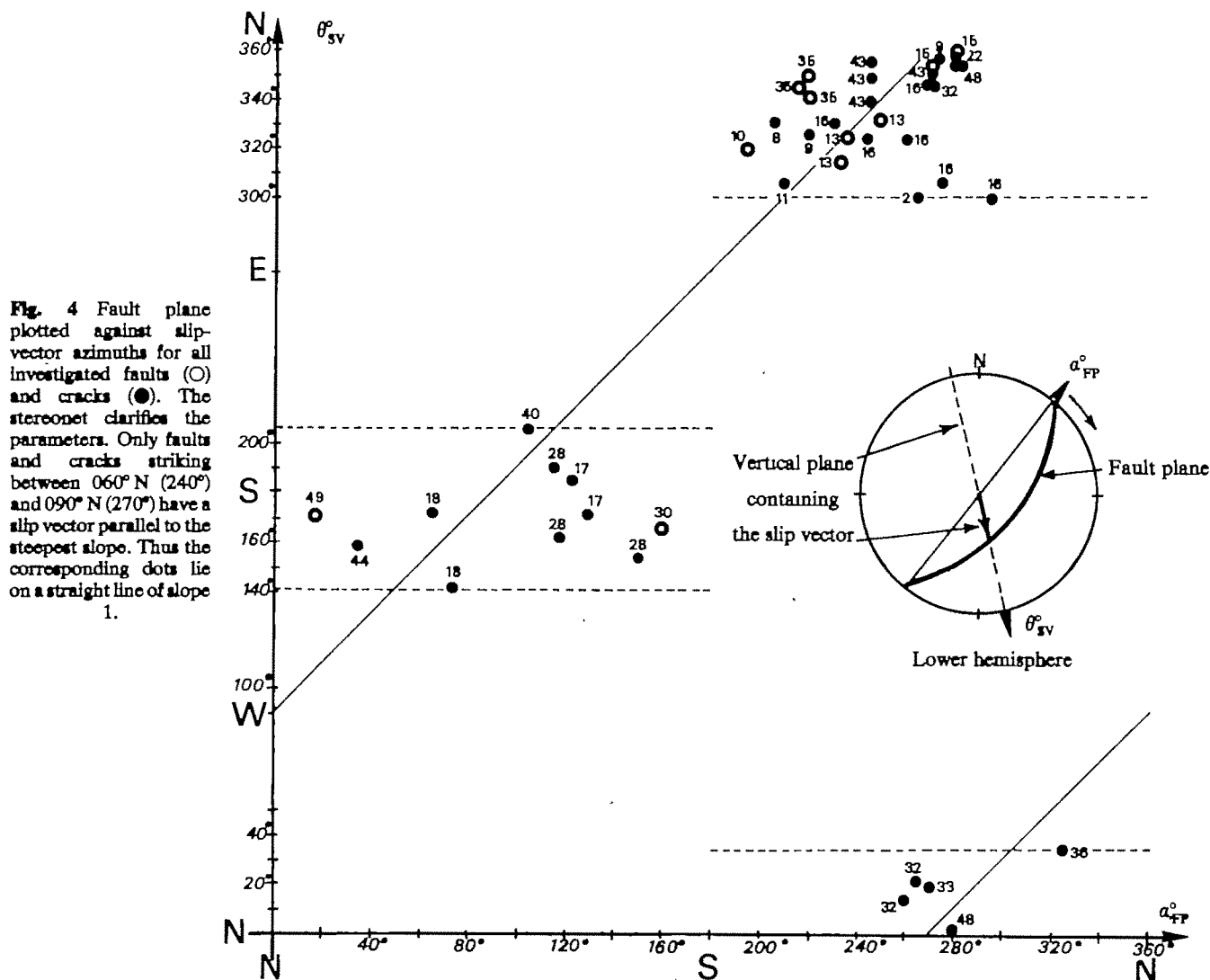


Fig. 4 Fault plane plotted against slip-vector azimuths for all investigated faults (○) and cracks (●). The stereonet clarifies the parameters. Only faults and cracks striking between 060° N (240°) and 090° N (270°) have a slip vector parallel to the steepest slope. Thus the corresponding dots lie on a straight line of slope 1.

NW Anatolia and to the sinistral faults of Macedonia^{3,11}. The deformation is, however, not purely planar so that extensive graben formation^{2,3} occurs near Thessaloniki.

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A doughnut-shaped pattern of seismic activity preceding the Shimane earthquake of 1978

AN earthquake occurred inland at shallow depth in Shimane, southwestern Japan (Fig. 1), on 3 June 1978 GMT. Its seismic magnitude was estimated as 6.1 by the Japan Meteorological Agency (JMA), which was larger than the body wave magnitude of 5.1 estimated by the US Geological Survey, (USGS). Some damage to construction was caused in the epicentral region. We show here that the doughnut-shaped active area and seismicity gap in its centre immediately preceding the earthquake indicated its region and likely magnitude.

The possibility of an earthquake occurring was inferred from the following reasons. First, the sequence of earthquakes during the past 50 yr suggested that a major earthquake with magnitude about 5 might occur in this region within a few years* (S. Asano, personal communication). Figure 2 shows that major events with magnitudes about 5 took place successively during 1950–55 and 1963–66. After a 10-yr quiescent period, an earthquake of $M = 5.3$ (JMA) occurred on 1 May 1977, adjacent to the focal region of the most recent event of $M = 6.1$. Considering that the active-inactive periods had already occurred twice in this region, the $M = 5.3$ event was suspected to indicate the onset of another seismically active period for a few to several years.

Second, the aftershock activity of the $M = 5.3$ event in 1977 had reached a new stage. Locations of the aftershocks of the $M = 5.3$ event had been limited to a small area until the autumn of 1977 when the microseismically active area started to spread into the adjacent areas (Fig. 3). Slight activity took place in the area north-west (October–November), east (November–December) and south (January–February) of the $M = 5.3$ event. Furthermore, from the beginning of 1978, the activity extended southwestwards to a somewhat broader area. No earthquakes had been observed in that area during the preceding half year. Although such changes in microseismicity do not always represent the possibility of the occurrence of a major earthquake, the fact that this region had entered a new stage in seismic activity may be significant from the viewpoint of earthquake prediction. For example, some of the major events of the Matsuhiro earthquake swarm in 1965–67, central Japan, were preceded by the spatial extension of microseismicity².

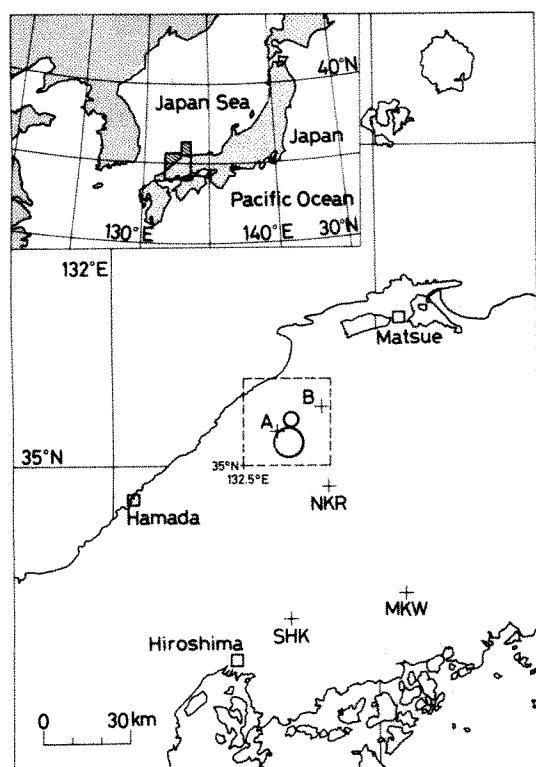


Fig. 1 Location of the $M = 6.1$ (larger circle) and $M = 5.3$ (smaller circle) earthquakes which occurred on 3 June 1978, and 1 May 1977, respectively. Crosses show the seismograph stations of the Shiraki Microearthquake Observatory (SHK, MKW and NKR) and the temporary stations (A and B) installed just before the $M = 6.1$ event. The area enclosed by the broken line is referred to in Figs 2 and 4.

Third, a doughnut-shaped pattern in microearthquakes had become apparent over a period of 5 months (Fig. 4). The microearthquakes which extended over the area south-west of the focal region of the $M = 5.3$ event had formed a doughnut-shaped active area and had left a circular aseismic area in its centre, suggesting a future earthquake, because such a pattern of seismicity had been seen before several other large earthquakes^{3,4}. The aseismic area was about 6 km in diameter. The extension and duration of this seismicity gap were considered to be too small for an event of $M = 7$ and probably 6, but large enough for a $M = 5$ event^{5,6}. The $M = 6.1$ event actually filled

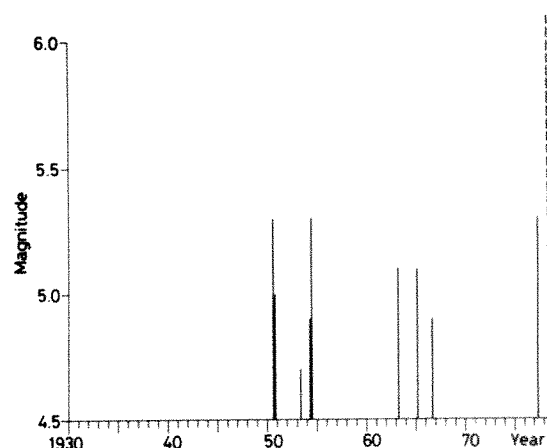


Fig. 2 A sequence of major earthquakes ($M > 4.5$) occurred within the area shown in Fig. 1 during the past 50 yr (data recorded after the Japan Meteorological Agency).

this seismicity gap, although the principal rupture seems to have started within the doughnut-shaped active area.

These seismological data which suggested the possible occurrence of major earthquakes were presented to the Coordinating Committee for Earthquake Prediction, Japan. The earthquake sequence of the last 50 years in this region was reported by JMA in the 37th committee, which was held in May 1977, just after the $M = 5.3$ event. After the aftershock area of this $M = 5.3$ event began to expand, the Shiraki Microearthquake Observatory reported this to the 40th and 41st committees in February and May 1978, respectively. These reports did not explicitly predict a major earthquake because of the lack of successful predictions in Japan.

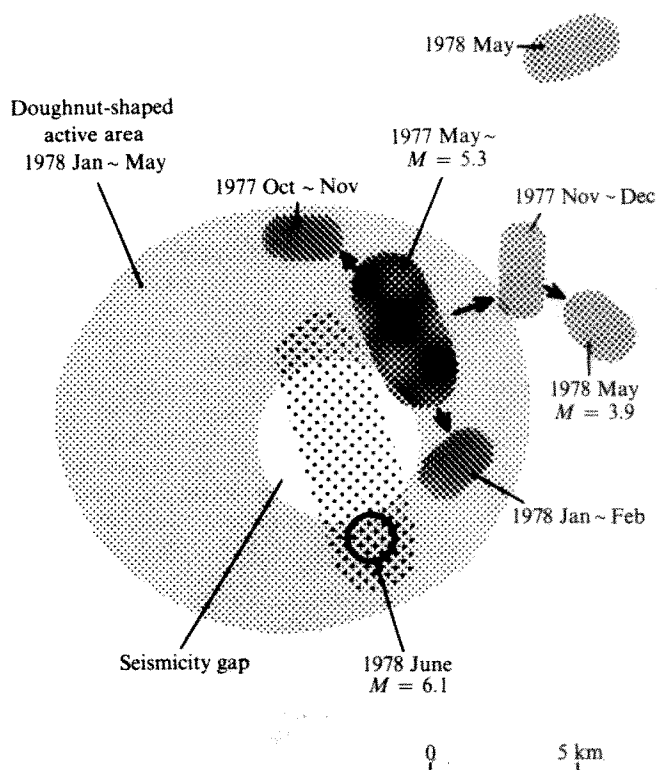


Fig. 3 Map of recent seismic activity around the focal region of the $M = 6.1$.

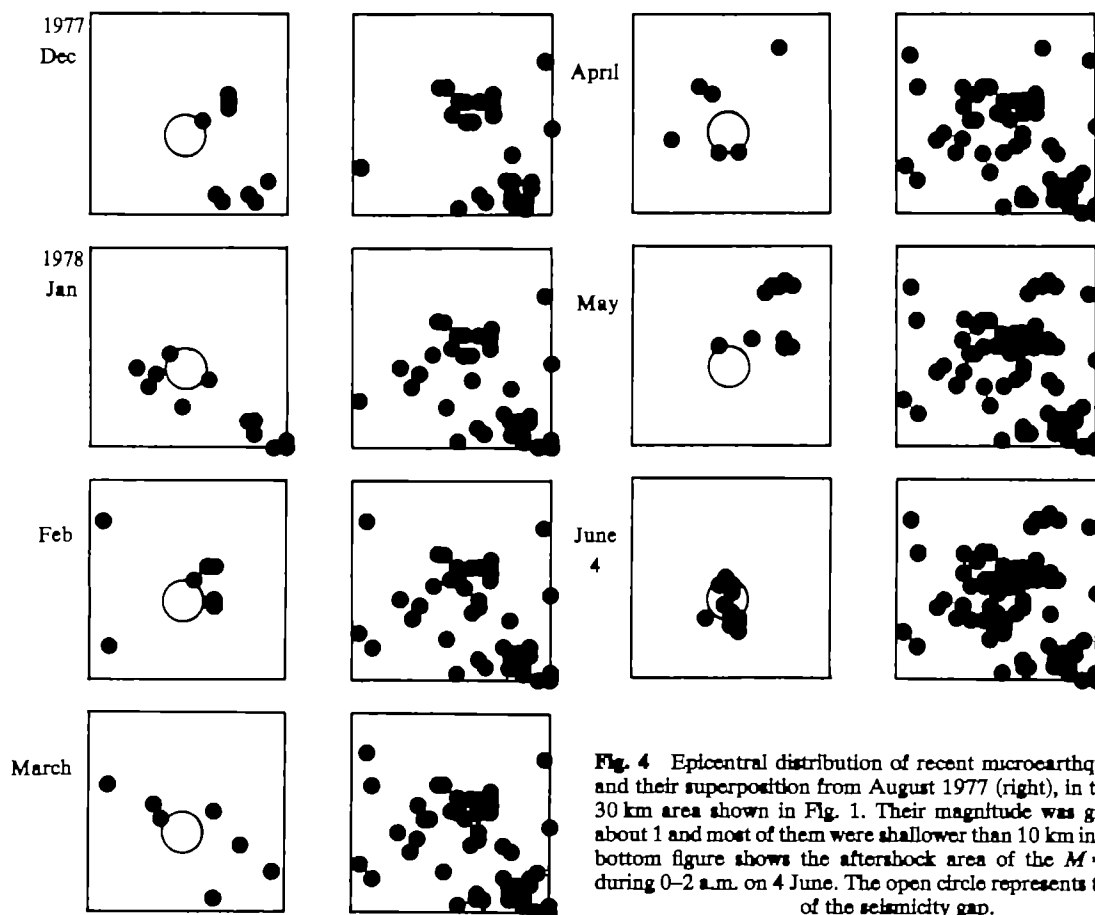


Fig. 4 Epicentral distribution of recent microearthquakes (left) and their superposition from August 1977 (right), in the 30 km $\times$ 30 km area shown in Fig. 1. Their magnitude was greater than about 1 and most of them were shallower than 10 km in depth. The bottom figure shows the aftershock area of the $M=6.1$ event during 0–2 a.m. on 4 June. The open circle represents the location of the seismicity gap.

Because of this anomalous seismic activity, we carried out temporary observations with seismometers enclosing the area of the seismicity gap from 5 to 2 days before the $M=6.1$ event. The temporary stations A (29 May–1 June) and B (30 May–1 June) are shown in Fig. 1. During this period, no events greater than about 0 in magnitude were observed in either the aseismic area or the doughnut-shaped active area. No events greater than about 1 in magnitude were also observed by the local network of the Shiraki Microearthquake Observatory until the $M=6.1$ event occurred on 3 June. Thus, the focal region of the $M=6.1$ event seems to have remained fairly inactive for several days just before the main shock.

The present $M=6.1$ Shimane earthquake was preceded by anomalous seismic activity in both time and space. A doughnut-shaped active area and a seismicity gap in its centre were apparent 5 months before the $M=6.1$ event. This suggested the location and the magnitude of the impending earthquake. The actual magnitude ($M=6.1$, JMA) was larger than expected ($M=5$). But a lesser magnitude ($M=5.1$) was also presented by USGS. The low activity around the focal region for several days just before the main shock, which was determined by the temporary observations, might have represented the imminent outbreak of the $M=6.1$ event. The microseismicity preceding the present event may be of interest as a way of predicting earthquake occurrence.

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Parallel changes in species diversity and palaeotemperature in the Lower Miocene

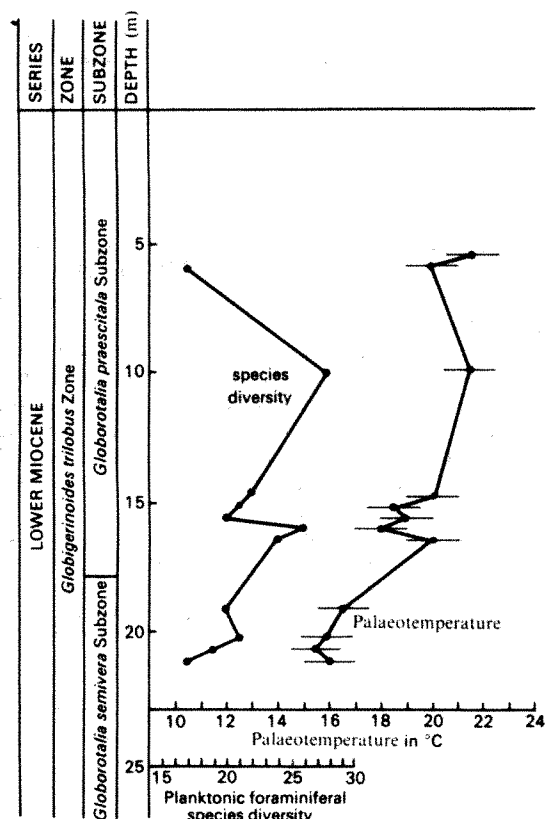
OXYGEN isotope analysis of planktonic foraminifera from 12 samples of deep-sea sediment of Lower Miocene age has been performed to estimate the sea-surface temperature at that time, and the results are reported here. The samples were taken from a borehole in the English Channel, 110 km south-west of the Isles of Scilly¹. The planktonic foraminifera have already been described in detail¹ and it has been shown that the fauna is from the Lower Miocene Burdigalian *Globigerinoides trilobus* zone. Comparison with samples of the same age from New Zealand suggests sea-surface temperatures in the range 16–20 °C or warmer, on the basis of oxygen isotope determinations in New Zealand² and on the presence in the present samples of *Globigerinoides sacculifer* (Brady). It has also been suggested from previous palaeoecological work on foraminifera of the Western Approaches, that there was only a small difference between the Miocene and present-day sea-surface August temperatures (which are 15–18 °C)³.

In the ocean both plankton tow⁴ and isotopic⁵ data suggest that *G. sacculifer* inhabits surface water and is therefore a suitable species to analyse for the estimation of isotopic palaeotemperatures. Specimens were selected from the samples

Table 1 Oxygen and carbon isotope data for *Globigerinoides sacculifer* (Brady) from the borehole

SA2 Sample	$\delta^{18}\text{O}$ PDB	$\delta^{13}\text{C}$ PDB
1700	-2.18	+2.48
1701	-1.89	+2.74
1702	-2.23	+2.93
1703	-1.86	+2.34
1704	-1.57	+2.49
1705	-1.63	+2.36
1706	-1.50	+2.59
1707	-1.86	+2.75
1708	-1.05	+1.26
1710	-1.02	+2.24
1711	-0.90	+1.80
1712	-0.99	+1.79

examined by Jenkins¹ and roasted *in vacuo* at 400 °C for 30 min before isotopic analysis, to volatilise possible organic contaminants. Carbon dioxide was generated by the action of orthophosphoric acid at 50 °C and analysed isotopically in a VG Micromass 602C mass spectrometer⁶. Overall reproducibility of the measurements is about $\pm 0.1\%$ (1σ). Measurements of δ_c (Table 1) have been converted to sea-surface temperatures assuming an oxygen isotopic composition δ_w for the Lower Miocene ocean, before the accumulation of the Antarctic ice sheet, of -1.2% on the PDB scale⁷. Although there is some uncertainty in the temperature derived using this estimate it probably does not exceed one or two degrees and is unlikely to contribute to the variability among the estimates. Temperatures were estimated using the temperature- δ relationship: $T = 16.9 - 4.4(\delta_w - \delta_c) + 0.1(\delta_c - \delta_w)^2$ (refs 8, 9). Figure 1 shows error bars corresponding to ± 2 standard deviations; these do not take account of possible systematic errors which would not contribute to the observed temperature changes within the sequence.

**Fig. 1** Measurements of palaeotemperature and species diversity of planktonic foraminifera in the Lower Miocene Sea Lab Trial borehole from the English Channel.

The isotope measurements (Fig. 1) show an estimated temperature range from 15.5 to 21.5 °C, with lower temperatures in the *G. semivera* subzone and higher in the succeeding *G. praescitula* subzone. Elsewhere *Globorotalia praescitula* first appears before the *G. trilobus* zone¹⁰ so that its local appearance in the English Channel may have been controlled by temperature.

Figure 1 shows species diversity of planktonic foraminifera¹ as well as temperature. In today's ocean there is a definite relationship between species diversity and temperature⁴ although other factors contribute to diversity patterns. Previous work has shown a broad correlation between species diversity and oxygen isotope palaeotemperature in the New Zealand Cainozoic^{11,12} but the comparison was done on the basis of total diversity of a section rather than on the diversity in individual samples. Figure 1 shows a remarkable detailed relationship between diversity and isotopic palaeotemperature among the individual samples studied.

Over geological time, species diversity is obviously likely to be controlled by evolutionary processes¹², as well as by changes in the competitive balance between different faunal groups. However, in the present situation we are studying changes within a single zone, and perhaps only over an interval of about a million years, so that neither factor is likely to contribute to varying species diversity. In this circumstance it is perhaps not surprising that the relationship between diversity and temperature is so clear. It is, therefore, of considerable interest because it opens up the possibility of estimating short-term changes in temperature in situations where the physical method of estimating palaeotemperature cannot be applied.

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Two separate photoreceptors control hypocotyl growth in green seedlings

PHOTOCONTROL of elongation growth has been extensively studied in dark-grown (etiolated) seedlings for some time. However, only relatively recently has detailed attention been given to green (de-etiolated) plants. One reason for this is that the photoreceptor, phytochrome, can easily be detected spectrophotometrically in non-green tissues but not in tissue containing chlorophyll. There is agreement that the photoreceptor operates in its low-energy mode (low irradiances or fluence rates, with red/far-red reversibility) in green seedlings as well as in dark-grown, non-green seedlings. In its high-energy

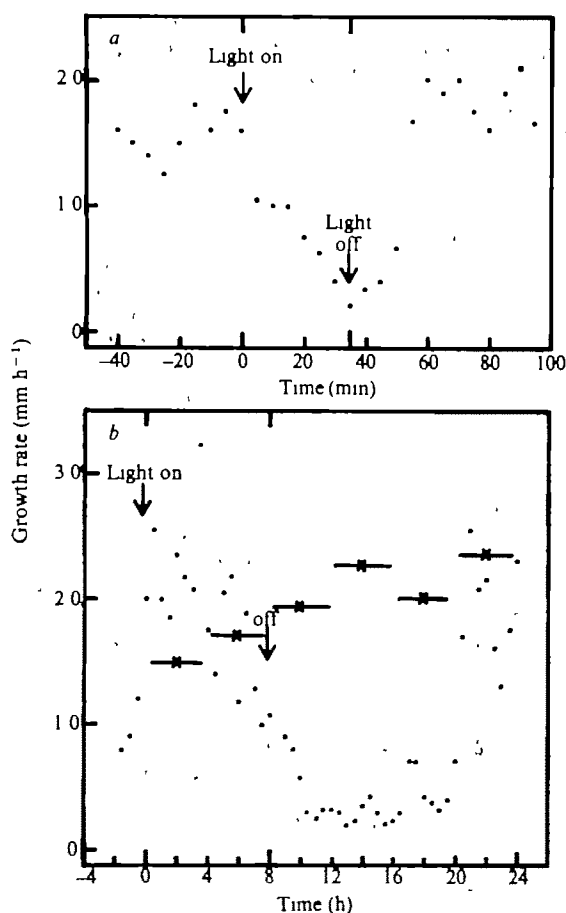


Fig. 1 Growth kinetics in response to illumination and darkness. *a*, Blue-light inhibition and recovery: blue light was provided by an opposed pair of collimators (to prevent phototropism) focused on the 5-mm region of the hypocotyl measured ~10 mm from the cotyledonary node. The light source consisted of 150-W quartz-halogen bulbs in projectors, and the light from each was passed through a layer of Cinemoid (deep blue no. 20) and 30 mm of 2.5% cupric chloride (to eliminate far-red) and an additional lens for focusing. This combination gives broad-band light, from 400–510 nm (peak 440 nm; half-band width ~45 nm). (Fluence rate as in Table 2.) *b*, Red light inhibition and recovery. Diffuse red light was provided by light from 20-W Gro-lux fluorescent tubes filtered through a layer of Cinemoid (ruby red no. 14). This combination gives broad-band red light from 600–710 nm (peak 660 nm; half-band width ~20 nm). (Fluence rate as in Table 2—saturation is given by ~1 W m⁻².) Crosses mark the growth rate of dark control seedlings (having had a similar pretreatment). Each cross represents the mean growth rate for a 4-h period, the limits of which are shown by the horizontal bar (measurements by ruler, $n = 12$).

mode (high irradiances and time dependency), exemplified by the action of prolonged irradiation with far-red, phytochrome might act only in etiolated seedlings¹⁻³. Photoregulation of stem extension in etiolated and de-etiolated seedlings is also achieved by blue light (400–500 nm), but it is still not clear if a photoreceptor other than phytochrome is responsible for this effect. Kinetic studies on etiolated seedlings suggest the operation of a separate blue-responsive photosystem². Notwithstanding this, it has recently been suggested that in green seedlings blue-light control of hypocotyl extension is due solely to absorption by phytochrome⁴. This claim, together with the evidence that changes in phytochrome photoequilibria can exert profound effects upon stem extension, may lead one to believe that this is the major photoreceptor controlling elongation growth in green plants. We report here, however, work that strongly indicates that two separate pigment systems control hypocotyl extension in green seedlings—phytochrome and a separate blue-light

receptor. Both systems produce the same response—inhibition—but we have found it possible to separate their effects by examining the times at which the response commences.

We have examined in detail the kinetics of the growth responses to light in seedlings of *Cucumis sativus* L which, 3.75 days after germination and growth in darkness (when hypocotyls were 30–40 mm long), were de-etiolated by exposure to white, fluorescent light ('daylight') for 8 h, followed by a 16-h dark period, that is, one 'short-day' pretreatment. At the end of the dark period plants were subjected to the experimental treatments. All plants were grown, and all experiments performed at 25°C. This de-etiolation treatment completely opens the hypocotyl hook, and causes greening and enlargement of the cotyledons. The seedlings are not morphologically identical to, say, the seedlings de-etiolated by 30 h of white light as used by Black and Shuttleworth¹, but they do have the same physiological characteristics as these, that is, phytochrome control of hypocotyl extension by the cotyledons, and loss of the far-red component of the high irradiance reaction¹. Responses with identical timings to those reported below have been obtained with seedlings de-etiolated for 30 h with white light. Returning such seedlings to darkness for several hours gives the same high growth rates as shown by the presently-used material. High growth rates are required for a detailed study of growth kinetics, especially the onset of photoinhibition. Hypocotyl extension in response to light and darkness was monitored using a linear-displacement transducer (ND2, Sangamo Weston Controls Ltd.) in which the counterbalanced, moveable armature was placed at the apex of the plant, between the two cotyledons. After a period of equilibration, extension was recorded continuously and transformed to growth rates (mm h⁻¹) computed at intervals over the growth period. (The few points before 'light on' in Fig. 1*b* illustrate the pre-equilibration rise in growth rate.) A good deal of variability in growth rates is encountered in measurements by transducers. We have found that although the absolute growth rate may vary considerably the timing of the photoresponses is remarkably constant (Table 1). The initial high growth rates of our seedlings allowed us to determine this timing with great definition. Results obtained with conventional measuring techniques are fully consistent with the data reported here which are concerned with the 'ultrastructure' of growth, as it were, obtainable only by means of a highly sensitive device such as a transducer.

Kinetics of light-induced inhibition and of the subsequent recovery in darkness clearly depend upon the spectral region (Fig. 1). The growth rate rapidly falls after about 5 min exposure to blue light, and inhibition reaches a maximum (that is a minimum growth rate) some 35 min later (Fig. 1*a*). On the other hand, red light-induced inhibition starts only 5–6 h after exposure to red light (Fig. 1*b*) and reaches a maximum at 10 h. That this is phytochrome-controlled inhibition is shown by the effectiveness of short periods of red light (10 min every hour), each period being far-red reversible. Thus, to produce photoinhibition several 'inputs' of P_E are required. In practice this is most easily achieved by continuous exposure to red light. The effects of continuous and intermittent red light are, however,

Table 1 Timing of photoresponses controlling hypocotyl extension

Treatment	Time to initial response (mean $\pm$ s.e.m.)	
	Inhibition	Recovery
White fluorescent light	4.25 $\pm$ 0.29 min ($n = 28$)	Short:
		18.9 $\pm$ 1.8 min ($n = 9$)
Red light	5.8 $\pm$ 0.31 h ($n = 21$)	Long:
		10.45 $\pm$ 0.49 h ($n = 19$)
Blue light	5.0 $\pm$ 0.55 min ($n = 9$)	10.0 $\pm$ 0.5 h ($n = 13$)
		18.9 $\pm$ 3.8 min ($n = 9$)

'Short' indicates those white light treatments where the irradiation was given for less than the time for the appearance of phytochrome-controlled inhibition (that is, <3 h). 'Long' refers to those light periods during which phytochrome-controlled inhibition sets in (that is, >8 h).

very close. Moreover, the input of P_{fr} over periods of 1 h and longer produce inhibition, always commencing about 6 h after the beginning of irradiation. We stress that blue light acts directly on the hypocotyl as already reported^{1,6,7}. We observe the same inhibition kinetics in both blue and white light (see below) whether or not the cotyledons are covered. In red light also, covering the cotyledons does not alter the time of onset of inhibition but the 'depth' of inhibition and the timing of recovery are both affected. These latter effects reflect the contribution to photoinhibition made by the cotyledons^{1,6}; the onset of inhibition is presumably due to the action of red light directly upon the hypocotyl^{1,6,7}.

At some time after the end of illumination plants begin to recover from photoinhibition and show a gradually increasing growth rate until a maximum 'dark' rate is obtained. The kinetics of these changes again depend on the spectral region used for previous illumination (Fig. 1a, b). In this and most experiments, recovery from blue light starts slowly, as soon as 5 min after the end of irradiation, with the major increase between 15 and 20 min of darkness, and is complete soon after; but in some experiments the start of recovery was delayed for up to 15–20 min. Red-light inhibition, induced through phytochrome, is much more persistent; about 8 h of darkness are required before the growth rate begins to recover. Thus, blue light inhibits virtually only during irradiation whereas phytochrome-mediated, red-light inhibition has a delayed onset and recovery. Such widely disparate kinetics are difficult to explain on the basis of one pigment system and we conclude, therefore, that hypocotyl extension in de-etiolated *Cucumis* is controlled by two photoreceptors, one being phytochrome and the other an as yet incompletely identified system, specific to blue light^{8,9}. That blue light does not operate through phytochrome in our experimental conditions is further substantiated by the inability of background far-red to alter either the extent or the timing of blue-light inhibition, even though $\phi(P_{fr}:P_{total})$ is reduced from 0.44 to 0.14 (Table 2). There is clearly no correlation between ϕ and the kinetics of photoinhibition.

What of the action of white light? White fluorescent light contains both blue and red wavebands and it therefore activates the specific blue light-sensitive system as well as phytochrome. Onset of inhibition is accordingly rapid (due to blue light) and recovery in darkness after exposure to several hours of white light is slow to develop, presumably because of the long-lasting inhibition caused by phytochrome. It is possible, however, by adjusting the length of the white-light period, to separate the timings of both sets of inhibition and recovery in a single experiment (Fig. 2). Periods of relatively short duration, for example 2–3 h as used in this experiment, produce the rapid inhibition typical of blue light. The characteristic phytochrome-controlled inhibition (red light) does not commence during the

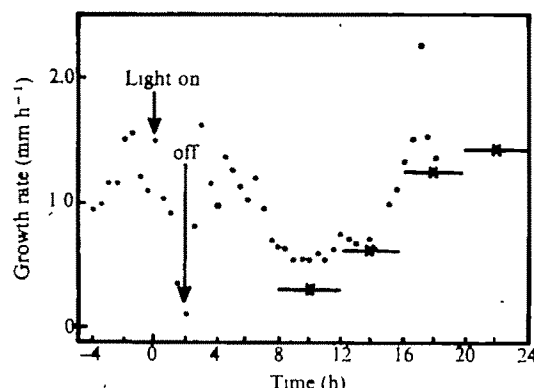


Fig. 2 Effect of a brief white light irradiation. The white light source consisted of 20-W 'daylight' tubes. (Fluence rate as in Table 2.) Crosses mark the mean growth rates calculated every 4 h (shown by the horizontal bar) of similarly treated seedlings, measured by ruler ($n = 12$). As in Fig. 1b these indicate that the seedlings merely recover their growth rate, showing no entrained rhythm.

Table 2 Effect of $P_{fr}:P_{total}$ on the kinetics of photoinhibition

Light source	Fluence rate	$\phi(P_{fr}:P_{total})^*$	Approximate time to start of inhibition (min)
White fluorescent	34 W m ⁻²	0.75	5
Red	8 W m ⁻²	0.75	300
Blue	24 W m ⁻²	0.44	5
Blue + far-red	24 + 6.5 W m ⁻²	0.14	5

*Measured in 15-mm long hypocotyl sections (including hooks) of 3.5-d-old dark-grown seedlings of *Cucumis scitovus* L. using a Perkin Elmer model 156 double wavelength spectrophotometer.

time that the plants are actually exposed to white light, as it requires 5 h for its development to begin (Fig. 1b). Twenty minutes after return to darkness a recovery of growth rate is apparent but ~2 h later this is followed by a second, transient inhibition which occurs in darkness. This comparatively short exposure to white light therefore produces dual kinetics and, we suggest, succeeds in revealing the temporal separation of blue light and phytochrome actions.

The two components of inhibition seem difficult to accommodate in a hypothesis which assigns a role to one pigment only—phytochrome—and appear to us to be best explained as the operation of two separate photosystems, each having different kinetics, in the following manner. The rapid inhibition is due to the blue light effect from which seedlings begin to recover at the end of the light period. The red component of white fluorescent light produces high levels of P_{fr} whose inhibitory action, beginning 5 h after the start of illumination, makes its appearance about 3 h after the plant has returned to darkness. The final recovery of growth rate is the delayed release from phytochrome-induced inhibition. In longer white light periods the actions of the two photosystems become superimposed. Inhibition quickly sets in but the rapid recovery from blue light is obliterated by the long-lasting, red light-induced inhibition, and the observed recovery upon return to darkness thus has only the kinetics which follow phytochrome photoinhibition.

The amount of inhibition caused by blue light depends strongly upon the irradiance, though the timing of the start of inhibition does not. Here we have used high irradiances (fluence rates) to produce substantial and clearly recognisable reductions in growth rate. Pronounced irradiance dependency with regard to the magnitude of inhibition is not found in the case of red light and saturation is achieved by relatively low fluence rates (about 1 W m⁻²). It therefore seems likely that in nature the dependency of hypocotyl and stem extension upon the fluence rate of white light is a response to the blue component. We further suggest that the blue-light system detects fluence rate differences while phytochrome is concerned more with light quality, particularly red/far-red ratios, a point which has been raised by other workers¹⁰. Morgan and Smith¹¹ reported rapid effects of white light on stem extension in de-etiolated *Chenopodium*. The similarity of some of their kinetics to those reported here suggest that the responses of *Chenopodium* to irradiance changes may be attributable to the blue light receptor.

Finally, we should add that the kinetics discussed above account for various growth patterns shown by seedlings held under different daily light regimes. Details of these will be reported elsewhere.

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In vitro duplication of the primary light-induced charge separation in purple photosynthetic bacteria

THE primary light-induced electron transfer events of both green plant and bacterial photosynthesis result in very rapid charge separation which subsequently generates a chemical potential within the organism. There is much information concerning the primary events of bacterial photosynthesis but for green plants the details of these processes are just beginning to become available. Strong evidence based mainly on electron spin resonance and electron-nuclear double resonance studies of oxidised bacterial reaction centres favours a dimeric bacteriochlorophyll *a* (BChl *a*) structure for the primary photochemical electron donor¹. The dimeric BChl *a* species absorbs light at 865 nm in reaction centres from *Rhodospseudomonas sphaeroides* yielding an excited state which rapidly transfers an electron to a molecule of bacteriopheophytin *a* (BPh *a*) in <10 ps (refs 2-4). Within about 150 ps the electron is transferred to a quinone molecule which in turn transfers an electron to a secondary quinone within a few microseconds^{5,6}. In reaction centres for which the quinones have been either extracted or

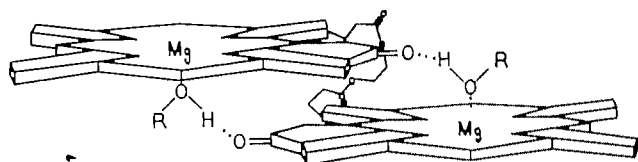


Fig. 1 PChl *a* dimer in its folded C_2 symmetric conformation.

chemically reduced before light excitation, BPh⁻ back transfers an electron to (BChl *a*)₂⁺ in 10-20 ns (ref. 7). Thus, the *in vivo* geometry of the reaction centre is such that the reverse electron transfer is $5-10 \times 10^3$ times slower than the forward reaction. We now report the first *in vitro* model that duplicates both the rapid light-induced charge transfer and the slow back reaction of the reaction centre.

Covalently linked dimers of BChl *a*, chlorophyll *a* (Chl *a*) and pyrochlorophyll *a* (PChl *a*) have been prepared which mimic the spectroscopic and redox properties of reaction centre chlorophylls⁸⁻¹⁰. The two chlorophylls are joined in each case at their propionic acid side chains through an ethylene glycol diester

linkage. The orientation of the chlorophyll macrocycles with respect to one another, and consequently their electronic properties, are highly solvent dependent. Treating solutions of these covalent dimers in dry non-nucleophilic solvents (such as benzene, toluene, CCl_4) with 10-100-fold molar excesses of a hydrogen-bonding nucleophile (such as water, primary alcohols, primary thiols) results in their folding into the C_2 symmetric conformation shown in Fig. 1. This structural assignment is based primarily on ¹H NMR spectral evidence^{8,10}. The folding is accompanied by shifts of both the longest wavelength absorption in their electronic transition spectra and their fluorescence maxima to longer wavelengths.

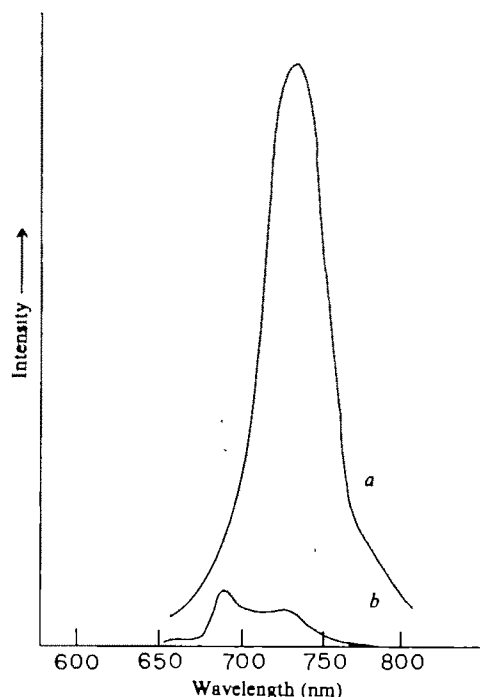


Fig. 3 *a*, Fluorescence spectrum of 0.2 mM PChl *a* dimer in toluene containing 20 mM ethanol. Excitation at 530 nm. *b*, Fluorescence spectrum of 0.2 mM PChl *a* dimer in toluene containing 0.8 mM PPhide *a*-OH. Excitation at 530 nm.

We used the PChl *a* dimer to produce a functional model reaction centre because of its inherent stability towards photo-degradation¹¹. The PChl *a* dimer was prepared by a method described elsewhere⁸. Folding this dimer into the conformation shown in Fig. 1 results in a shift of its absorption maximum from 663 nm to 694 nm, whereas its fluorescence maximum shifts from 670 nm to 735 nm. To position pheophytin *a*, which serves as the electron acceptor, close to the dimer, we have used the strategy of replacing the hydrogen-bonded short-chain alcohol which secures the conformation of the dimer shown in Fig. 1 with a primary alcohol derivative of pheophytin *a*. The pyropheophorbide *a* ethylene glycol monoester (PPhide *a*-OH), a precursor of the PChl *a* dimer, proves to be ideal for this.

When dry 0.2 mM solutions of PChl *a* dimer in toluene are treated with as little as a fourfold molar excess of PPhide *a*-OH at room temperature or a stoichiometric twofold molar excess at

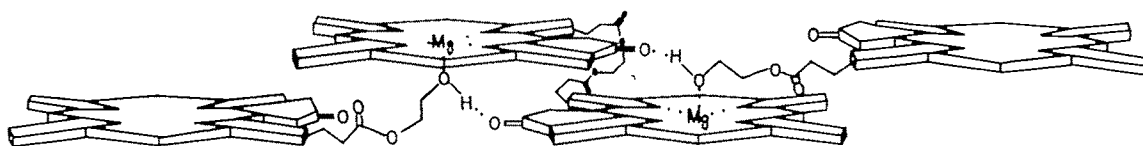


Fig. 2 Proposed structure of the (PChl *a*)₂-(PPhide *a*-OH)₂ reaction centre model.

0°C, the dimer readily folds into the structure shown in Fig. 2 (^1H and ^{13}C NMR structural evidence, M.R.W., unpublished). This folding is accompanied by a shift of the dimer electronic absorption maximum to 694 nm. More importantly, the 735 nm fluorescence of the dimer is almost completely quenched (Fig. 3). If a similar experiment at the same concentrations is carried out with PChl *a* dimer folded with ethanol while unbound pheophytin *a* is present in the solution, the fluorescence quantum yield of the dimer actually increases by about 10% over that seen in the absence of pheophytin *a*. This effect may be attributed to excitation energy transfer from excess pheophytin *a* in solution to the folded dimer which acts as an energy trap.

To determine the mechanism and kinetics of the quenching of the PChl *a* dimer fluorescence by PPhide *a*-OH in our model reaction centre, we have determined the excited state difference spectrum of the model as a function of time on the picosecond time scale. The experimental apparatus and techniques used to obtain the data have been described elsewhere¹². Fig. 4 shows the difference in optical density between the excited state and the ground state following a 6-ps flash at 530 nm.

The rise time for all ΔA changes is <6 ps. These changes persist for 10–20 ns with the subsequent return of the ground state absorption spectrum. The spectrum in Fig. 4 displays a positive ΔA change at 800 nm and strong bleaching at 660 nm.

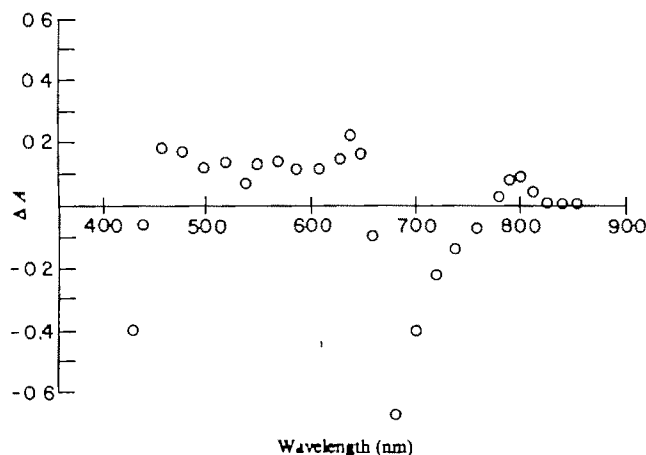


Fig. 4 Initial absorption changes following 6 ps excitation with 530 nm light of 0.2 mM PChl *a* dimer and 0.8 mM PPhide *a*-OH in toluene at 20°C.

Positive ΔA changes dominate the 440–630-nm region of the spectrum. The 800-nm feature in the spectrum is characteristic of a Chl *a* cation radical¹³. The one electron oxidation product of the PChl *a* dimer folded with ethanol displays a broad electronic absorption near 800 nm. Similarly, P_{700} in green plants undergoes light-induced oxidation to yield a species which absorbs light near 800 nm (ref. 14). The strong bleaching at 660 nm and the smaller bleachings at 500 nm and at 530 nm can be ascribed to the disappearance of ground state PPhide *a*-OH. One electron reduction of pheophytin *a* results in very similar spectral changes¹⁵. Thus, the overall spectrum in Fig. 4 may be adequately described as a superposition of the PChl *a* dimer cation radical spectrum and the PPhide *a*-OH anion radical spectrum. Whether the complex exists as a radical pair or as a charge transfer state has not yet been ascertained. The overall spectral features strongly parallel the type of spectral changes observed following flash excitation of bacterial reaction centres even though the wavelengths of the ΔA changes are different for the *a*-series plant chlorophylls^{2–4}. Based on the fluorescence quenching data and assuming reasonable values for the oscillator strength of the 800-nm transition in (PChl *a*)₂⁺, the quantum efficiency of electron transfer in our model approaches

unity. Thus, our model reaction centre channels most of the light energy it absorbs into an electron transfer process which displays the same asymmetric kinetics as observed *in vivo*.

It is clear from our experiments that positioning the PPhide *a*-OH electron acceptor close to the PChl *a* dimer electron donor is important for efficient light-induced electron transfer. However, the kinetics of both the forward and reverse transfers depend critically on the energies of the states involved. Excitation of the PChl *a* dimer into its first excited singlet state raises its energy to ~1.8 eV above the ground state. Charge separation is then an energetically 'downhill' process with the resultant ion pair state ~1.5 eV above the ground state (estimated from the difference between the one electron oxidation potential of the folded PChl *a* dimer, 0.50 V compared with SCE, and the one electron reduction potential of PPhide *a*-OH, -1.02 V compared with SCE, in methylene chloride). The ~0.3-eV energy difference between the singlet and ion pair states is sufficient for a rapid forward transfer rate, but results in the reverse electron transfer being an activated process. Thus, the rate of an electron transfer leading back to the first excited singlet state should be substantially slower than the charge separation rate.

The only reasonable pathway remaining for the ion pair state is to cross over to a highly vibrationally excited state of the ground electronic state. As $\Delta G \ll 0$, ~-1.5 eV, the Franck-Condon factors for this process are small and thus the rate of return to the ground state is relatively slow.

Because the PPhide *a*-OH molecules may exhibit some conformational freedom due to rotation about the single bonds of their alkyl chains, the role of these changes following excitation and their effect on the reverse electron transfer rate need to be further explored. In conclusion, we have successfully modelled the primary light-induced charge separation chemistry in reaction centres of purple photosynthetic bacteria by using *in vitro* reaction centre models based on chlorophyll derivatives.

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Antigen specific T-cell helper factor cross reacts idiotypically with antibodies of the same specificity

THE recognition system of bone marrow-derived (B) cells and their products is based on the interaction of the active site of immunoglobulins with the specific antigenic determinants. However, the molecular nature of the recognition component of thymus-derived (T) lymphocytes is not yet established. Studies on antigen specific T-cell helper or suppressor factors¹⁻⁵ and isolation of the antigen receptor directly from T cells, or from serum^{6,7} are examples of approaches to elucidate this problem. The synthetic polypeptide poly(Tyr,Glu)-poly(DLAla)-poly(Lys) [abbreviated (T,G)-A--L] has been extensively used in studies on the molecular basis of antigenicity⁸ and genetic regulation of immune responsiveness⁹⁻¹¹. Studies on the antibody response to (T, G)-A--L have revealed the involvement of several cell types and a T-cell replacing soluble factor which are antigen specific and genetically controlled¹²⁻¹⁴. An important feature of the T-cell factors is their antigen specificity. The specificity of a T-cell factor produced to (T, G)-A--L was demonstrated by the removal of its activity on specific antigen-Sepharose columns¹⁵ and also by demonstrating that the *in vivo* helper effect was specific to the antigen used in the T cell education^{1,16}. It is very likely that the specific T-cell factor represents the molecular recognition component of T cells—the T-cell receptor. If this is the case, the molecular identification and characterisation of the T-cell factor may answer one of the most controversial questions in immunology. It has been suggested that antigen-specific molecules of B and T cells share idiotypic determinants^{17,18}. It was, therefore, of interest to find out whether the (T, G)-A--L specific factor will share idiotypic determinants with antibodies of the same specificity. The results of the present study demonstrate that B-cell products (antibodies) and T-cell products (factors) possess cross-reacting idiotypic determinants. This strongly suggests the presence of similar variable regions in both antigen binding entities, which are probably coded by the same variable region genes. Cross-reactive idiotypic determinants are also present on (T, G)-A--L specific factors obtained from low responder C3H/DiSn T cells confirming previous reports on the activity of these factors.

The successful production of anti-idiotypic antisera in guinea pigs to C3H.SW anti-(T, G)-A--L antibodies has been recently reported¹⁹. Idiotypic determinants recognised by these sera were present in anti-(T, G)-A--L antibodies from different individuals of C3H.SW mice. A linkage between the expression of the cross-reactive idiotypes and heavy chain constant region allotypes was demonstrated¹⁹. This characterised anti-idiotypic serum was used in the present study to look for the presence of idiotypic determinants on the (T, G)-A--L specific T-cell factor. Indeed, the activity of a (T, G)-A--L specific factor was removed after passage through an immunoadsorbent of Sepharose-coupled anti-idiotypic serum.

The (T, G)-A--L specific T-cell factors were produced by 'educated' T cells of C3H.SW, CWB and C3H/DiSn inbred mouse strains as described elsewhere²⁰. The helper activity of the factors was assayed by transferring them into irradiated mice together with high responder (C3H.SW) bone marrow cells and (T, G)-A--L²⁰. The immune response was followed by determining the anti-(T, G)-A--L haemagglutination titres in the sera of the recipients²⁰. The anti-idiotypic serum prepared in guinea pigs against C3H.SW anti-(T, G)-A--L antibodies was the same as previously reported¹⁹. The IgG fraction (10 mg) of the anti-idiotypic serum was coupled to 1 g of Sepharose²¹.

As shown in Table 1, a factor produced by C3H.SW (high responder) educated T cells replaced the helper function of thymocytes in eliciting an antibody response to (T, G)-A--L. The activity of this factor was completely removed by passage through the anti-idiotypic-Sepharose immunoadsorbent,

whereas no effect on the activity of the factor was observed when normal guinea pig IgG or guinea pig anti-mouse IgG were used as immunoadsorbents.

C3H/DiSn (H-2^b) low responder mice are capable of producing a (T, G)-A--L specific factor which cooperates with high responder (H-2^b) B cells for the production of antibodies (see Table 1 and ref. 20). The factor produced by C3H/DiSn mice was removed by the anti-idiotypic antibodies. As with the (T, G)-A--L specific factor from C3H.SW mice, the activity of the factor from C3H/DiSn mice was completely retained after passage through guinea pig anti-mouse IgG immunoadsorbent.

Table 1 Effect of anti-idiotypic antibodies on the helper activity of (T, G)-A--L specific T-cell factors

C3H.SW bone marrow cells and (T, G)-A--L transferred with:	No. of animals	No. of experiments	log ₂ haemagglutination titres
No addition	31	4	0.77 ± 0.2
10 ⁸ C3H.SW thymocytes	19	4	4.21 ± 0.6
C3H.SW factor	30	4	4.37 ± 0.03
C3H.SW factor passed on an anti-idiotypic-Sepharose column	30	4	1.03 ± 0.21
C3H.SW factor passed on a normal guinea pig-Sepharose column	19	3	4.74 ± 0.35
C3H.SW factor passed on a guinea pig anti-mouse IgG-Sepharose column	6	1	3.7 ± 0.34
C3H/DiSn factor	11	2	4.0 ± 0.22
C3H/DiSn factor passed on an anti-idiotypic-Sepharose column	11	2	1.09 ± 0.39
C3H/DiSn factor passed on a guinea pig anti-mouse IgG-Sepharose column	12	2	3.75 ± 0.18
CWB factor	6	1	4.0 ± 0.36
CWB factor passed on an anti-idiotypic-Sepharose column	4	1	4.0 ± 0

C3H.SW recipient mice were irradiated (850 R) and reconstituted with 2×10^7 syngeneic bone marrow cells, 10 µg of (T, G)-A--L and either thymocytes or (T, G)-A--L specific T-cell factors produced by one spleen equivalent of educated T cells. Mice were bled 12 d after transfer and their sera assayed by passive haemagglutination with (T, G)-A--L coated sheep red blood cells. Values are means ± s.e.m.

CWB (H-2^b, Ig-1^b) mice are high responders to (T, G)-A--L. They are congenic with C3H.SW (H-2^b, Ig-1^a) mice and differ only in their heavy chain allotypic markers. Anti-(T, G)-A--L antibodies of CWB mice do not share idiotypic determinants with antibodies of C3H.SW mice¹⁹. It was, therefore, of interest to find out whether a factor of CWB mice would react with the anti-idiotypic antibodies. As demonstrated in Table 1, the CWB factor was not affected by the anti-idiotypic antibodies against C3H.SW anti-(T, G)-A--L.

As shown for antibodies, the ability to produce a (T, G)-A--L specific factor which cross reacts with C3H.SW anti-(T, G)-A--L antibodies is linked to the Ig-1 allele of the mouse strain.

Note that the anti-idiotypic serum bound only 30% of the idiotypes (C3H.SW anti-(T, G)-A--L antibodies)¹⁹ whereas in this study we have demonstrated that the activity of the factor was completely removed by the anti-idiotypic immunoadsorbent. As the activity of the factor is lost on dilution (at fourfold or higher dilutions, data not shown) it is possible that most but not all of the factor present was removed by the anti-idiotypic. It is, of course, possible that the particular type of the gene product

that is expressed in the anti-(T, G)-A-L antibodies to the extent of 30% and bears the cross-reactive idiotype determinants, is expressed to a higher extent in the population of the factor molecules of the same specificity. This, however, does not exclude the possibility that T- and B-cell products have potentially the same repertoire but that regulation processes determine a different expression of a particular type in one or the other type of recognition entities. Indeed, it is clear that antibodies of different classes with the same specificity share the same V region repertoire but that antibodies of the IgG class reach a higher affinity upon maturation than those of the IgM class²². It has also been shown recently in another system that the T-cell receptor is restricted to only part of the idiotypes expressed by antibodies²³.

In contrast to our findings in mice, it has been reported that an antigen-specific T-cell factor produced in rabbits did not show the presence of V_H markers²⁴, namely a locus allotype, whereas a receptor directly isolated from rabbit T cells did show the presence of V_H-related allotypic determinants²⁵.

It is noteworthy that the antigen-specific T-cell helper factors have been shown to possess Ia determinants^{1,2,26}. Thus, these factors are coded by two unlinked genes. A similar conclusion has been drawn from genetic studies with an anti-idiotypic serum to a T-cell receptor²⁷. The molecular basis of the combination between I region gene products and immunoglobulin variable region gene products is not yet understood. Preliminary experiments in our laboratory suggest that the two determinants are part of one entity since combination of effluents passed separately on anti-Ia and anti-idiotypic immunoadsorbents did not result in an active helper factor.

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Is synaptic facilitation caused by presynaptic spike broadening?

FACILITATION is a characteristic of many synapses whereby the efficacy of synaptic transmission increases following presynaptic activity. This form of neuronal plasticity has been implicated in behavioural modifications such as habituation¹ and learning². Facilitation has been shown to be due to a presynaptic increase in transmitter release³, but its biophysical mechanism remains obscure. Although it is often suggested that facilitation is due to the action of a residual increase in intracellular free calcium⁴, an alternative hypothesis, that facilitation may be caused by action potential prolongation, has recently been proposed⁵⁻⁷. In molluscan neural somata, spike broadening occurs in a train of impulses, due to non-inactivating calcium channels and cumulatively inactivating potassium channels⁷. This might result in an increased calcium influx associated with prolonged spikes⁸, which would lead immediately to facilitated transmitter release⁹. We show here that at crustacean neuromuscular junctions, spike broadening is not primarily responsible for facilitation.

In previous work on neuromuscular junctions, no presynaptic spike broadening was observed, and facilitation could be evoked by repeated terminal depolarisation^{10,11}. However, the extracellular recording method used is particularly insensitive to changes in spike duration¹², and a cumulative potassium inactivation would invalidate the interpretation¹³ of the effect of extracellular stimulation on presynaptic nerve polarisation. Moreover, the effect of caesium on neuromuscular transmission is consistent with the spike broadening facilitation hypothesis. Caesium prolongs nerve terminal potentials (n.t.ps) (probably by blocking potassium channels), increases the number of quanta released by an impulse, and reduces facilitation at crab neuromuscular junctions^{14,15}. Similarly, in molluscan somata, when the inactivating outward current is blocked pharmacologically, the spikes are all broadened, but progressive spike prolongation in a train is prevented.

An alternative explanation for the caesium effect¹⁰ is that the longer n.t.ps may simply be releasing all the transmitter immediately available for release, so that the transmitter release is saturated. Then, whatever processes are responsible for facilitation may still be operable in caesium, but are not expressed, as facilitation of the already saturated release of transmitter is impossible.

These alternatives may be distinguished by soaking neuromuscular junctions in a caesium-substituted potassium-free saline, and then reducing calcium and raising magnesium

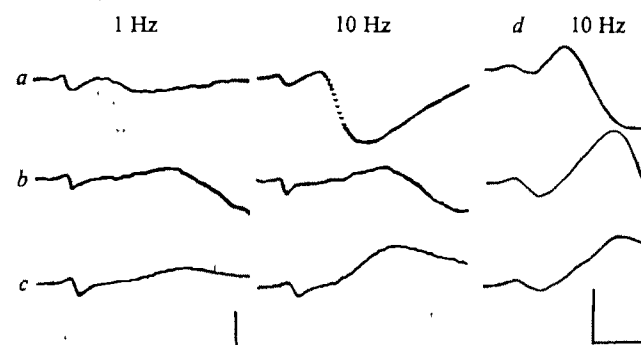


Fig. 1 Extracellular recordings of nerve potentials and synaptic currents from synaptic sites. a-c Are from three separate preparations and show responses at 1 and 10 Hz in normal saline (a), 5.4 mM potassium replaced by caesium with normal calcium and magnesium (b), and 5.4 mM caesium with 1 mM calcium and 15 mM magnesium (c). d, Responses from a single preparation stimulated at 10 Hz are shown in the three experimental solutions. All traces are computer-averaged responses to 64 stimuli. Calibration bars 0.1 mV, 2 ms

Table 1 Effects of caesium or 4-aminopyridine in normal and low-calcium media on synaptic transmission and facilitation

Experiment	Solution	e.j.p. amplitude (mV) at frequency of		
		1 Hz	10 Hz	Facilitation
1	Control	0.19	1.08	4.68
	Ca	0.95	3.37	2.54
	Ca low-Ca	0.36	1.67	3.64
2	Control	0.15	1.37	8.13
	Ca	15.58	37.97	1.44
	Ca low-Ca	0.12	0.95	6.92
3	Control	1.50	8.89	5.93
	Ca	4.75	15.50	3.26
	Ca low-Ca	0.38	4.00	10.53
4	Control	0.64	2.44	2.81
	Ca	2.33	4.48	0.92
	Ca low-Ca	0.25	0.77	2.08
5	Control	0.60	2.30	2.83
	Ca	5.00	12.50	1.50
	Ca low-Ca	1.00	4.50	3.50
6	Control	0.16	0.78	3.84
	Ca	1.80	6.00	2.33
	Ca low-Ca	0.60	2.80	3.67
7	Control	0.19	0.55	4.50
	AP	3.55	5.00	0.49
	AP low-Ca	0.30	0.85	1.83
8	Control	0.10	0.44	3.40
	AP	2.10	5.00	1.38
	AP low-Ca	0.08	0.30	2.75
9	Control	0.23	0.45	0.96
	AP	3.36	5.73	0.71
	AP low-Ca	0.04	0.12	2.00
10	Control	0.19	0.28	0.47
	AP	4.44	5.48	0.32
	AP low-Ca	0.07	0.18	1.57
11	Control	0.10	0.44	3.40
	AP	1.01	2.34	1.32
	AP low-Ca	0.06	0.20	2.33
12	Control	0.07	0.35	4.00
	AP	1.18	2.50	1.12
	AP low-Ca	0.12	0.80	5.67

'Control', normal saline (as in ref. 3, except that 10 mM HEPES buffer is used to hold pH to 7.3), 'Ca', 5.4 mM caesium substituted for potassium, 'AP', saline plus 25 μ M 4-aminopyridine; 'Ca low-Ca', 5.4 mM caesium substituted for potassium, calcium reduced from normal (13.5 mM) to 1 mM, and magnesium raised from normal (2.5 mM) to 15 mM, 'AP low-Ca', saline with 25 μ M 4-aminopyridine, 1 mM calcium and 15 mM magnesium. In the last two solutions, the calcium and magnesium concentrations were buffered with EDTA¹⁶ and the calculated calcium concentration was confirmed with an Orion ion-selective electrode. Excitatory junctional potential (e.j.p.) amplitudes were corrected for the nonlinear relation between transmitter release and e.j.p. amplitude, using Martin's revised correction procedure²², which is applicable to crustacean muscles because their multi-terminal innervation and short electrotonic length make them nearly isopotential²³. The membrane time constant was estimated from the e.j.p. falling phase and the synaptic current time course was derived from the e.j.p. time course²⁴ or measured from the extracellularly recorded synaptic current. Caesium and low calcium had little effect on the synaptic current time course, so errors in estimating transmitter release due to changes in release synchrony were averted^{25,26}. Each e.j.p. amplitude given is the average of at least 10 measurements. The standard errors were lower than 11% in each case (typically 5%) and are not shown. Facilitation is expressed as the fractional increase in transmission at 10 Hz compared with that at 1 Hz. A facilitation index of unity signifies a doubling in transmitter release.

until transmitter release is restored to near the control level in normal saline. As either calcium reduction or blockade of potassium currents prevents spike broadening in molluscan somata, both treatments combined ought to be doubly effective in preventing facilitation due to spike broadening. In contrast, if spike broadening does not normally occur at crustacean nerve terminals and has nothing to do with facilitation, reducing calcium and elevating magnesium should return transmitter release to a non-saturated level and facilitation should reappear. It was shown previously¹⁶ that changes in calcium concentration alone do not affect facilitation.

Table 1 shows the results of six experiments in which we recorded intracellularly from crayfish (*Procambarus clarkii*) claw opener muscles while stimulating the opener motor neurone, using procedures described elsewhere^{3,10}. Replacing potassium with caesium increased transmission 3–100-fold. Facilitation was reduced to 18–61% of its control value. On

reducing the free calcium and increasing the free magnesium concentration, transmission is reduced and facilitation restored, the degree of restoration of facilitation corresponding roughly to the extent to which transmission is restored to the control level.

Caesium and low-calcium-high-magnesium might have unexpected effects on spike duration that would argue against our interpretation of their effects on facilitation. From previous observations of n.t.ps at different frequencies³, we would expect the presynaptic action potential duration to be unchanged at 10 Hz relative to 1 Hz in each of the experimental solutions. Figure 1a–c confirms this prediction. Moreover, we would expect the n.t.p. to be prolonged in the presence of caesium and that this effect would not be reversed by reducing the calcium-to-magnesium concentration ratio. This expectation was also confirmed (Fig. 1d).

To generalise our results, we chose to determine whether similar effects would be obtained using a different pharmacological agent, 4-aminopyridine (4-AP), for blocking potassium channels¹⁷. To obtain effects quantitatively comparable with those of 5.4 mM caesium, we treated neuromuscular junctions with 25 μ M 4-AP (the half-maximal dose of 4-AP is about 50 μ M). We found that, as with caesium, facilitation was markedly reduced and that this effect was largely reversed by reducing the calcium concentration. The results of six experiments are included in Table 1.

In squid axon, both caesium and 4-AP block voltage-dependent potassium channels^{17,18}. In molluscan somata, caesium blocks voltage-dependent channels, whereas 4-AP has little effect on delayed potassium activation^{19,20}. Neither agent seems to block calcium-activated potassium channels. It seems probable that both agents are blocking a voltage-dependent potassium current in crayfish nerve terminals. As these voltage-dependent channels are thought to be the ones subject to progressive inactivation in dorid nudibranchs⁷, blocking them should prevent spike broadening, especially in low calcium, because a persistent calcium current is also required for the expression of progressive spike broadening⁷. The large facilitation we observe in the presence of either 4-AP or caesium and reduced calcium argues against the notion that spike broadening due to potassium inactivation is responsible for synaptic facilitation. Rather, the block of facilitation by caesium and 4-AP in normal calcium seems to be due to a saturation of transmitter release.

There is also some evidence that in snails the calcium-activated potassium channels are subject to inactivation, contributing to spike broadening⁶. Such channels should not be activated in low calcium^{6,21}. The persistence of facilitation in 1 mM calcium in these experiments and even in 10 and 100 μ M calcium (ref. 16 and R.S.Z., unpublished) argue against the notion that spike broadening due to inactivation of calcium-activated potassium channels could underlie facilitation. Thus, it seems that facilitation at crayfish neuromuscular junctions arises in some step of depolarisation-secretion coupling following the generation of the presynaptic electrical signal.

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Striatal dopamine receptors become supersensitive while rats are given trifluoperazine for six months

OF all the side effects of drugs used to treat psychotic illness such as schizophrenia, chronic tardive dyskinesias are the most disturbing. They appear after months or years of therapy, and persist when the offending drug is withdrawn in about half of the cases. Such tardive dyskinesias resemble in character abnormal movements produced by levodopa in patients with Parkinson's disease. They can be controlled by drugs that antagonise the cerebral actions of dopamine (DA)¹. Thus, they seem to be due to overstimulation of cerebral DA receptors, although they are caused by neuroleptic drugs which, in acute experiments, antagonise cerebral dopamine receptor action^{2–5}. This paradox has been resolved by suggesting that such drugs, by binding to and antagonising cerebral DA receptors, may eventually actually increase receptor sensitivity. Indeed, following treatment for a few weeks with phenothiazines or butyrophenones and withdrawal of the drugs, animals exhibit behavioural and biochemical supersensitivity to DA agonists which persists for some time^{6–13}. However, tardive dyskinesias usually appear while the patient continues to take the offending drug, so we have studied the effect on striatal DA receptor activity of continuous 6-month administration to rats of a potent neuroleptic drug in common clinical use (trifluoperazine). At intervals the animals were tested for a behavioural response to the DA agonist apomorphine, and were killed for biochemical assessment of DA turnover and DA receptor activity in that part of the brain (the corpus striatum) believed to be the site responsible for production of tardive dyskinesias. We report here that the initial behavioural and biochemical evidence for striatal DA receptor blockade by trifluoperazine disappears within a few weeks of starting therapy, to be replaced by supersensitivity after 6 months of drug administration, despite continued drug intake.

Trifluoperazine hydrochloride (Smith, Kline and French, 2.5–3.5 mg per kg per day) was administered in the drinking water for 6 months to male Wistar rats (Olac International; weight 200 g at the start of the experiment). The drug was made up freshly in distilled water (with ascorbic acid added) each day in darkened bottles. Control animals were given distilled water alone. All animals were kept in the same environment at 21 ± 3 °C on a regular 12-h night/day cycle. At intervals (usually after 1 and 2 weeks, and 1, 3 and 6 months) trifluoperazine-treated and control rats were assessed as follows: spontaneous locomotor activity, presence of catalepsy, and incidence and severity of stereotypy induced by apomorphine hydrochloride (Evans Medical; 0.5 mg per kg, subcutaneously (s.c.), given 15 min previously). At the same intervals, groups of trifluoperazine-treated and control rats were killed by decapitation and the paired corpus striatum was rapidly dissected out for: (1) fluorimetric assay of DA and its major metabolites homovanillic acid

(HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) according to the techniques of Westerink and Korf¹⁴; (2) assay of basal and dopamine (1–150 μM) stimulated adenylate cyclase according to the technique of Miller, Hearn and Iverson¹⁵ (ED₅₀ values for dopamine stimulation varied between runs due to inherent changes in assay procedure. This problem was overcome by running control samples and samples from drug-treated animals in strict parallel throughout the entire procedure. Attention should be paid to changes in relative ED₅₀ values); (3) specific ³H-spiroperone (20 Ci mmol⁻¹; Radiochemical Centre) binding using a range of 0.125–4.0 nM radioactive ligand and DA (10⁻⁴ M) to define specific binding as modified from the technique of Creese, Burt and Snyder¹⁶ (³H-spiroperone binding in striatum is to DA receptors¹⁷). The results are summarised in Tables 1 and 2.

After an initial period of weight loss in the first week the animals tolerated trifluoperazine well, gaining weight at the same rate as control animals, and regularly ingesting between 2.5 and 3.5 mg per kg per d. For the first week spontaneous locomotion was reduced in trifluoperazine-treated animals which were moderately cataleptic. Thereafter, these rats regained normal activity and catalepsy disappeared so that they were indistinguishable in these respects from control animals by 3 and 6 months.

Administration of apomorphine (0.5 mg per kg, s.c.) to control animals at each testing period resulted in the production of mild stereotypy consisting of discontinuous locomotion and sniffing. Apomorphine-induced stereotypy was antagonised by trifluoperazine in the first month of treatment, had returned to control levels by 3 months, and was enhanced after 6 months, the animals exhibiting pronounced licking, gnawing or biting (Table 1). From consideration of the proportion of animals continuously exhibiting this behaviour to a range of doses of apomorphine (0.125–2.0 mg per kg, s.c.), the dose-response curve for maximum stereotypy was found to have shifted to the left in the animals treated with trifluoperazine for 6 months, compared with control rats.

Striatal DA concentrations were unaltered, whereas HVA and DOPAC levels were increased in the first weeks of drug administration, but thereafter the increase in DA metabolites in the striatum disappeared rapidly, as has been observed by others^{18,19} (Table 1). Striatal DA, HVA and DOPAC levels after 6 months of trifluoperazine treatment were not consistently different from those in age-matched control rats.

The increase in striatal adenylate cyclase activity from basal levels was linearly related to added DA concentration between 1 and 150 μM in control animals. In the first month of trifluoperazine intake, more DA was required to produce an equivalent stimulation of striatal adenylate cyclase above basal

Table 1 Effect of 6 months of oral administration of trifluoperazine (2.5–3.5 mg per kg per d) on apomorphine-induced stereotypy and striatal DA, HVA and DOPAC levels compared with controls

Time	Apomorphine-induced stereotypy score		% Of control		
	Control group	Drug-treated group	DA	HVA	DOPAC
1–2 weeks	2.0 ± 0.0	0.0 ± 0.0†	98 ± 7	234 ± 31†	238 ± 34†
1 month	2.0 ± 0.0	0.3 ± 0.2†	—	122 ± 15	107 ± 9
3 months	2.3 ± 0.2	1.6 ± 0.2	130 ± 9*	124 ± 5	88 ± 5
6 months	2.1 ± 0.1	3.1 ± 0.1*	89 ± 10	118 ± 7	110 ± 10

Apomorphine-induced (0.5 mg per kg, s.c.) stereotypy was assessed according to the scale: 0 = normal, 1 = continuous locomotor activity, discontinuous sniffing, 2 = discontinuous locomotor activity, continuous sniffing, 3 = discontinuous biting, licking and gnawing, 4 = continuous biting, licking or gnawing. The results are expressed as the mean ± 1 s.e.m. for 6 animals in each group. Striatal DA, HVA and DOPAC levels were measured by the technique of Westerink and Korf¹⁴, in 6 control and 6 drug-treated animals at the time intervals shown. Values for control animals for DA were 11.1 ± 0.9 μg per g, for HVA 93 ± 57 ng per g and for DOPAC 850 ± 63 ng per g (± 1 s.e.m.) and showed no change with age. All data obtained in drug-treated animals were compared with those obtained in age-matched untreated animals kept in otherwise identical conditions and studied at the same time.

* $P < 0.05$, † $P < 0.005$, compared to control animals, Student's *t* test for parametric data; Mann-Whitney *U* test for non-parametric results.

Table 2 Effect of 6 months of oral administration of trifluoperazine (2.5–3.5 mg per kg per d) on dopamine stimulation of striatal adenylate cyclase and specific striatal ^3H -spiperone binding compared with controls

Time	Stimulation of adenylate cyclase caused by 50 μM DA (pmol cAMP per 2 mg tissue per 2.5 min)		Total specific ^3H -spiperone binding (pmol per mg)	
	Control group	Drug-treated group	Control group	Drug-treated group
1–2 weeks	107 $\pm$ 6	41 $\pm$ 19*	11.7 $\pm$ 0.4	11.0 $\pm$ 0.5
1 month	106 $\pm$ 18	51 $\pm$ 27*	11.3 $\pm$ 0.8	4.7 $\pm$ 0.2*
3 months	48 $\pm$ 11	61 $\pm$ 19	—	—
6 months	55 $\pm$ 15	90 $\pm$ 24*	8.1 $\pm$ 0.2	9.8 $\pm$ 0.4*

The DA stimulation of striatal adenylate cyclase caused by 50 μM DA was calculated from the linear portion of the concentration (1–150 μM DA)–response curve, obtained by regression analysis, as determined by incubating striatal tissue from 3 control or drug-treated rats with the range of concentrations (each determination being carried out in duplicate). Total specific ^3H -spiperone binding (as judged by DA 10^{-4} M displacement) was determined at nonsaturating substrate concentration (0.25 and 0.50 nM). Data were obtained by using pooled tissue from at least 6 control or drug-treated animals with each determination being carried out in triplicate. All data obtained in drug-treated animals were compared with those obtained in age-matched untreated animals kept in otherwise identical circumstances and studied at the same time.

* $P < 0.005$ Student's t test.

levels, and the concentration of DA required to produce a 50% increase in adenylate cyclase from basal levels (ED_{50}) was increased to 87 μM , compared with 6 μM in age-matched control animals. After 3 months of trifluoperazine treatment the ED_{50} for added DA was barely different from that required in control animals (66 μM and 72 μM , respectively). However, by 6 months, 50 μM produced a greater percentage increase in adenylate cyclase from basal level compared with control animals and the ED_{50} was less in drug-treated than control animals (4 μM and 32 μM , respectively) (Table 2).

Total specific binding of ^3H -spiperone to striatal membranes was less in trifluoperazine-treated animals compared with controls at nonsaturating ligand concentrations after 1 month of drug intake (Table 2). Scatchard analysis showed this to be due to a reduced receptor affinity in animals treated for 1 month relative to control animals (K_D 1.04 $\pm$ 0.13 nM compared with 0.32 $\pm$ 0.05 nM for controls; $P < 0.005$) whereas total number of binding sites was unchanged (15.1 $\pm$ 1.7 pmol per g compared with 19.3 $\pm$ 2.6 pmol per g for controls). However, by 6 months total specific ^3H -spiperone at ligand concentrations below saturation was greater in trifluoperazine-treated than in age-matched control animals (Table 2). Scatchard analysis revealed a tendency for increased receptor affinity (K_D 0.50 $\pm$ 0.06 nM compared with 0.67 $\pm$ 0.06 nM for control animals; $P < 0.08$) with no obvious change in number of binding sites (23.4 $\pm$ 2.5 pmol per g, and 20.7 $\pm$ 1.7 pmol per g for control animals).

The effect of chronic trifluoperazine administration in increasing receptor affinity was seen more clearly by comparing the dissociation constant after 1 month of drug treatment with that after 6 months (K_D 1.04 $\pm$ 0.13 nM and 0.50 $\pm$ 0.06 at 1 and 6 months, respectively; $P < 0.01$). Thus, the dissociation constant fell between 1 and 6 months of trifluoperazine therapy, indicating an increased receptor affinity. By contrast, in control animals the reverse occurred, namely an increased dissociation constant (K_D 0.32 $\pm$ 0.05 nM and 0.67 $\pm$ 0.06 nM at 1 and 6 months, respectively; $P < 0.007$), indicating a decrease in receptor affinity with normal ageing. Furthermore, comparison of the number of binding sites at 1 and 6 months shows a small increase in trifluoperazine-treated animals (15.1 $\pm$ 1.7 pmol per g to 23.4 $\pm$ 2.5 pmol per g; $P < 0.02$), but no change in control animals (19.3 $\pm$ 2.6 pmol per g, 20.7 $\pm$ 1.7 pmol per g; $P < 0.05$).

Our results indicate a fairly consistent general conclusion, supported by a variety of different behavioural and biochemical

observations. Trifluoperazine administration in the first days or weeks caused antagonism of DA-mediated behaviour, a compensatory increase in striatal DA turnover, decreased capacity for DA stimulation of striatal adenylate cyclase, and decreased striatal ^3H -spiperone binding. All these effects may be attributed to the acute or subacute actions of these drugs in brain tissue. Even the *in vitro* adenylate cyclase and ^3H -spiperone binding changes probably represent the effects of residual drug in the tissues studied.

Over the next 6 months, trifluoperazine intake remained constant and we assume that brain levels of the drug did not change in a manner that might explain our results. A gradual accumulation of the drug might occur, but not a progressive decrease in brain trifluoperazine concentration. In any event, the changes we observed over the months of chronic trifluoperazine intake were not a gradual decline of the acute effects of the drug, but a reversal of these actions.

After 1 to 3 months of trifluoperazine intake, spontaneous locomotor activity, stereotypy induced by apomorphine, striatal dopamine turnover, adenylate cyclase and ^3H -spiperone binding had all returned to normal, as judged by age-matched control rats. After 6 months, drug-treated animals were supersensitive to apomorphine in respect of maximum stereotypy, and showed increased sensitivity for DA stimulation of striatal adenylate cyclase and increased ^3H -spiperone-defined receptor affinity. Thus, a drug which initially caused behavioural and biochemical antagonism of DA effects in the striatum produced the opposite effects after 6 months' administration, namely enhanced striatal dopamine action.

Does this reversal of trifluoperazine's effect on DA action during chronic administration apply to other neuroleptic drugs? So far, we have obtained similar results for thioridazine (30–40 mg per kg per day) showing a reversal of all these behavioural and biochemical indices of DA action over 6 months' intake.

We consider our results to indicate that as far as striatal DA receptors are concerned, the initial antagonistic effects of neuroleptic drugs are replaced by some evidence of supersensitivity despite continued drug intake. This would explain why drug-induced parkinsonism may disappear and why tardive dyskinesias may appear during chronic neuroleptic therapy. Furthermore, this type of experiment may provide a realistic animal model for testing drugs for their capacity to induce tardive dyskinesias.

Finally, it is reasonable to wonder whether changes similar to those observed in the striatum occur in the mesolimbic and mesocortical DA areas of the brain. If so, and the necessary experiments are under way in our laboratories to study this, the whole concept of antipsychotic drugs acting primarily by antagonising cerebral DA receptors may require revision.

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Effects of insulin on insulin-binding components extracted from rat fat cell membranes

WE have previously shown the presence of two distinct insulin-binding species (designated species I and II) in a Triton X-100 extract of fat cell membranes¹. They were separable electrophoretically and displayed different insulin-binding characteristics. Scatchard analysis of the insulin-binding data for species I indicated a complex, non-classical association, illustrated by a curvilinear plot. The characteristics of this curve are quite similar to those of the binding curves obtained with unfractionated detergent extract or with intact plasma membranes¹. In contrast to these findings, insulin-binding to species II was characterized by a relatively low affinity and a linear Scatchard plot. Although these findings indicate different insulin-binding and electrophoretic properties for the two species, the possibility remains that they are related structurally. For example, the K_d values for the low-affinity portion of the peak I Scatchard plot and for insulin binding to peak II are similar. Furthermore, following the incubation of isolated peak I with ¹²⁵I-labelled insulin and subsequent electrophoresis, a small amount of radioactivity migrated in the position of peak II (ref. 1). We report here that interconversion occurs between the two binding species and that the conversion is mediated by insulin.

Soluble insulin-binding material was prepared by treating a purified plasma membrane fraction from rat adipocytes with 1% (v/v) Triton X-100 as described by Cuatrecasas². Particulate material was removed by centrifugation of the detergent suspension at 150,000g for 90 min at 4 °C. ¹²⁵I-labelled insulin (100-200 µCi per µg) was prepared by the chloramine-T

method as previously described¹. Specific binding of ¹²⁵I-labelled insulin by the soluble material was measured by the polyethylene glycol precipitation method², modified slightly to increase the sensitivity of the assay¹.

The soluble insulin-binding material was subjected to electrophoresis on 5% polyacrylamide gels (12.5 cm in length) using the system of Davis³ in conditions we have previously reported¹. Following the electrophoresis, the gel was sliced and the insulin-binding activity eluted from the various gel segments as previously described¹. The binding activity was assayed in the eluates by the polyethylene glycol method² and then concentrated by ultrafiltration¹. Results were obtained using the concentrated preparations of the isolated insulin-binding species.

In the absence of insulin at 4 °C the two insulin-binding species migrate as single peaks of activity with no spontaneous formation of any significant amounts of the other binding species (Fig. 1). Thus, in these conditions both binding species are relatively stable and migrate during electrophoresis in their customary positions.

Figure 2 shows the effect of insulin. Addition of the hormone (50 ng ml⁻¹) to isolated species I (Fig. 2a) causes the appearance of a significant amount of insulin-binding material in the region occupied by species II. There is a small amount of insulin-binding material located beyond species II but this small insulin-binding peak is inconsistently found and its significance is not clear. In contrast to the findings with isolated species I, addition of insulin to isolated species II (Fig. 2b) fails to cause any formation of species I.

Figure 3 shows the sensitivity of the insulin-mediated conversion of species I to species II. The loss of insulin-binding activity in the position of species I is paralleled by the appearance of insulin-binding activity in species II. A significant amount of conversion is elicited by an insulin concentration as low as 0.5 ng ml⁻¹, which is well within physiological insulin levels. These findings demonstrate a direct and highly sensitive effect of insulin on conversion of the high-affinity insulin-binding activity to a low-affinity form which has a different electrophoretic mobility from the parent species.

Ginsberg *et al.*⁴, using material solubilised from turkey red cells, showed an insulin-mediated conversion of a large-form insulin-binding peak to a small form. These studies differ from our findings in rat fat cells by the demonstration that the small peak could be converted back to the large peak by insulin. Our failure to find a conversion of species II to species I either in the presence or absence of insulin may be related to the conditions used. All procedures were carried out at 4 °C, a temperature at

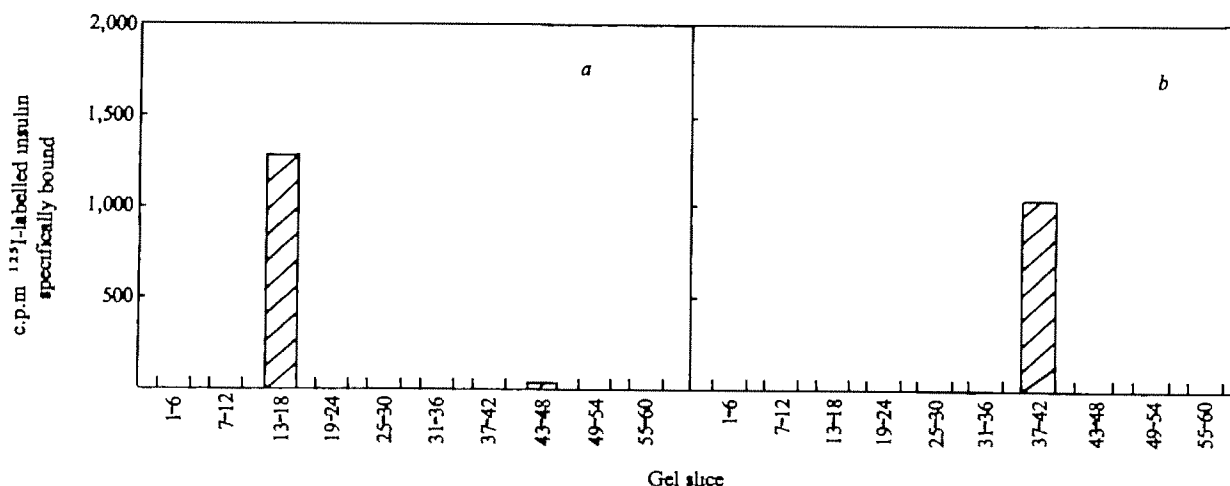


Fig. 1 Electrophoresis of the isolated insulin-binding species. Species I (a) and species II (b) were isolated from the Triton X-100 extract of fat cell plasma membranes and concentrated as previously described¹. Polyacrylamide-gel electrophoresis of the separate species was carried out for 3 h following a 16 h incubation period at 4 °C (ref. 1). The gels were then sliced and the gel sections eluted¹. The amount of insulin-binding activity was determined in the eluates by the polyethylene glycol method⁴.

which changes in the size of the turkey insulin receptor are slow⁴. However, the insulin-binding species isolated from rat fat cells may be quite different from those isolated from turkey red cells. For example, we isolate two insulin-binding species from the adipocyte plasma membrane, whereas only one species is initially isolated from the avian cell. In the red cell system, insulin exposure is needed before the second species is detected. The fat cell system also seems to be more sensitive to the effects of insulin. A hormone concentration of 0.5 ng ml^{-1} causes significant binding-species conversion ($\sim 30\%$) in our system, whereas a concentration of 25 ng ml^{-1} is required in the red cell extract to effect an 18% conversion to the small form. Moreover, $\sim 70\%$ of the total binding by adipocyte species I could be converted to species II, compared with a 30% conversion for the avian cell preparation.

Maturo and Hollenberg⁵ have shown the presence of two distinct insulin-binding activities in a detergent extract of rat liver plasma membrane. The large species isolated by Sepharose-6B chromatography had complicated insulin-binding properties, whereas the smaller form demonstrated a simple insulin-binding isotherm. The insulin-binding activity isolated by insulin-agarose affinity chromatography corresponded to the small form, and the large form was regenerated by the addition of a glycoprotein fraction which by itself failed to bind insulin specifically. The two forms of insulin-binding activity may correspond to species I and II which we have also demonstrated in rat liver membranes⁶. Maturo and Hollenberg⁵ and Jacobs *et al.*⁷ have found that the insulin-binding activity isolated by insulin-agarose affinity chromatography of liver extracts has hormone-binding characteristics which are similar to the binding characteristics of species II (ref. 1). This observation supports our present finding that the interaction of species I (high affinity, complex insulin-binding kinetics) with insulin (and presumably with insulin-agarose) causes a conversion to species II (low

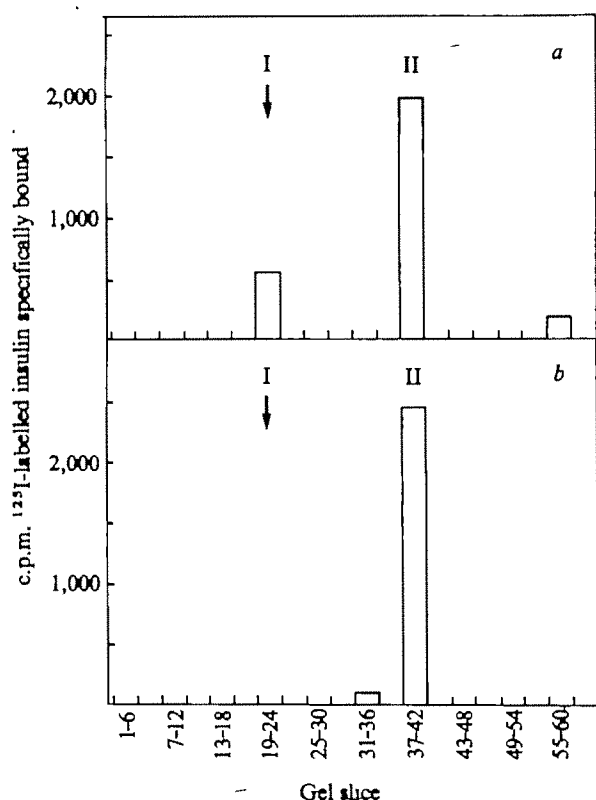


Fig. 2 Electrophoresis of the isolated insulin-binding species in the presence of insulin. Species I (a) and species II (b) were incubated with insulin (50 ng ml^{-1}) for 16 h at 4°C . The incubation mixtures were then subjected to gel electrophoresis for 3 h. The gels were cut, eluted and insulin-binding activity determined as described in Fig. 1.

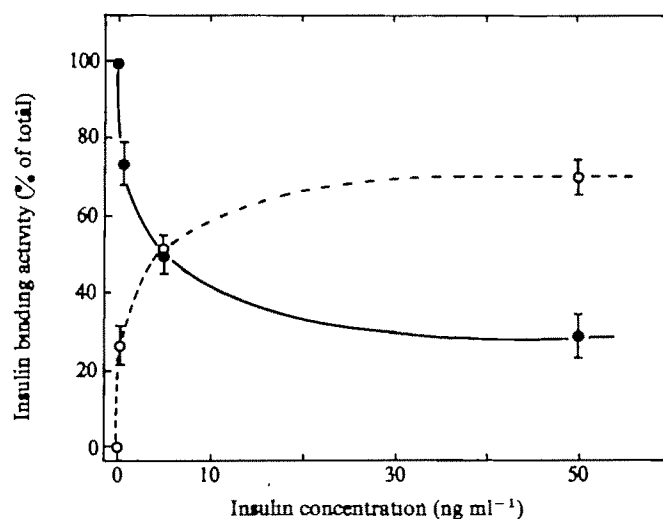


Fig. 3 Effect of insulin on the conversion of species I to species II. Species I was incubated with the indicated concentrations of insulin for 16 h at 4°C . The amount of species II formed and the disappearance of species I were determined as indicated in Fig. 1. Each point on the graph represents the mean $\pm$ s.e. of three separate experiments. The total amount of insulin-binding eluted from the gel was taken as 100%. ●, Peak I; ○, peak II.

affinity, simple insulin-binding kinetics). Thus, it may be impossible to purify the high-affinity insulin-binding component by the use of affinity columns that contain insulin.

The findings reported here emphasise the complexities which surround the interaction of insulin with the insulin receptor. The ability of insulin to induce the formation of a low-affinity component from a high-affinity binding species may help to explain the mechanisms underlying certain phenomena found in studies of insulin-binding to target cells and subcellular particles (for example, 'negative cooperativity', curvilinear Scatchard plots). In addition, the loss of insulin binding associated with high plasma insulin levels in insulin-resistant states of man and animals⁸⁻¹³ may in part be related to the insulin-mediated conversion of species I to species II. Finally, as the conversion of the high-affinity species to the low-affinity form is highly sensitive to insulin, this molecular event may represent an early step in insulin action which follows binding of the hormone to the receptor.

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Effect of tetrodotoxin on adrenaline secretion in the perfused rat adrenal medulla

EXTRACELLULAR Ca ions are required for the secretion of many hormones¹. In the adrenal medulla catecholamine secretion evoked by acetylcholine (ACh) is completely blocked by omitting Ca from the medium^{2,3}. The mechanism for Ca entry has been studied in a pheochromocytoma cell line (PC12) which originated from a rat adrenal medullary tumour^{4,5}. Two alternative ways of Ca entry were considered, voltage-dependent Ca channels and ACh channels. By analysing dopamine secretion⁴ and radioactive Ca influx⁵ in various ionic environments it was concluded that more than 80% of the Ca ions enter through the voltage-dependent Ca channels during stimulation by cholinergic agonists. Action potentials mediated by Na^{6,7} and Ca⁷ have been demonstrated in primary cultures of rat adrenal chromaffin cells. Furthermore, extracellular recording techniques can detect spontaneous action potentials in these cells. The frequency of these action potentials is modified in a dose-dependent manner by ACh (3×10^{-7} M to 10^{-4} M)⁷, and the same range of ACh concentrations stimulates adrenaline secretion in the perfused rat adrenal medulla^{3,8}. Thus modulation of action potential frequency by ACh may be a mechanism for controlling the amount of Ca entry. The potential change during a Na action potential may activate voltage-dependent Ca channels, thereby increasing Ca influx. Also, spontaneous action potentials are blocked by 6 μ M tetrodotoxin (TTX)⁷, a specific inhibitor of Na channels⁹. Therefore, if the Na action potential is involved in secretion, TTX should reduce Ca entry which in turn should decrease catecholamine secretion. We tested this hypothesis in the perfused rat adrenal medulla and report here that TTX decreases a portion of the adrenaline secretion evoked by either high KCl or ACh. Thus, Na action potentials, by facilitating the activation of Ca channels, may be involved in the regulation of adrenaline secretion from adrenal chromaffin cells.

The technique for perfusion of the isolated rat adrenal medulla has been described by Ishikawa *et al.*³. Male rats (Sprague-Dawley, Simonsen) weighing 180–300 g were used. After ether anaesthesia an adrenolumbar vein entering the left adrenal gland was cannulated and arteries and other veins connected to the gland were ligated. The adrenal gland was then removed from the rat and maintained at room temperature

Table 1 Adrenaline release stimulated by acetylcholine (2 μ M)

Conditions during second response	Second response as a % of initial response	Third response as a % of initial response
ACh control	71 $\pm$ 3 (6)	47 $\pm$ 11 (6)
ACh + TTX (6 μ M)	41 $\pm$ 4 (6)	37 $\pm$ 6 (6)
	$P < 0.0001^*$	NS at $P = 0.2^*$
ACh + CoCl ₂ (5 mM)	0 (2)	216 (2)

Experiments were carried out as described in the legend to Fig. 1a, b except that release was evoked by three successive challenges to 2 μ M ACh. The response to the second ACh challenge is expressed as per cent of the response to the initial ACh challenge after subtraction of basal secretion. As in Fig. 1, the first and third responses were evoked in ACh alone. The total initial response to ACh was $120 \pm 13(14)$ pmol min⁻¹. The basal secretion was $30 \pm 7(14)$ pmol min⁻¹. Data are expressed as the mean $\pm$ s.e.m. with the number of adrenals indicated in parentheses. The inhibition of the response to ACh by TTX (second response) was reversible (third response). The response to ACh after removal of CoCl₂ was not only reversed but inexplicably enhanced. To prevent ACh hydrolysis eserine sulphate (1.5 μ M) was added to the saline of all experiments involving ACh.

*Student's *t*-test for comparison with the ACh control, two-tailed.

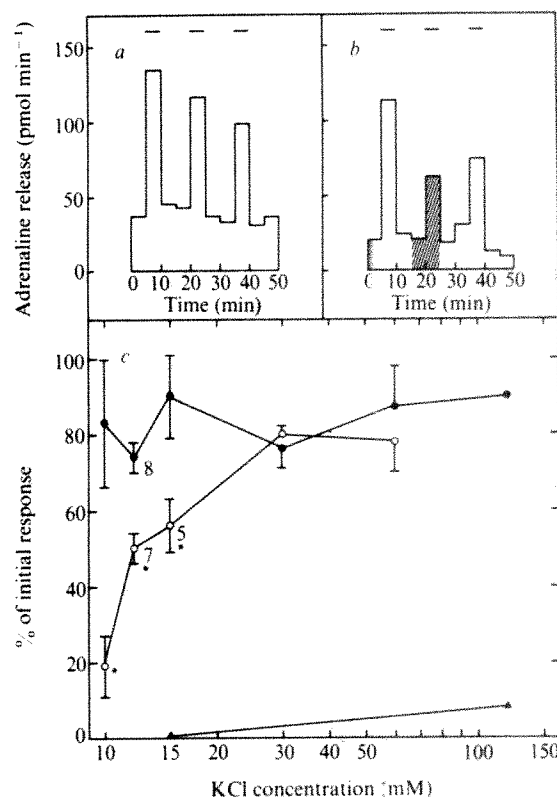


Fig. 1 a, Adrenaline release during three successive challenges with 12 mM KCl (indicated by the horizontal bars at the top of the graph). Each KCl challenge and collection period lasted 5 min. Between KCl challenges the adrenal was perfused for 10 min with normal saline. At the end of the 50 min the adrenal gland was discarded. b, Effect of TTX (6 μ M) on adrenaline release stimulated by 12 mM KCl. Conditions were identical to those of a except that TTX was present during the period indicated by cross hatching. c, Effects of TTX and CoCl₂ on adrenaline release stimulated by different concentrations of KCl. Experiments were carried out as for a and b. After subtraction of basal secretion from the stimulated secretion, the net response to the second KCl challenge is expressed as per cent of the net response to the initial KCl challenge. The second challenge contained: KCl alone (●), 6 μ M TTX (○) or 5 mM CoCl₂ (▲). The KCl concentration is plotted on a log scale. Each point with standard error bars represents the mean result from four different adrenals, except where *n* is specified by the number adjacent to the point. Points without standard error bars represent single samples. Statistically significant (Student's *t*-test two-tailed, $P < 0.05$) depression of the KCl response in the presence of TTX is indicated by an asterisk. This effect of TTX was completely reversible, as there were no statistically significant differences ($P > 0.07$) in response to the third KCl challenge between controls and adrenals which received TTX during the second challenge. The effect of 6 mM CoCl₂ was also reversible. CoCl₂ (5 mM) interfered with neither the extraction nor fluorescence of adrenaline standards or samples.

(21–23°C). Saline was perfused retrogradely through the vein and collected from a small incision on the adrenal cortex. The speed of perfusion was controlled by a roller pump at 0.15 ml min⁻¹.

Adrenaline was assayed by using a trihydroxyindole fluorometric assay¹⁰ after previous butanol extraction¹¹ of the samples. The assay is specific for adrenaline. All values are based on adrenaline standards which were carried through the entire extraction procedure for each experiment.

The normal solution used for perfusion had the following composition: NaCl 150 mM, KCl 5.6 mM, CaCl₂ 2 mM, MgCl₂ 1 mM, NaH₂PO₄ 1 mM, HEPES-NaOH 10 mM and glucose 5.0 mM. The saline was bubbled with 100% O₂. The concentration of KCl, CaCl₂ or CoCl₂ was increased by replacing an osmotically equivalent amount of NaCl.

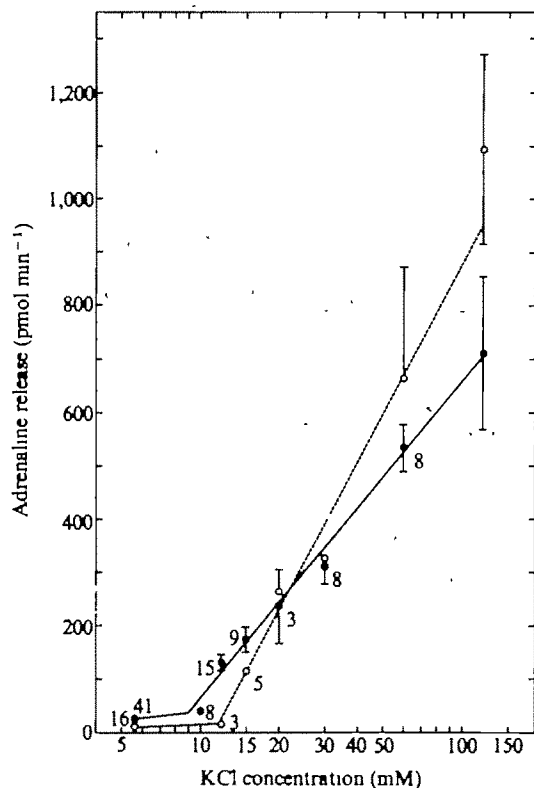


Fig. 2 Adrenaline secretion by adrenals perfused with different concentrations of KCl and the effect of Ca. The duration of the KCl challenge and each collection period was 5 min. Adrenaline secretion at each concentration of KCl (plotted on a log scale) is presented as the amount of secretion per min. The concentration of CaCl_2 was 2 (●) or 10 mM (○). Each point represents the mean result from four different adrenals unless a different n is indicated by the number adjacent to the point. Standard error bars are also given for each point except when the standard error is less than 10 pmol min^{-1} . Basal secretion is represented by the amount of release at 5.6 mM KCl. The lines were drawn by eye.

When the adrenal gland was perfused with normal saline the basal secretion varied between preparations from <15 to 68.8 pmol min^{-1} ($24 \pm 2 \text{ pmol min}^{-1}$, mean $\pm$ s.e.m., $n = 41$). As the saline was switched to a high KCl solution the rate of adrenaline secretion increased, then returned to the original level when the perfusion solution was returned to normal saline. However, the rate of adrenaline output tended to decrease as the KCl challenges were repeated (Fig. 1a). The rate of adrenaline secretion was greater with higher concentrations of KCl (Fig. 2). The lowest concentration to evoke adrenaline secretion above the basal level was ~10 mM KCl ($39 \pm 7 \text{ pmol min}^{-1}$, $n = 8$). The adrenaline release increased exponentially to 120 mM KCl ($610 \text{ pmol min}^{-1}$ for a 10-fold KCl concentration change).

CoCl_2 (5 mM), a blocker of voltage-dependent Ca channels¹², inhibited KCl-evoked release. The inhibiting effect of CoCl_2 on KCl-stimulated catecholamine secretion has been previously reported^{4,13,14} and suggests that voltage-dependent Ca channels mediate Ca entry.

An increase in the external Ca concentration raises the activation threshold for voltage-dependent Na and Ca channels¹⁵⁻¹⁸. If voltage-dependent channels are involved in KCl-induced catecholamine secretion, raising the external Ca should shift the lowest concentration of KCl required to evoke secretion. When CaCl_2 in the saline was increased from 2.0 mM to 10 mM, the lowest KCl concentration necessary to evoke adrenaline secretion shifted from ~10 mM to between 12 and 15 mM and the slope increased to 940 pmol min^{-1} for a 10-fold KCl change (Fig. 2, dotted line). The greater slope in 10 mM Ca saline can be explained by an increased Ca influx through individual Ca channels. The shift in the lowest KCl concen-

tration was significant, as the rate of secretion was lower at 12 and 15 mM KCl in the presence of high Ca.

The participation of Na action potentials in KCl-evoked secretion is demonstrated by the effect of TTX on secretion. When 6 μM TTX was added to saline, KCl-evoked release was depressed to various degrees depending on the concentration of KCl (Fig. 1b, c). The effect of TTX is present only at lower KCl concentrations.

High KCl concentrations may stimulate the cholinergic nerve terminals which innervate the chromaffin cells and thus cause subsequent release of ACh. To rule out such an indirect effect of KCl, atropine-sulphate (10 μM), an inhibitor of the muscarinic ACh receptor, was added to the saline of all experiments involving stimulation by KCl. This concentration of atropine completely blocked stimulation of adrenaline release by 2 μM ACh (see below) and ACh stimulation of action potential frequency⁷. Although the ACh receptors on the rat adrenal chromaffin cell are predominantly of the muscarinic type⁷, we have additionally confirmed that the above inhibitory effects of TTX on 15 mM KCl stimulation of adrenaline secretion still occur in the presence of high concentrations of both nicotinic and muscarinic antagonists (500 μM hexamethonium bromide + 50 μM atropine sulphate). Thus, the effect of TTX on KCl-evoked adrenaline release is due to a direct effect on the chromaffin cell.

The natural stimulus for adrenaline secretion is ACh, which is released from the splanchnic nerve terminals. We therefore tested the effects of TTX on ACh-stimulated secretion. The addition of ACh (2 μM) to saline increased adrenaline secretion ($120 \pm 13 \text{ pmol min}^{-1}$, $n = 14$). This ACh-stimulated secretion was blocked by 10 μM atropine sulphate. When TTX (6 μM) was added with ACh (2 μM), the rate of adrenaline secretion decreased to 58% of the control (Table 1).

A TTX-resistant, Co^{2+} -sensitive component of adrenaline secretion is present during stimulation by 2 μM ACh and at all levels of KCl stimulation. This stimulation is probably due to the direct activation of voltage-dependent Ca channels. The effect of TTX on KCl-evoked and ACh-stimulated adrenaline secretion is likely to be due to the specific effect of TTX on Na channels in the chromaffin cells. One possible explanation for the involvement of Na channels in secretion is that Ca ions may enter the cell through the Na channel¹⁹. As CoCl_2 (5 mM), an inhibitor of the Ca^{2+} but not the Na^{20} channel, completely blocked KCl (Fig. 1c) and ACh (Table 1) stimulated release, this seems unlikely. Alternatively, a relatively small depolarisation by KCl or ACh probably stimulates Na action potentials which serve as a trigger for further activation of voltage-dependent Ca channels.

The effect of TTX on secretion is larger at low KCl concentrations and is eventually lost as the KCl concentration is increased. One explanation is provided by the different inactivation characteristics of the Na and Ca channels. Voltage-dependent inactivation of Na channels generally occurs rapidly²¹ whereas Ca channels allow Ca ions to enter the cell for prolonged periods even at large levels of sustained depolarisation^{22,23}. Second, analysis of cultured adrenal chromaffin cells indicates that a low concentration of KCl (11.6 mM) produces an increase in action potential frequency which is sustained for the duration of perfusion with KCl (5 min). With a higher concentration of KCl (15.6 mM) the frequency of action potentials increases initially then gradually decreases to basal level⁷. Thus, the total number of action potentials during a 5-min application of KCl may be larger in 11.6 mM than in 15.6 mM KCl. Such an effect would diminish the relative contribution of the Na action potential as the KCl concentration is increased.

These results conflict with previous studies on the PC12 clonal cell line. PC12 cells have Ca^{2+} and Na^{24} (TTX-sensitive) action potentials. Although direct activation of voltage-dependent Ca channels by KCl or cholinergic agonists contribute to Ca influx⁵ and catecholamine secretion^{4,25}, TTX has no effect on either Ca influx or secretion. As cells *in situ* may be under the influence of

circulating hormones and other factors²⁶, their properties could be different from those of cell lines.

In view of the partial inhibition by TTX of adrenaline release from the adrenal medulla we propose that the Na action potential, by facilitating the activation of a voltage-dependent Ca channel, may have a regulatory role in catecholamine release. The facilitatory effect of the Na action potentials on secretion occurs at the level of the chromaffin cell membrane and is only detectable at low levels of depolarisation. Na action potentials seem to have a similar function in pancreatic β cells, where 37% of the first phase of insulin secretion evoked by glucose is blocked by TTX²⁷.

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Thyroid hormone induces competence for oestrogen-dependent vitellogenin synthesis in developing *Xenopus laevis* liver

OESTROGEN-DEPENDENT synthesis of vitellogenin, the precursor of the major yolk proteins, in the liver of egg-laying vertebrates is an attractive model for investigating regulatory mechanisms of gene expression. Induction of vitellogenin synthesis by oestrogen occurs not only in the liver of females but also in males which normally do not produce this protein, thus showing that responsiveness to oestrogen is not linked to sex (for review see ref. 1). However, Follet and Redshaw² reported that

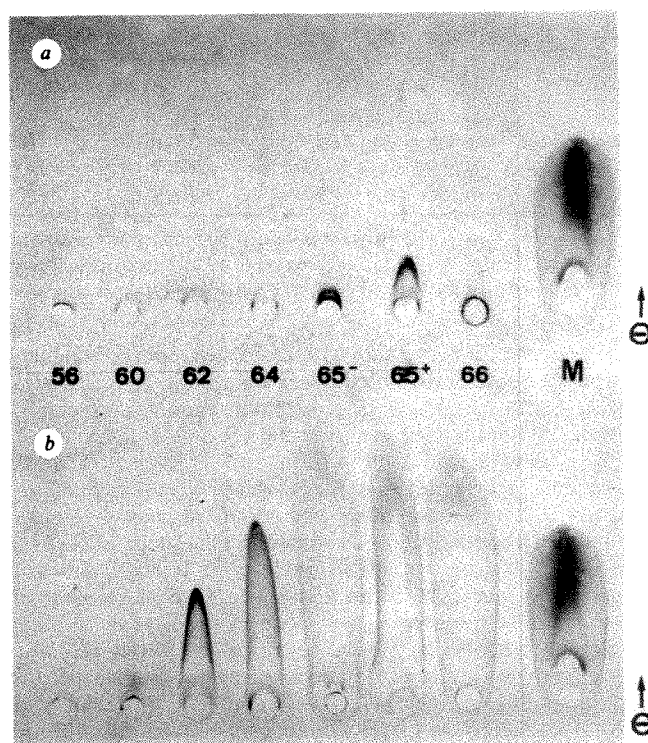


Fig. 1 Detection of vitellogenin in the blood plasma of normal *Xenopus laevis* larvae by rocket immunoelectrophoresis. Blood samples were collected from normal (a) and oestrogen-treated larvae (b) after anaesthesia with 1:1,000 MS 222 solution (Tricaine methanesulphonate; Sandoz) by heart puncture. For each stage, designated according to the scale of Nieuwkoop and Faber⁶, 3-5 μ l samples from four or five animals were pooled, centrifuged and the plasma used for immunological analysis. Rocket immunoelectrophoresis⁵ was performed in 55 $\times$ 205 $\times$ 1 mm 1% agarose gel (Sigma) in 0.02 M barbital buffer (pH 8.6) containing monospecific antivitellogenin antibody diluted 1:15. Vitellogenin antibody was raised in a rabbit by subcutaneous injection of 1 mg purified vitellogenin and Freund's complete adjuvant, followed by two boosters of 0.5 mg vitellogenin each 10 and 75 days respectively after the first injection. By adsorbing the rabbit antiserum with blood plasma from unstimulated male *Xenopus* it was possible to eliminate nonspecific components and to obtain a monospecific antivitellogenin antibody, as revealed by the Ouchterlony test (data not shown). To each well 10 μ l of blood plasma, diluted 1:1 with barbital buffer, were applied and electrophoresis performed at 4°C, beginning at 10 V for 30 min, and thereafter at 30 V for 17.5 h; after the initial 2 h 10 μ l of barbital buffer were added to each well. After electrophoresis the gels were washed successively in 0.9% NaCl and distilled water. After drying they were stained for 10 min in 0.5% Coomassie brilliant blue and destained in the solvent containing ethanol, distilled water and acetic acid. Arabic numerals give stages according to Nieuwkoop and Faber⁶, representing prometamorphic tadpoles (56/57), actual transformation of tadpoles (66-66) and postmetamorphic froglets (>66). M, vitellogenin marker. The broad and diffuse rockets obtained with the marker protein and blood plasma of later developmental stages are caused by excessive amounts of vitellogenin and do not reflect heterogeneity of the proteins.

liver of *Xenopus laevis* larvae is refractory to oestrogen, becoming responsive shortly after completion of metamorphosis. As amphibian metamorphosis is known to be obligatorily dependent on thyroid hormones (for review see ref. 3), the question arises as to whether acquisition of competence to respond to oestrogen by the *Xenopus* liver cells is part of the hormonally regulated metamorphic changes or merely reflects age-dependent maturation. Here we present evidence that during normal development of *Xenopus laevis* the liver cells become responsive to oestrogen during metamorphosis and before vitellogenin synthesis has been initiated by endogenous oestrogens. Moreover, we demonstrate that responsiveness to oestrogen fails to appear when metamorphosis is suppressed, but readily appears after induction of metamorphosis by thyroxine, suggesting that acquisition of competence to respond to oestrogen by the *Xenopus* liver cells is controlled by thyroid hormones.

Responsiveness to oestrogen was tested by the appearance of vitellogenin in the blood of tadpoles stimulated by oestrogens either continuously or for periods of 7 days, as described in

Table 1 First appearance of vitellogenin in the blood and of the response to estrogen by liver cells during normal development of *Xenopus laevis*

	Stage; age	Vitellogenin-positive samples as % of (total)						
		56/57; 39	60; 46	62; 49	64; 53	65 ⁻ ; 54	65 ⁺ ; 56	≥66; 58-92
Control		0% (6)	0% (6)	0% (10)	0% (8)	50% (8)	80% (10)	25% (20)
Oestradiol-3, 17 β		0% (3)	0% (3)	15% (6)	100% (5)	100% (3)	100% (8)	100% (15)
Ovocyclin		0% (3)	0% (3)	15% (6)	100% (4)	100% (4)	100% (8)	100% (15)

Tadpoles of *Xenopus laevis* were obtained by induced breeding and reared in standard conditions⁷. To test for responsiveness to oestrogen they were exposed either continuously from stage 49 onwards or for 7 days to oestradiol-3,17 β (Merck) or Ovocyclin (1 part oestradiol-3-benzoate + 3 parts oestradiol-3-benzoate-17-acetate) (Ciba-Geigy) each at a concentration of 5 μ g per 100 ml water. Age refers to days after hatching.

Table 1 legend. In agreement with an earlier report of Gallien⁴ continuous treatment resulted in sex reversal in genetic males, yielding 100% females as judged from a random sample of 22 autopsies. Without oestrogen a normal sex ratio of tadpoles was found as shown by the proportion of 32 females:31 males determined in a randomly selected sample. Vitellogenin was detected in the plasma of pooled blood samples by rocket immunoelectrophoresis⁵, as described in Fig. 1 legend.

In a first experiment, involving three different batches of tadpoles, we investigated the first appearance of vitellogenin and the earliest response to oestrogen by the liver in normally developing tadpoles. A typical result of the immunoelectrophoretic analysis is given in Fig. 1, which shows the specificity of the immunological reaction. The height of the precipitation peaks is an indication of the vitellogenin concentration in the blood plasma. The results of these analyses are summarised in Table 1. During normal development and in the absence of extraneous oestrogen vitellogenin is already detectable during

late metamorphosis, stage 65/66 (ref. 6). As there is a 50%:50% distribution of males and females in this group, pooling of blood samples results in a dilution of vitellogenin. Therefore the possibility that synthesis of this protein might be initiated at an even earlier stage is not excluded. In contrast to Follet and Redshaw² who considered the appearance of vitellogenin synthesis to be a post-metamorphic event, our results provide good evidence that this change in biosynthetic activity of the larval liver cells is part of the metamorphic transformation. We assume that this discrepancy might be caused by differences in sensitivity of the methods used for detection of vitellogenin. Figure 1a and Table 1 show reduced levels of vitellogenin and a decrease in the relative number of vitellogenin-positive blood samples from 80% to 25% in newly metamorphosed froglets, suggesting vitellogenin utilisation by the first generation of growing oocytes.

To determine the stage of onset of competence to respond to oestrogen in the larval liver, blood samples of oestrogen-stimulated tadpoles were analysed. Except for sex reversal in genetic males, oestrogen treatment had no visible morphogenetic effect nor did it alter the rate of development. A representative experiment is shown in Fig. 1b, demonstrating precocious appearance and higher levels of vitellogenin in the blood of oestrogen-treated tadpoles. The corresponding results, summarised in Table 1, clearly reveal that oestradiol-3,17 β and Ovocyclin applied in water were equally efficient in inducing vitellogenin synthesis as early as stage 62. No differences in timing and extent of response were noted between continuous and short-term treatment by oestrogen. Thus during normal development of *Xenopus laevis* the competence of larval liver cells to respond to oestrogen is apparently established during metamorphosis, before vitellogenin synthesis has been induced by endogenous oestrogen.

To establish whether the onset of responsiveness of the larval liver to oestrogen is dependent on thyroid hormone, the response to oestrogen was followed in thyrostatic tadpoles, developmentally arrested at stage 56, between 65 and 85 days after hatching and in a group of thyrostatic tadpoles, induced to metamorphosis by L-thyroxine at 78 days after hatching. Typical immunological tests are shown in Fig. 2 and a summary of the analyses is presented in Table 2.

Thyrostatic tadpoles in which metamorphosis had been suppressed failed to respond to effective levels of oestrogen, even at an age when normally developing animals had become fully responsive to this hormone. However, treatment of thyrostatic tadpoles with 10⁻⁸ M L-thyroxine at an age at which normally-developing sibling larvae (Table 1) had already completed metamorphosis not only induced morphological transformation but also elicited responsiveness to oestrogen. As in normal development, vitellogenin synthesis is detectable earlier in oestrogen-treated animals than in controls, suggesting that also during thyroxine-induced metamorphosis, competence to respond to oestrogen in liver cells is established before effective concentrations of oestrogen are secreted.

To our knowledge these results are the first demonstration that acquisition of competence for oestrogen-dependent synthesis of vitellogenin by the larval *Xenopus* liver is obligatorily dependent on thyroid hormone. However, it is not yet clear whether this maturative event reflects a direct or an indirect effect of the hormone. As it is generally assumed that

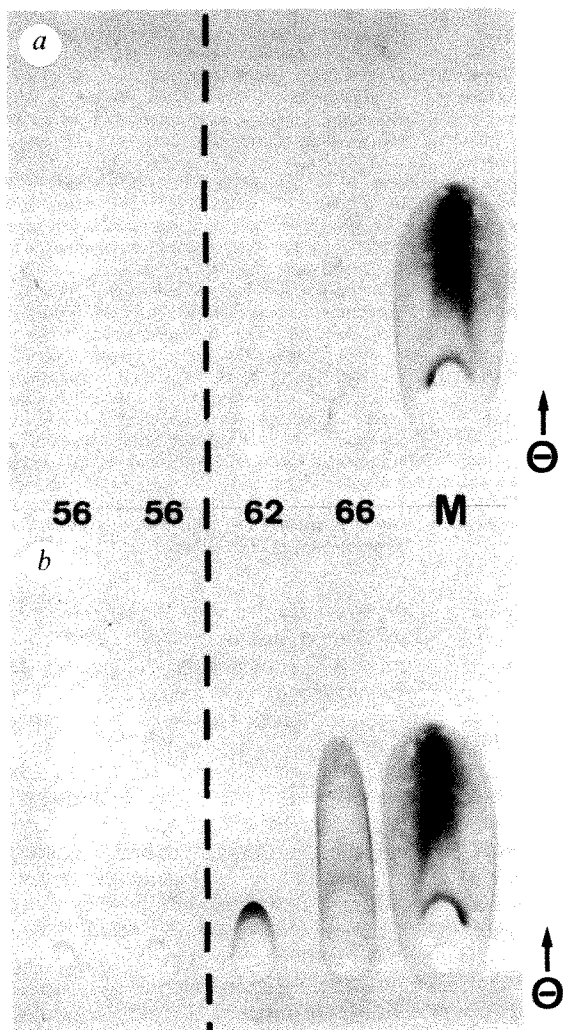


Fig. 2 Detection of vitellogenin in the blood plasma of thyrostatic tadpoles (left) and during thyroxine-induced metamorphosis (right). a, No oestrogen; b, with oestrogen. Further explanations as for Fig. 1.

Table 2 Absence of response to oestrogen in thyrostatic tadpoles and its induction by L-thyroxine

Thyrostatic-tadpoles				
	Vitellogenin-positive samples			
	Stage; age	56; 45-58	56; 64-68	56; 75-85
Control		0% (10)	0% (5)	0% (10)
Oestradiol-3, 17 β		0% (10)	0% (10)	0% (10)

Thyroxine-induced metamorphosis				
	Vitellogenin-positive samples as % of (total)			
	Stage; age	60; 85	62; 97	65/66; 105
Control		0% (5)	0% (5)	33% (18)
Oestradiol-3, 17 β		0% (5)	100% (5)	100% (10)

Thyrostatic animals were obtained by raising tadpoles from stage 52 onwards in a solution containing 0.4% thiourea which causes developmental arrest at stage 56 without inhibiting further growth⁸. To induce metamorphosis thyrostatic tadpoles were transferred to 10⁻⁸ M thyroxine (sodium salt, Fluka), which causes a slightly delayed but otherwise normal metamorphosis. Other details as for Table 1.

the effects of steroid hormones are mediated by cytoplasmic and nuclear binding proteins^{9,10}, the larval *Xenopus* liver because of its hormone-dependent maturation of oestrogen sensitivity, provides an ideal model for study of the molecular basis of competence and the primary events of the interaction between oestrogens and target cells.

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Note added in proof: After this paper had been submitted, a similar study was published by Knowland¹¹ indicating that liver explants of *Xenopus* larvae can be induced *in vitro* to synthesise vitellogenin as early as stage 57, but that the appearance of responsiveness is not influenced by oestrogen.

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Identification and change of collagen types in differentiating myoblasts and developing chick muscle

COLLAGEN is the major structural protein in the supporting and connective tissues. In addition to its mechanical properties it has been considered to be involved in developmental processes¹⁻³. Previous studies on muscle development have concentrated on the contractile components but more recently there has been a realisation of the significance of connective tissue in this process. For example, *in vitro* studies have demonstrated the importance of a collagen substrate in promoting myoblast

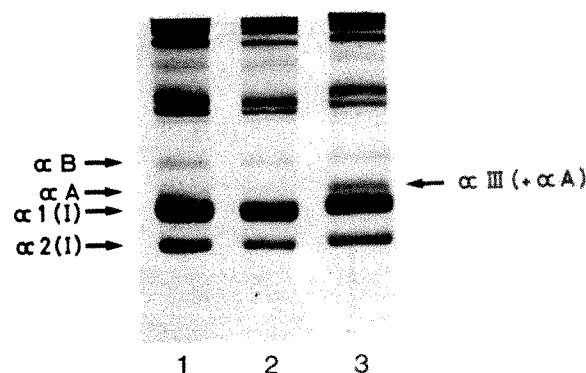


Fig. 1 SDS-polyacrylamide gel electrophoresis patterns showing the presence of type I, type III and type V collagen in 1 M NaCl precipitate from pepsin-solubilised intramuscular collagen from 1-week-old chick limb. Track 1 depicts type I (ratio $\alpha 1/\alpha 2 = 2.2$) and type V collagen; track 2 shows an increase in the $\alpha 1(I)$ band (ratio $\alpha 1/\alpha 2 = 2.8$) due to the presence of $\alpha(III)$ following incubation with dithiothreitol; track 3 shows resolution of $\alpha 1(I)$ and $\alpha(III)$ using a modified interrupted electrophoresis technique¹⁹.

differentiation⁴, and more recently it was shown that all types of collagen were equally effective⁵. Mayne *et al.*⁶ demonstrated that clones of chick muscle cells were capable of synthesising type I collagen. Ketley *et al.*⁵ confirmed the synthesis of type I collagen and also reported that the collagen in intact chick muscle was solely of this type. On the other hand, Duance *et al.*⁷ and Bailey and Sims⁸ investigated the distribution of the genetically different collagen types within bovine muscle connective tissue and showed by biochemical and immunofluorescent techniques a specific distribution of the isomeric forms of collagen in this tissue. Here we report our studies on the development of chick skeletal muscle which were designed to investigate some pertinent points: (1) the distribution of collagen types in chick muscle; (2) the role of collagen in the development of muscle as a functional tissue; (3) the cellular derivation of these different collagens. Chick muscle was chosen, first, because much is known about its development and differentiation *in vitro*, and second, because it is possible to manipulate its early development in the embryo *in vivo*^{9,10}. We have isolated three isomeric forms of collagen from chick muscle, and using immunofluorescent techniques demonstrate a specific distribution of these collagen types within this tissue. Finally we have used *in vitro* muscle cell culture to examine how this distribution is achieved by temporal transitions in synthesis. These results suggest that collagen has a subtle role in the development of muscle.

Seven-day-old chick leg muscle was homogenised and the myofibrillar protein was removed by extensive washing. The insoluble intramuscular collagen was digested with pepsin and the solubilised collagens separated by fractional salt precipitation as described previously^{7,8}. Identification of the collagen types was achieved by SDS-polyacrylamide gel electrophoresis (PAGE). Antibodies to the collagen types I and III were raised in New Zealand White rabbits following injection at fortnightly intervals in Freund's adjuvant. Type-specific antibodies were obtained using immunoabsorbent columns to remove nonspecific antibodies as described previously⁷. The sections cut from the leg muscles of chicks were stained with anti-type I or III chick collagen, followed by fluorescein-conjugated anti-rabbit IgG. Rabbit anti-human type V antibodies were found to cross react with chick type V and were therefore used in this study. (We use the term type V to refer to the new type of collagen described by Burgeson *et al.*¹¹ which consists of αA and αB chains, although the molecular compositions as $[\alpha B]_2\alpha A$ (ref. 12) or $[\alpha B]_3$ and $[\alpha A]_3$ (ref. 13) have not been resolved.)

Myoblasts obtained from the thigh muscles of 13-d chick embryos were cultured for 10 d at 37°C on collagen-coated (rat tail tendon collagen) Sterilin dishes. The synthesis of collagen was demonstrated by the incorporation of ³H-proline

Table 1 Detection of collagen types by haemagglutination titres

Day	0	1	2	3	4	5	6,7	8	9,10
Type I collagen									
Haem titre	0	0	0	0	1/2	1/2	1/4	1/8	1/8
Type III collagen									
Haem titre	0	0	0	1/2	1/2	1/4	1/16	1/6	1/16
Type V collagen									
Haem titre	0	1/32	1/32	1/64	1/32	1/64	1/512	1/128	1/128

Myoblast culture: 13-d embryo thigh muscle was dissociated using 0.25% trypsin in Earle's balanced salt solution (Flow) for 40 min at 37 °C. After centrifugation the pellet was resuspended in minimal essential medium supplemented with 10% horse serum, 5% chick embryo extract, 500 IU ml⁻¹ penicillin, 500 µg ml⁻¹ streptomycin (Flow), and 50 µg ml⁻¹ ascorbic acid (BDH). Fibroblast contamination was minimised by the 'selective settling' procedure of Yaffe¹⁷ using a type I collagen (rat-tail tendon) coated vessel and incubating at 37 °C for 40 min. Cells were plated at 9.0 × 10⁵ cells ml⁻¹ on 60-mm Nunclon dishes (3 ml) or 50-mm type I collagen (rat tail tendon) coated Sterilin dishes (2 ml) and incubated at 37 °C in a 5% CO₂/95% air atmosphere. The culture medium was replaced every 24 h. Haemagglutination: the culture medium was diluted 1:5 with phosphate-buffered saline and bound to tannic acid-treated sheep red blood cells by the method of Herbert¹⁸. Types I, III and V collagen coated cells were similarly prepared using 10 µg collagen per ml 3% cells. 50 µl of a 2% suspension of coated cells was incubated at 4 °C overnight with anti-type I, III and V collagen. Uncoated cells were used as controls.

into the medium and the determination of ³H-hydroxyproline on the amino acid analyser. The radioactive collagens isolated from the cultures following pepsin treatment were analysed for collagen types by SDS-PAGE. Analysis for the presence of the different types of collagen was also carried out by both the passive haemagglutination and haemagglutination-inhibition techniques.

In these studies we have established that chick muscle is similar to human and bovine muscle in containing collagen types I, III and V (Fig. 1). Immunofluorescence localisation studies have demonstrated the presence of type I mainly in epi- and perimysium, type III in the perimysium and type V in the endomysium (Fig. 2).

The initial studies on cultures of myoblasts *in vitro* using a ³H-proline label demonstrated the synthesis of collagen and that the majority of the collagen was in the medium, in agreement with studies by Lipton¹⁴. Analysis by SDS-PAGE demonstrated the presence of type I collagen but the ³H-activities of the type III and type V collagen bands on the gel were too low to confirm their presence. However, by using the more sensitive haemagglutination and haemagglutination-inhibition assays we were able to demonstrate the presence of all three types of collagen in the medium. The myoblasts fused to form myotubes on the fourth or fifth day in culture. By sequential analysis from day 1, we were able to demonstrate temporal transitions in the nature of the collagen in the medium (Table 1). Thus type I and type III collagen could not be detected until after myoblast fusion, whereas the type V collagen appeared during growth of the myoblasts before myotube formation. This suggests that the presence of type V is probably involved in the sequence of events leading to myoblast differentiation and could serve as a marker of differentiating myoblasts.

Similar temporal transitions of the different forms of collagen have also been observed during normal development *in vivo* at the electron microscope level. Thus, patchy areas of basement membrane appear round the myotubes when they first form in chick limbs after 6 d of incubation, but organised bundles of collagen fibres do not appear in the epi- or perimysium until after approximately 10 d of incubation¹⁰.

The cellular derivation of the different collagen types is not clear. *In vivo* the collagen could be derived from a variety of cells, and even *in vitro* the contribution from a small proportion of contaminating fibroblasts cannot be discounted. Lipton¹⁴ used several rigorous procedures to produce pure myogenic cultures and observed in the electron microscope a patchy basement membrane around myofibres only when fibroblasts

were present, although fibroblasts on their own were incapable of forming basement membrane. Therefore, either the myotubes can proliferate their own basement membrane sheath, or, as the studies of Lipton suggest, a fibroblast-myotube interaction is necessary. This interaction deserves closer examination in view of its possible role in the early development of muscle where a similar interaction between muscle and non-muscle tissue has a major role in the formation of the pattern of muscles^{10,15}. Although it has been suggested that pattern formation could be controlled by diffusible molecules¹⁶, collagen may also have a regulatory role.

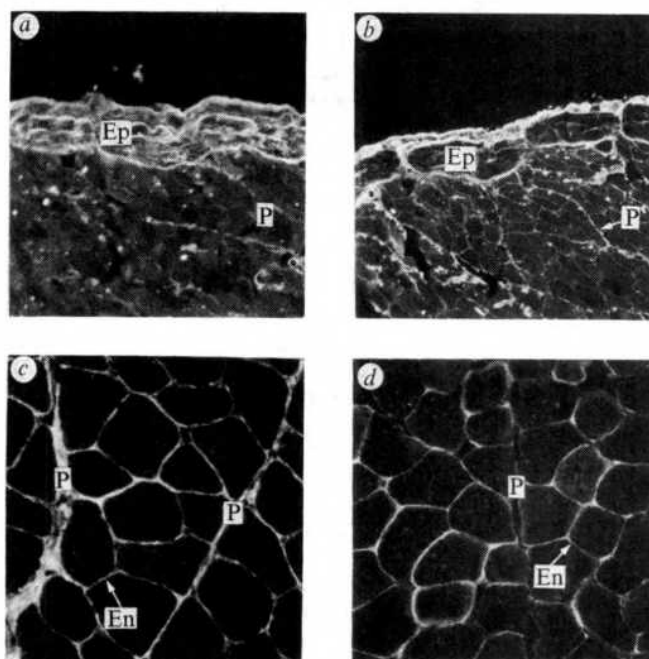


Fig. 2 Immunofluorescent staining of longitudinal sections of chick muscle with anti-type I (a) and anti-type III (b) (×48). Anti-type I stains the epimysium (Ep) and to a lesser extent the perimysium (P). Anti-type III also stains the epimysium but with a different distribution of the stain from anti-type I. Internal staining of the perimysium and slight staining of the endomysium (En) is also observed with the anti-type III. Transverse sections of the chick muscle stained with anti-type III (c) and anti-type V (d) (×96). Anti-type III stains predominantly the perimysium and to a lesser extent the endomysium (En). Anti-type V staining is confined to the endomysium.

We have demonstrated a spatial distribution of the isomorphic forms of collagen in chick muscle. Furthermore, we have shown temporal transitions of these isomorphic forms *in vivo* and *in vitro*. These studies strongly suggest that there is a role for collagen in muscle development. Therefore we feel that a more detailed investigation of muscle-collagen interactions should now be undertaken to elucidate the precise role of collagen in muscle development. We are now using specific collagen antibodies to study the spatial distribution and temporal synthesis of the collagen types in embryonic muscle *in vivo*.

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Calcium does not inhibit active sliding of microtubules from mussel gill cilia

CALCIUM ions are probably a general regulator of the assembly state and function of most cytoplasmic microtubules, the organelles upon which processes such as cell division, transport and secretion, as well as ciliary motility, may finally depend. Ciliary activity is the best model we have of microtubule function and the cilium is the only microtubule-based system allowing extensive systematic dissection at present. We are therefore interested in the mechanism by which increasing cytoplasmic Ca^{2+} causes changes in ciliary beat parameters, particularly an arrest response in the mussel (*Elliptio* or *Mytilus*) gill^{1,2}. It is well known that the beat in cilia is the result of a sliding interaction between doublet microtubules in the axoneme^{3,4}, as can be demonstrated directly when axonemes are treated briefly with trypsin⁵. On addition of ATP the trypsin-treated axonemes no longer reactivate to beat normally; instead, they telescope apart. We demonstrate here that, although all sliding of the ciliary microtubules stops during ciliary arrest *in vivo*, the sliding interaction seen after trypsin treatment is not directly inhibited by Ca^{2+} .

The response of intact mussel gill epithelium to increasing cytoplasmic Ca^{2+} can be reproduced experimentally with isolated gill cells and also with detergent-treated models and groups of ciliary axonemes ('eyelashes')^{6,7}. We are able to identify eyelashes that originate from three ciliated cell types: lateral (L), latero-frontal (LF) and frontal (F) cells. When ATP and Mg^{2+} are added, such cell models and axonemes reactivate at about 50% efficiency. We have demonstrated previously⁶ that at 10^{-2} M, Ca^{2+} inhibits 97% of all such motility, that 50% inhibition occurs at about 10^{-4} M Ca^{2+} , and that the L-cilia are differentially inhibited at 10^{-6} M Ca^{2+} .

In this study, cilia from *Elliptio* and related species were mechanically detached from the epithelium and from isolated cells. This suspension was filtered several times through cheese-cloth and the cilia were separated from the cells in a clinical centrifuge at 600g. Cilia were then pelleted in a Sorvall HB4 rotor at 10,000g and resuspended in 1% Triton X-100 in extraction solution (30 mM HEPES, 50 mM KCl, 2 mM EDTA, pH 7.0) for 10 min. They were sedimented again, resuspended in wash solution (30 mM HEPES, 50 mM KCl) and stored on ice for further use. Axonemal protein was determined by the method of Schaffner and Weissmann⁸ using bovine serum albumin as a standard. Trypsin digestion was carried out at room

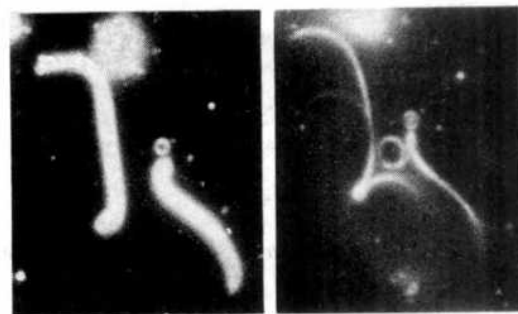


Fig. 1 Dark field images of trypsin-treated ciliary axonemes: left, before ATP addition; right, after disintegration in medium without added Ca^{2+} . $\times 2,900$.

temperature; the trypsin:protein ratio was always $\sim 1:500$. Digestion was stopped after 3 min with excess soybean trypsin inhibitor. Disintegration was followed in a drop of the solution containing trypsin-digested axonemes on a glass slide, covered with a cover slip. Reactivation solution (30 mM HEPES, 50 mM KCl, 4 mM MgCl_2 , 2 mM EGTA, 2 mM ATP, pH 7.0) was drawn in from the side by capillary action, while the axonemes were observed under dark field. Axonemes were photographed before and after ATP reached them. This procedure was carried out in the same way when 10^{-2} M CaCl_2 was substituted for EGTA in the reactivation solution.

In dark field, the axonemes resemble long bright sticks of variable length (Fig. 1). When reactivation solution was added to axonemes not treated with trypsin, they reactivated normally and beat occurred over a long period (10 min observed). Disintegration of axonemes was never found in the presence of ATP if they were treated only with detergent. However, trypsin-digested axonemes twitched briefly when the front of the ATP-containing reactivation solution reached them. Then, they either disintegrated into bundles of thinner sticks (banana peel) (Fig. 1) or elongated (Fig. 2). In many cases, the fragments curved as this occurred. Afterwards, no further movement could be observed. Elongation unequivocally represents active sliding of microtubules, but disintegration might also occur if ATP plasticises the axoneme to permit passive unrolling. Both effects can occur on one axonemal fragment. Figure 2 is the first direct dark field demonstration of sliding in a trypsin-treated metazoan somatic cell cilium.

By a differential count, we quantitated the percentage of trypsin-treated axonemes which underwent either type of movement in various conditions (Table 1). When reactivation solution without ATP was drawn through the preparation, there was almost no effect, whereas about 40% of the axonemes disintegrated or elongated in the presence of 2 mM ATP. It is difficult to separate accurately disintegration and elongation but we estimate that roughly 10% of the axonemes elongate; this varies greatly between preparations. Before addition of ATP, the longer axonemes in the field are approximately the length of L-cilia, that is 14 μm . However, it is not yet possible to determine precisely the origin of the isolated axonemes. To investigate the effect of Ca^{2+} on the sliding in these preparations, 10^{-2} M Ca^{2+} was used in the reactivation medium. The overall extent of disintegration and elongation in the preparation was

Table 1 Effect of calcium addition on amount of axonemal disintegration and sliding

	% (Disintegration plus sliding) $\pm$ s.d.		
	No ATP	ATP alone	ATP, Ca^{2+}
Mussel 1	5 $\pm$ 2.0	43 $\pm$ 10	39 $\pm$ 7.1
Mussel 2	3 $\pm$ 1.0	42 $\pm$ 8	38 $\pm$ 8.1
Average*	6 $\pm$ 2.0	41 $\pm$ 8	39 $\pm$ 8.0

2 mM Mg^{2+} , ATP alone or with 10 mM Ca^{2+} added to trypsin-treated axonemes.

* 20 Experiments.

unaffected by the presence of high Ca^{2+} (Table 1). No obvious change in the relative amounts or the velocities of either of these processes was detectable. Specifically, elongation of axonemes was not inhibited. Photographic and dark field observation of digested axonemes revealed that neither the form nor the final extent of elongation (Fig. 2) was affected. Similar results were obtained when Ca^{2+} replaced both Mg^{2+} and EGTA in the reactivation solution, but preliminary work indicates that the efficiency of sliding is somewhat reduced when minimal Mg^{2+} is present.

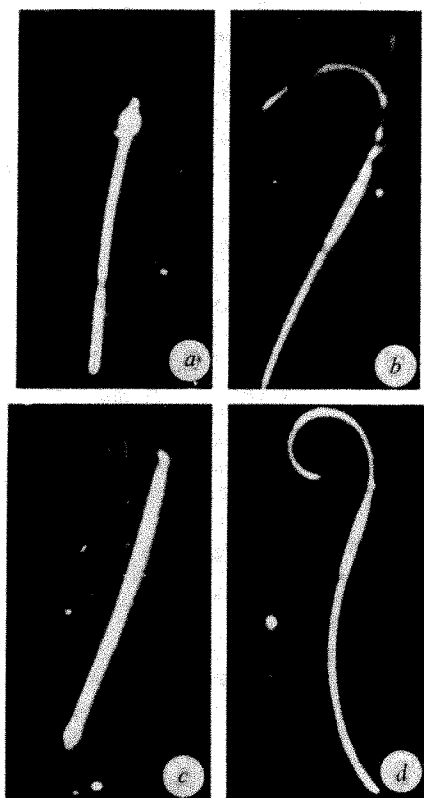


Fig. 2 Dark field images of trypsin-treated ciliary axonemes. Sliding in medium without Ca^{2+} before (a) and after (b) addition of ATP. Sliding in medium with $\sim 10^{-2}$ M Ca^{2+} : c, axonemal length ~ 13 μm before ATP addition; d, after addition of ATP. $\times 3,100$.

These results clarify the effect of Ca^{2+} on the ciliary axonemes. The major bonds that hold the trypsin-treated axoneme together in the absence of ATP are thought to be dynein-tubulin 'rigor' type interactions, described by Gibbons and Gibbons⁹. It is known that ATP will plasticise these bonds, and this may be responsible for the disintegration seen when ATP and Mg^{2+} are added to the trypsin-treated preparations⁴. Because the amount of disintegration seen is relatively unaffected by the presence of high Ca^{2+} , we conclude that Ca^{2+} does not significantly stabilise such bonding. More importantly, Ca^{2+} , at least in concentrations approaching 10^{-2} M, does not affect form or extent of the sliding elongation seen in the trypsin-treated axonemes in the presence of ATP and Mg^{2+} . Also, the overall number of axonemes which slide in the absence compared with the presence of Ca^{2+} does not seem to change. These results make it unlikely that a small subset of doublets or arms in each axoneme is specifically inhibited by Ca^{2+} , although we cannot yet exclude this possibility. In any event, a significant proportion of mussel gill axonemes which do elongate in the presence of Ca^{2+} are likely to be L-cilia axonemes which implies that sliding is little affected by raising the free Ca^{2+} about four orders of magnitude above the concentrations at which it arrests beat. We conclude that the trypsin-resistant dynein-tubulin interactions responsible for microtubule sliding are insensitive to changes in Ca^{2+} that physiologically account for ciliary arrest.

Summers and Gibbons⁵ originally demonstrated that in trypsin-treated sea urchin sperm axonemes, reactivation with Ca^{2+} and ATP would produce sliding, but less effectively than Mg^{2+} and ATP. In *Tetrahymena*, in experiments similar to ours, Sale¹⁰ has shown that trypsin-treated axonemes slide in the presence of 1 mM Ca^{2+} and Mg^{2+} . Zanetti *et al.* (personal communication) have shown that sliding also occurs in *Tetrahymena* when $\text{Ca}^{2+} < 10^{-3}$ M is substituted for Mg^{2+} . Therefore, although these experiments are not as stringent as ours, as in life Ca^{2+} does not cause ciliary arrest in these systems, they essentially confirm our results that in trypsin-treated axonemes high Ca^{2+} has little or no effect on sliding. This is perhaps not surprising as the absence of complete arrest as a physiological effect of increasing cytoplasmic Ca^{2+} implies continued microtubule sliding. For example, in *Paramecium*, increasing intracellular Ca^{2+} to greater than 10^{-6} M causes ciliary reversal with continued beat¹¹. In *Tetrahymena*, despite such possible ciliary reversal, the direction of active sliding is unaffected by increased Ca^{2+} (ref. 10).

We also conclude that the Ca^{2+} -sensitive system responsible for ciliary arrest originally present in isolated gill cilia is destroyed by brief treatment with trypsin. Concomitantly, there is a change in ciliary behaviour such that the normal constraints on microtubule sliding disappear and the axoneme telescopes apart rather than beating. It is possible that these two effects are correlated. Any or all of three morphological components of the axoneme might be implicated in either Ca^{2+} -sensitive or Ca^{2+} -insensitive aspects of the normal control of sliding: the dynein arms themselves; the spoke, central sheath complex; and the interdoublet (nexin) links. Spokes and interdoublet links are degraded by trypsin¹², although spokes at least do not completely disappear⁴. Despite the relative stability of the dynein arms, and their demonstrable function after trypsin digestion, some regulatory component of these structures might also be degraded. Detailed study of the biochemical effects of trypsin treatment has not been undertaken and might prove profitable. Of these components, the spoke-central sheath interaction has been most directly implicated in the control of sliding, both on morphological grounds by Warner and Satir¹³, and in mutant studies¹⁴. The detailed interaction of this system with Ca^{2+} is not understood. Also, the precise mechanisms by which sliding is converted into bending and otherwise restricted or synchronised in the intact axoneme in $\text{Ca}^{2+} < 10^{-7}$ M are still unknown. When these features are more accurately specified, it should be possible to localise the site(s) of interaction of Ca^{2+} and to detect how appropriate changes in axonemal behaviour are produced by increasing axonemal Ca^{2+} .

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Effect of modification by *N*-acetoxy-*N*-2-acetylaminofluorene on the level of DNA methylation

DNA METHYLATION in prokaryotes as well as in eukaryotes is a widely distributed phenomenon but its biological role is still unknown. In prokaryotes, DNA methylation has been related to restriction recognition mechanisms^{1,2}; no such correlation has yet been found in eukaryotes. Some investigators have suggested that cell differentiation and embryogenesis might be related to DNA methylation³⁻⁵. The recent results obtained by Christman *et al.*⁶ indicating that inhibition of DNA methylation may promote the differentiation and expression of globin genes in Friend erythroleukaemia cells, support this theory. In their observations, inhibition of DNA methylation was obtained after treatment of the cells with the methionine analogue L-ethionine which is also a carcinogen. We have used another approach where the change of levels of *in vitro* 5-methyl-³H-cytosine formation in DNA following DNA modification with a carcinogen is observed. We have chosen *N*-acetoxy-*N*-2-acetylaminofluorene (*N*-AcO-AAF) which reacts covalently *in vitro* and *in vivo* with nucleic acids and proteins⁷⁻⁹ and which, after administration to animals, induces tumours¹⁰.

The DNAs substituted mainly in position 8 of guanine moieties by this aromatic compound were used as substrates for DNA (cytosine-5) methyltransferase from rat brain tissue, an organ which exhibits high levels of methyltransferases during early postnatal development (ref. 11 and unpublished results), and has been compared to non-substituted DNA for the methyl acceptance capacity.

Table 1 The methylation of DNA modified by *N*-AcO-AAF or *N*-AcO-AAIF

Substrate	% Methylation*
Non-modified DNA	100.0
DNA-AAF; 4.0%	46.8 ± 5%
DNA-AAIF, 4.5%	37.0 ± 2.4%
DNA-AAIF, 5.8%	32.2 ± 2.2%

Unmodified or carcinogen-modified chicken erythrocyte DNAs (40 µg) were obtained as described in the legend to Fig. 2. DNA from chicken erythrocyte was modified by *N*-AcO-AAIF following the procedure previously described¹⁸. DNA substrate was incubated with DNA methyltransferase from rat brain in conditions as described in the legend to Fig. 1.

* Percentages of methylation were calculated using unmodified chicken erythrocyte DNA as control (100%). Values are from experiments made in triplicate.

The optimal conditions for the methylation of DNA by *S*-adenosyl-L-methyl ³H-methionine in the presence of rat brain DNA (cytosine-5) methyltransferase, using DNAs from chicken erythrocyte or *Micrococcus lysodeikticus* (Sigma), were first established. The substrate concentration which gave a maximal reaction velocity with 0.6 mg of brain enzyme protein was 40 µg of DNA. At this DNA concentration the maximal level of methylation was obtained after ~90 min of incubation (Fig. 1). 5-Methyl-³H-cytosine was the only methylated base found to be present in DNA reacted with the brain enzyme (data not shown).

DNAs from chicken erythrocyte or *Micrococcus lysodeikticus* were then reacted with *N*-AcO-AAF resulting in the production of carcinogen covalently bound to the guanine residue of DNA¹². By using the appropriate conditions DNA could be modified *in vitro* by the carcinogen to a varying extent. Calculations to establish the percentage of modified bases have been described elsewhere^{13,14}.

Figure 2 shows the results obtained if carcinogen-modified DNAs (DNA-AAF) were methylated in the presence of rat

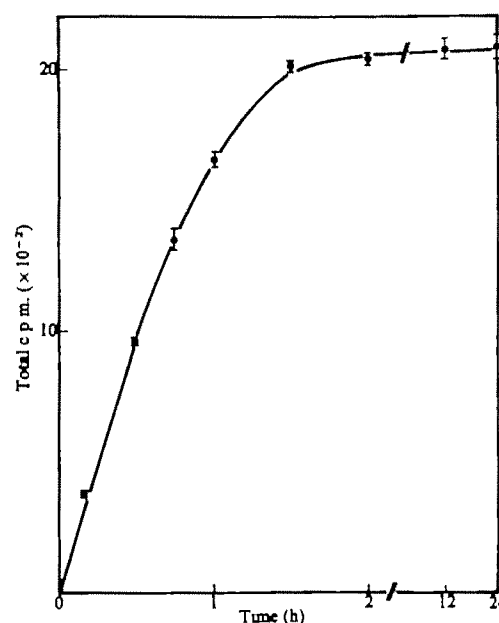


Fig. 1 Methylation of chicken erythrocyte DNA by rat brain DNA (cytosine-5) methyltransferase. The standard assay mixture contained in a final vol. of 0.2 ml: 40 µg DNA, 0.35 µg pancreatic RNase, 0.4–0.6 mg enzyme protein, 2 µCi *S*-adenosyl-L-methyl-³H-methionine, 50 mM Tris-HCl pH 7.8 and 0.5 mM dithiothreitol. After incubation at 37°C for various times 0.6 ml H₂O and 1 ml of carrier calf thymus DNA solution (1 mg ml⁻¹) were added to the incubation mixture. This was then deproteinised with SDS sodium perchlorate and extracted with a mixture of chloroform-isooctyl alcohol as described elsewhere²⁵. The final DNA pellet was dissolved in 0.1 M NaOH (0.2 ml) by incubation at 90°C for 10 min. The clear solution was transferred to a scintillation vial and the radioactivity measured after complete mixing with Unisolve 1, scintillator (Koch-Light). Blank values were subtracted in each case. Averages with s.d. are from two experiments made in duplicate. Brain enzyme was prepared as follows: Sprague-Dawley rats (7-day-old) were decapitated and the brain cortices quickly removed and homogenised with buffer solution containing 2.4 M sucrose and 0.001 M MgCl₂ in a glass-Teflon Potter-Elvehjem homogeniser to a final volume of 10 times the tissue weight. The remaining procedure was similar to that described by Pogo *et al.*²⁶. All operations were carried out at 4°C unless otherwise stated. Protein concentration was determined according to Lowry *et al.*²⁷ using bovine serum albumin as standard. Chicken erythrocyte DNA was obtained using the procedure described elsewhere²⁸.

brain DNA methyltransferase. An inverse relationship between levels of DNA methylation and the percentage of modified bases residues in the DNA structure was observed. The inhibition of DNA methylation cannot be attributed to the presence of free carcinogen in the reaction mixture as any unreacted carcinogen was removed from the DNA solution.

Similar results obtained using carcinogen-modified DNAs from two sources having a different GC content showed that inhibition of DNA methylation is independent of the nature of DNA used.

We also modified DNA with *N*-AcO-AAIF (DNA-AAIF) which is the 7-iodo derivative of *N*-AcO-AAF. Fuchs *et al.*¹⁵⁻¹⁸ reported that in DNA-AAIF the iodo-fluorene ring lies along the phosphate-sugar backbone of the double helix (outside binding model) whereas in DNA-AAF the fluorene ring is inserted between the adjacent base pairs (insertion-denaturation model). Therefore, we tested DNA-AAIF to see whether the different positioning of the fluorene residue could diversely affect the methyl accepting properties of nucleic acid. As shown in Table I, the iodo derivative substituted DNA behaved in a way similar to DNA-AAF, showing that different positioning of the fluorene ring within the DNA molecule does not change its inhibitory properties.

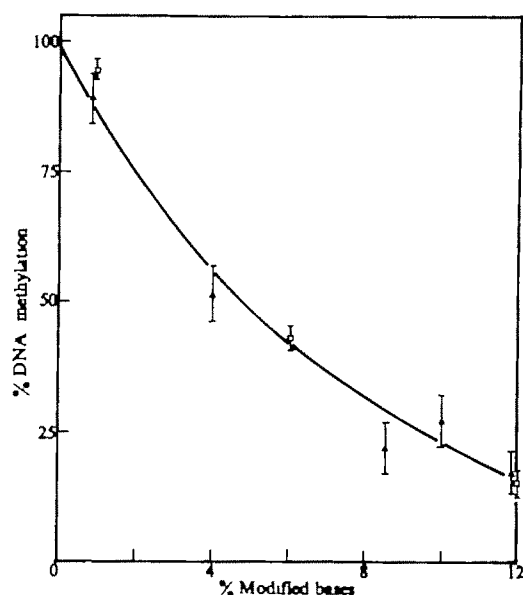


Fig. 2 The methylation of *in vitro* modified DNA-AAF: *N*-AcO-AAF was synthesised by a modification of the original procedure²⁹ consisting of direct hydrogenation and acetylation of 2-nitrofluorene¹⁸. The purity of the *N*-AcO-AAF obtained was checked by thin-layer chromatography and the final product was stored at -20°C as alcoholic solution. DNA bound to the carcinogen was prepared as described elsewhere¹⁴. The *in vitro* methylation reaction of carcinogen modified chicken erythrocyte or *Micrococcus lysodeikticus* DNAs were done as described in the legends to Fig. 1. The results are expressed as percentage relative to control (100%) determined with non-modified DNA. Blank values were subtracted in each case. Δ , Chicken erythrocyte DNA; $\square$, *Micrococcus lysodeikticus* DNA. Averages with s.d. are from four experiments made in triplicate except for the experiments with 11, 3 and 13% modified bases, which are in duplicate.

Arylamidation of the C8 of guanine by some aromatic amines induces a rotation of the bases involved in the double helix, altering its normal conformation and carrying a local destabilisation of the DNA^{13,19}. As shown previously, this local denaturation may change some biological properties of nucleic acids by inducing cell transformation¹⁰, premature termination of transcription at (or near) the site of DNA modification²⁰ and more recently *in vitro* inhibition of DNA synthesis²¹. Our results show that modification of guanine residues in position 8 impairs another biological property of the double helix.

The carcinogen mediated effect on DNA methylation can be explained as follows: binding of methyltransferase near or at the position of methylation in DNA may be hampered by the presence of carcinogen-modified guanines paired to cytosine residues susceptible to methylation. Consequently, DNA methylation is now restricted to cytosine residues paired to guanines which are recognised by the enzyme but which are not modified. Alternatively, it can be proposed that binding of the enzyme to DNA is not affected by the presence of carcinogen, but, subsequent walking of the enzyme on the DNA helix is interrupted by the carcinogen. The last alternative agrees with the proposed mechanism of action described for DNA (cytosine-5) methyltransferase²²⁻²⁴ as well as with the model explaining the premature termination of DNA transcription in DNA-AAF²⁰.

Further work is required to establish whether DNA under-methylation caused by aromatic amides *in vitro* can also be obtained *in vivo*, and to demonstrate the effect of DNA hypomethylation on cell differentiation and/or carcinogenesis.

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Corrigenda

In the letter 'Speciation of dissolved iodine in estuarine waters' by J. D. Smith and E. C. V. Butler, *Nature* **277**, 468-469, the values for iodine in the Yarra River estuary in the text and Fig. 1 should all be multiplied by 0.73.

In the letter 'Opposing effects of tumour promoters on erythroid differentiation' by R. M. Miao *et al.*, *Nature* **274**, 271 (1978), all concentrations of 12-*O*-tetradecanoyl-13-acetate (TPA) should be 1,000-fold lower than stated; for example, 10^{-7} M should read 10^{-10} M.

Errata

In the letter 'Immunological properties of the surface of parasitic nematodes' by C. D. Mackenzie, P. M. Preston and B. M. Ogilvie, *Nature* **276**, 826-828 (1978), the following acknowledgement was omitted: 'P. M. Preston was financed by a grant from the World Bank/UNDP/WHO Special Programme for Research and Training in Tropical Diseases.'

In the letter 'Measurement of carbon tetrafluoride in the atmosphere' by R. A. Rasmussen *et al.* *Nature* **277**, 549-551, for % read p.p.t. (parts per 10^{12}), and in paragraph 8, line 8, for p.p.b. read p.p.t.

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By the time the ink has dried

David W. Hughes

WHAT use is a textbook? The answer to this question depends to an extent on which side of the desk you are sitting. Cynically we could say that the main aim of the student is simply to pass the final examination. He judges the worth of the recommended textbook by its ability to help him achieve this aim with the minimum of effort. The book should contain the course material to the depth required and hopefully little other additional material and too much rigour leading to confusion. There should be a reasonable selection of examination-type questions at the end of each chapter plus answers and wherever possible worked solutions.

The lecturer approaches the book from a different standpoint. He passed all the exams a long time ago. For him the book should fill in the background to the subject, giving it roots so to speak. It should be a guide to his lectures so that the student can turn to it to clarify the difficult or unclear bits. Also it should be a stepping stone for the mind, leading the student on to more detailed studies and to the boundaries of the subject, the regions which are uncertain, the outstanding problems, the next steps in the pursuit of knowledge and understanding.

Starting near the intellectual top end of the market we have the second edition of *The New Cosmos* by Albrecht Unsöld (Springer: New York and Berlin; paperback DM37, \$18.50). This is an English translation of the 1974 German work. The book has two main audiences, the first being the final-year astronomy and astrophysics student who is looking for an overview of his subject. The fact that the book only has 451 pages is an enormous advantage here as it saves the student, especially near exam time, from the task of wading through the larger monographs. It is also an excellent kick-off point for the mathematics or physics graduate who has turned to astronomy, astrophysics, space science or cosmology as a research field. It is a comprehensive but concise introduction to these topics. The book has an abundance of clear figures and takes pains to stress the delicate balance between the advances in observational work and in theoretical studies. It is geared to the numerate student and does not shirk the responsibility of tackling properly such subjects as radiation theory, stellar interiors, galactic rotation, degenerate matter and galactic and stellar evolu-

tion. Many astronomers and astrophysicists today grew up in their subjects clutching the first edition. This new edition is for the next generation and it can be classified simply as essential reading, a Godsend for student, lecturer and research worker alike.

Moving down the scale of complexity we come to *University Astronomy*, by J. M. Pasachoff and M. L. Kutner (W. B. Saunders: Philadelphia and London; £13.50), a book which includes basic algebraic and trigonometric equations but excludes infinitesimal calculus. The authors are aiming at the science student during his first year at university and they are well on target. The book describes our modern knowledge of astronomy, fitting the latest discoveries alongside the established results. It is refreshing to notice how the authors discuss the whole spectral range of observations as they go along and don't for example relegate all the radio work to a separate chapter. It was a book full of surprises, the large majority of them pleasant ones. It was heartening to come across time and again examples of the authors' desire to stretch the student by introducing him to the problems of experimentation and theorising. The book contained many thought-provoking sections, a few examples taken randomly includes a discussion of the errors of observation and standard deviation, Occam's Razor, the futility of studying UFOs and a Martian's investigation of life on Earth. The authors don't turn their backs on the basic physics which underlies many of the facets of astronomy but explain physical concepts in a clear and concise way. Each chapter is rounded off by a summary and outline section, a collection of multiple choice review questions, discussion topics and suggestions for additional reading. The authors are to be congratulated on producing such an excellent résumé of the subject and one that, considering the self-imposed mathematical limitations, never deviates from stressing the underlying scientific principles of astronomy.

J. M. Pasachoff and M. L. Kutner have very thoughtfully produced a *Teacher's Guide to University Astronomy* (W. B. Saunders: Philadelphia and London). This contains the answers to all the questions posed in their main textbook (considering my initial comments a more appropriate title might have been *Student's Guide*). It also has a set of seven

laboratory exercises together with the addresses of the suppliers of the relevant planetary, solar and sky photographs some sample exam papers, possible syllab and lists of audiovisual aids and stockists. It is obviously geared for the American market, but don't let this put off the non-American readers.

Both *University Astronomy* and the next book under review *Astronomy Now* (by J. M. Pasachoff; W. B. Saunders: Philadelphia and London; \$11.95) come from the same stable. *University Astronomy* contains more about the Earth and relativity, and does use basic mathematical equations. *Astronomy Now* is a shorter non-mathematical version ideal for the innumerate and the mathematicophobes. It is difficult to advise you which one of these books to choose. I know that a student who does not need the algebra would automatically pick the book without it and thus avoid the confusion. But I like mathematics and physics; they add spice to astronomy and I would advise the students to buy *University Astronomy* in the hope that some of the joys of maths and physics rubbed off.

This review continues by considering four, more descriptive astronomy textbooks which have been tailored to the needs of the American university humanities and social science students who have usually to take two one-year courses (each of about 72 lectures and lasting two semesters) in natural science subjects. I need not go into details as to why astronomy should be and is in many cases an obvious choice. Many books are produced at the required level and I have often felt apprehensive about this market being flooded. But this worry is groundless because the market is enormous. R. H. Garstang (*J. Br. Astr. Assoc.* **88** 407; 1978) gives two examples. Of the 20,000 students at the University of Colorado, 1,050 are taking the astronomy course. If we select another university more or less at random we find that 2,000 students at the University of Texas are also doing elementary astronomy. It's little wonder that a fair armful of these books are published each year considering that the publishers break even point is in the low thousands.

I am confronted with *Discovering Astronomy*, by Robert D. Chapmar (Freeman: San Francisco; hardback £12.60, paperback £7), *The Restless*

Universe: An Introduction to Astronomy, by Harry L. Shipman (Houghton Mifflin: Boston and London) and *Cosmic Evolution: An Introduction to Astronomy*, by G. B. Field, G. L. Verschuur and C. Ponnampertuma (Houghton Mifflin: Boston and London). They are all the same size; one is red, the other blue, the third brown; they contain the regulation collection of colour plates (where would one be without NASA and the Hale Observatory?). They are beautifully illustrated, well indexed, glossaried and spattered with review questions, thought questions, chapter summaries, and the like. They each have their 'special way of approaching the subject'. Chapman uses the historical angle starting from Earth and the view Earth dwellers have of the sky and moving out to the Moon, Sun, planets, stars, Galaxy and Universe. Shipman states that he differs from similar texts by stressing contemporary astronomy—"the events that readers have probably heard about from the media" and by indulging in a detailed inter-comparison of the 'geological' processes that have taken place on different planets. Field, Verschuur and Ponnampertuma concentrate on the 'evolution of the Universe from the primordial big bang, through the origin of galaxies, stars and planets to the emergence of intelligent life on Earth and the search for this life elsewhere. Even with these 'different approaches' in mind I was continually struck by the overall similarities. The differences are at best marginal and in most cases simply seem to be a difference in the ordering, much more than in content. In my experience it is a very rare lecture course that starts at the beginning of a textbook and methodically works its way through chapter by chapter, so the ordering is fairly immaterial. So I have a problem, which one should I recommend? I shudder to suggest that you should resort to 'eeny, meeny, miney, mo' but this is one way out. The other is simply to buy the cheapest.

My final book is *Exploration of the Solar System* by William J. Kaufmann III (Macmillan: New York; Collier Macmillan: London; £0.00). A book mainly about planets with a little on stars and a sister to Kaufmann's 1977 book *Astronomy: The Structure of the Universe*, which was mainly about stars with a little on planets. Kaufmann's books are aimed at the same non-mathematical audience as the three mentioned above. In *Exploration of the Solar System* one feels that Kaufmann has room to spread himself. He is not restricted by space, he studies the problems, the spacecraft, the explorations, the data, the planetary features and models in detail and with thoroughness.

In general I was extremely impressed by the high standards of these books. Writing an astronomical textbook is no easy task because topic after topic is in a state of rapid development. To quote

R. D. Chapman "it is inevitable that some parts of any book will be out of date by the time the ink has dried". It must be frustrating to write a line such as "ratio of the mass of Pluto to that of the Earth = 0.1(?)" months before the mass was accurately measured due to the discovery

and observation of a satellite. But still the job needs to be done and it is refreshing to see how well it is done time after time. □

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Mathematical cosmology

Mathematical Cosmology. By P. T. Landsberg and D. A. Evans. Pp. 306. (Clarendon/Oxford University Press: Oxford, 1978.) £6.95.

ALTHOUGH the number of students taking formal courses in cosmology is tiny, most physical scientists find room in their activities to read up on it from time to time. The stumbling block to penetrating the technical literature is usually the general theory of relativity, with its mind bending concepts of curved spacetime, event horizons and singularities, not to mention punishing (as well as non-linear) mathematics. The solution to this barrier is, according to P. T. Landsberg and D. A. Evans in their *Mathematical Cosmology*, a "stage II" book, defined in their introduction to be one which goes beyond the broad qualitative outlines, but uses only simple mathematical arguments. And this means no general relativity. Although other books, such as Michael Berry's *Principles of Cosmology and Gravitation* (Cambridge University Press, Cambridge, 1976), also attempt this, Professors Landsberg and Evans give probably the first comprehensive and systematic non-relativistic treatment of cosmology.

Students familiar with Bondi's little book *Cosmology* will know how fairly elementary Newtonian ideas can lead effortlessly to the main features of relativistic cosmological models. The authors of *Mathematical Cosmology* follow the same path, but in a much more elaborate and detailed

fashion. All the basic conceptual structure is kept simple, and individual chapters can be read in isolation without difficulty. The standard Friedmann models are thoroughly discussed, and even the steady state theory gets a mention. Each chapter is terminated with a set of questions, the answers to which are provided.

Eventually, perhaps inevitably, general relativity refuses to be excluded. It starts to intrude during the discussion of light propagation, and the appearance of distant astronomical objects. However, in this limited context, without the field equations of the dynamics of the space-time itself, there is nothing terribly formidable about it, and students should be able to follow the lucid discussion easily. When horizons are finally discussed, the approach is physical rather than geometrical, and indeed, this is true of the book as a whole.

For scientists and students with a serious interest in cosmology who do not want to invest a lot of time learning differential geometry and topology, *Mathematical Cosmology* gives an excellent, clearly written and coherent account of the standard theory which is very economical on intellectual investment. It is also economical on the pocket, because at £6.95, it is rather cheap for a 306-page textbook. For students taking cosmology the hard way, it is still worthwhile background reading, and of course most of the results are identical to those of the relativistic treatment.

Paul Davies

Paul Davies is Senior Lecturer in the Department of Mathematics at King's College, University of London, UK.

Vibrations and waves

Vibrations and Waves in Physics By I. G. Main. Pp. 336. (Cambridge University Press: Cambridge and London, 1978.) Hardback £17.50; paperback £4.95.

THIS is an excellent book which can be thoroughly recommended either for use as a text for an undergraduate course introducing the student to many of the widely-pervading notions connected with vibrations and waves, or indeed for anyone who feels the need

to broaden his knowledge in this sphere. The material covered starts with free vibrations and continues through forced vibrations, anharmonic and coupled vibrations, to non-dispersive waves. After a short chapter on Fourier theory the author looks at dispersion, water waves, electromagnetic waves, and de Broglie waves, and finally covers basic ideas in the reflection, refraction, and diffraction of waves.

The book is well and carefully constructed. An example of this is the useful flow diagram near the front, showing clearly which are the parts containing the coherent development

of the basic theory and which are what the author, in his preface, terms "excursions into physics". These "excursions" demonstrate the way in which many useful results and an increased understanding of many physical phenomena may be obtained by simple order-of-magnitude calculations.

One of the best features of the book is the choice of problems at the end of each chapter. These span a wide variety of topics and put the material of the preceding chapter firmly in a physical context. In the penultimate chapter, for example, the problems include such apparently disparate topics as reflection of electromagnetic waves from a plasma, reflection and refraction of shallow water waves at a change in depth, evaluation of lattice heat capacity at low temperatures, and calculation of the Fermi energy for conduction electrons in a metal. Almost every chapter includes at least one ingenious and non-run-of-the-mill problem illustrating the application of previously presented principles in an

everyday situation. Hints for solution are given for some of the problems, and answers are provided to all numerical questions.

The similarity of both the chapter headings and the length of the book invite comparison with Pain's *The Physics of Vibration and Waves* (Wiley: London, 1976). In fact the books are very different in character, Main spending much more time on explanation, whereas Pain covers a wider variety of material with, therefore, much less explanation. The difference between the books is well illustrated by the fact that the summaries that occur at the end of each chapter in Pain are primarily lists of formulae, whereas the summaries at the end of each section in Main are primarily words.

S. J. Flockton

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Classical mechanics

Mechanics and Motion. By L. Mackinnon. Pp. 161. (Clarendon/Oxford University Press: Oxford 1978.) Hardback £6; paperback £3.25.

A SOUND knowledge of classical mechanics is a valuable asset to anyone studying physics, and this book attempts to deal with those branches of the subject which are most relevant to the modern physics in a university degree course. The book is based on a first-year lecture course given by the author at the University of Essex. The basic concepts of mechanics are discussed in the first chapter, after which there are chapters on rotary motion, vibratory motion, wave motion and central force motion. Finally, there is a chapter entitled "Analytical Classical Mechanics" in which the concepts of Lagrangian and Hamiltonian mechanics are introduced.

The amount of material seems to have been limited to what can reasonably be included in a lecture course of approximately 30 one-hour lectures, and within this constraint the choice of material is reasonably satisfactory. However, the level of presentation is sometimes rather superficial. For example, there is no precise definition of the centre of mass of a system of particles, and there is only the briefest mention, and then only for the particular case of the centrifugal force, that the inertial reactions on a body act through the centre of mass. The

concept of reduced mass is not discussed at all, beyond a brief statement in the conclusion to the chapter on central force motion that the source of the force is free to move. Also, the theorems of parallel and perpendicular axes for moments of inertia are quoted without proof, the proof of the parallel axes theorem being left as an exercise for the reader. As a further example in a more advanced topic, the Coriolis force is introduced by considering a very specific problem; and the general form of the force is then stated without proof, as a rigorous treatment of the equations of motion in a rotating frame of reference is not given. Even if such a detailed treatment is considered to be unsuitable in a lecture course, it should be included in a book based on that course. A similar objection can be levelled at the discussion of Fourier analysis in the chapter on vibratory motion.

Apart from these deficiencies the book is quite well written in a style which is rather less formal than that of most books on classical mechanics. Unfortunately the standard of production is lower than one would have expected from the Oxford University Press. The book is printed directly from the type-written manuscript and some of the equations are rather badly composed. This technique may reduce the cost of production slightly, but the result is visually far less attractive than a type-set book.

J. W. Humberston

J. W. Humberston is a Lecturer in Physics at University College, London, UK.

Fluid mechanics experiments

Fluid Mechanics: A Laboratory Course. By M. A. Plint and L. Bosworth. Pp. 186. (Charles Griffin: London and High Wycombe, UK, 1978.) Paperback £5.95.

THIS book describes, in a clear and interesting manner, a series of fluid mechanics experiments using low and high speed air jets, low speed and supersonic wind tunnels and a pipe flow apparatus. The low speed experiments in air include the pressure distribution around a cylinder, the lift on an aerofoil, boundary layer traverses on a flat plate and jet and pipe flow traverses. The compressible flow experiments measure mass flow and pressure distributions in convergent-divergent nozzles and look at the supersonic flow past a double wedge aerofoil. Lubrication properties of an oil wedge using a Michell bearing rig, and the properties of an air bearing are also treated.

In cases where theory depends primarily on the physics, relevant equations are derived; otherwise the necessary equations are quoted. The authors present their own results in detail and explain the reasons for any differences that occur between theory and experiment. They argue quite rightly that details of this type can help students to look critically at their own experimental results even though the apparatus may be different. The experiments are typical of those in undergraduate courses in aeronautical, mechanical and civil engineering and in some physics courses. However, they represent only a selection of the vast number of valuable undergraduate experiments in fluid mechanics and it is unlikely that the experiments at a particular university or polytechnic will coincide exactly with the authors' choice. Even so the book would be helpful as additional reading for undergraduate engineers. The book would be most useful to those who, on entering the teaching profession, require to establish a series of fluid mechanics experiments.

The book is well presented with good diagrams, and it is not in competition with other books. For completeness it could have included more on the estimation of experimental errors but this can be found in other textbooks on experimental techniques.

T. N. Stevenson

T. N. Stevenson is Senior Lecturer in the Department of the Mechanics of Fluids at the University of Manchester, UK.

A detached view of the Earth

Remote Sensing: Principles and Interpretations. By F. F. Sabins, Jr. Pp. 426. (Freeman: San Francisco and Reading, 1978.) \$25; £14.50.

To the casual or unwary would-be customer the title of this large new volume suggests a comprehensive treatment of the explosively expanding modern science, remote sensing of the environment. This is an impression which would not be properly rectified by a reading of the Preface, in which the author confesses that "... because I am a geologist by training and early experience, there is a tendency (in this book) to emphasise the geologic aspects of image interpretation". Nor would it be clear from the nearby statement that "This book is designed for a one-semester or one-quarter course for upper-class or graduate students with no previous training in remote sensing" that the treatment is, in fact, very heavily biased towards the needs of the student of geology, as distinct from his colleagues in environmental science, geography, and allied fields.

To some potential purchasers, this could prove to be a very valuable volume, selecting and synthesising smoothly from the physical bases on which environmental remote sensing rests, by way of the wide variety of means by which different statements of the targets of remote sensors are acquired and analysed, to some of the chief spheres of remote sensing data applications. However, if one's interests are not predominantly concerned with the ways analyses of spacecraft (especially Landsat) data may supplement the longer-established practices of photogeology using images from aircraft and balloons, purchasing this book could be a costly mistake.

My own view is that the confessed "tendency to emphasise ... geologic aspects" is an exceptional understatement, given the breadth of cover suggested by the title. Certainly, the author does not spend all his time discussing geological phenomena, for there are useful sections on water and ice, but other classes of phenomena with which a more broadly-based student would be significantly concerned—including other surface features such as snow, land use and crops, natural vegetation, and settlements, and atmospheric phenomena such as energy budgets, cloud organisations and winds—are treated very cursorily indeed. One notable example concerns applications of remote sensing

techniques in land-use studies: this vitally-important field, full of economic and humanitarian potential, is credited with less than three pages of text. The atmosphere is ignored to such an extent that thermal infrared imagery from meteorological satellites is said to be useful for many applications—but none of those mentioned are atmospheric. Indeed, it is stated that "Clouds and surface winds ... produce confusing patterns" in such images, and that such factors must be considered (as complicating factors) by the

(surface) interpreter. Such a treatment is scarcely fair when the primary purposes of these satellites are recalled.

Had this textbook been entitled *Satellite Remote Sensing for the Geologist*, I would have applauded it; as it is, I cannot do so for, as a teacher in geography, I find the contents much too restricted for general courses in remote sensing of the environment.

Eric C. Barrett

Eric C. Barrett is Reader in Climatology and Remote Sensing at the University of Bristol, UK.

Global geology

Earth. By F. Press and R. Siever. Second edition. Pp. 649. (Freeman: San Francisco and Reading, 1978.) Hardback \$16.95, £9.90, paperback (Europe and Africa only) £7.20.

This is an excellent book which is specifically designed as a teaching text, but could equally well be read without supervision. It is intended for students with no previous science background and who may not necessarily be intending to follow a geological career—though this text may well encourage them to do so. The reader is not treated as an imbecile, but is assumed to be a reasonable, intelligent being to be treated as an equal. The care that must have been taken in the wording does not show and, while American in spelling, the language is the best form of English-American.

It is arranged in three basic sections: (1) the Earth as an evolving planet and how it is studied, (2) surface processes and (3) internal processes and their surface effects. The individual chapters are planned to be read in any order, which could be dangerous as such a versatility means that repetitions are inevitable. In this case, however, the repetitions are extremely well done, usually comprising a review from a slightly different angle from the way in which the original version was presented.

Each chapter has its own brief introduction and summary, together with a few exercises and a bibliography. The summaries are too brief to act as 'crib-sheets', but the exercises show a healthy versatility, sometimes requiring calculations to be made but usually calling for a real assessment of some particular problem—unlike exercises in some books that merely require the student to find the appropriate section for regurgitation without further thought.

Some difficulties could arise if the student is not following the actual order of the chapters as occasionally relevant information has appeared in previous chapters. Specific sections within a

chapter are boxed off for more detailed consideration, usually at a slightly higher level than the main text, and it is a pity that the bibliography is not similarly indicated as being at a complementary or more advanced level.

The reviewer's standby of printer's errors appear to be absent and the whole volume is very well produced, usually with two text columns per page (25½ × 21 cm) with a wide margin often utilised for figure legends. The illustrations are well chosen, though almost entirely North American, and plentiful, both in terms of photographs and two-tone diagrams—the brown tone allowing attention to be brought to the more salient points. There is a good glossary and index, which will be essential for the new student or for later reference.

The only minor murmurs must be in the coverage of palaeontology in general and of sedimentary structures. In both cases, discussion of the use of fossils for stratigraphical purposes is surprisingly inadequate for any potential geology student. Detailed consideration of the evolution of a trilobite's eye can well be assigned to postgraduate studies, but the real value of fossils, in numerous ways, must be incorporated into a student's thinking at a very early stage. This also gives rise to some uncertainty about the author's use of stratigraphical time periods, certainly within the more advanced 'boxes', when the stratigraphical table only appears as a small diagram within the text, rather than an appendix, and the periods are not defined in the glossary. It is also unfortunate that the opportunity was not taken to give SI units in addition to the American metric unit conversion table.

To carp, however, at such a comprehensive text, so ably presented, should not detract from an unreserved recommendation of this book for either the first-year non-scientific undergraduate or layman. It is extremely good value, even if the dollar recovers!

D. H. Tarling

D. H. Tarling is Senior Lecturer in the Department of Geophysics and Planetary Physics at the University of Newcastle upon Tyne, UK.

Zoonoses and the Origins and Ecology of Human Disease

Richard N. T-W-Flennes

March/April 1979, xvi + 196pp., £9.20/\$19.00 0.12.256050.7

Current works on zoonoses tend to be catalogues of diseases which are interchangeable between man and animals. In this book, a new dimension is added to the question of zoonoses by viewing them against their proper background of ecology, and it is suggested that man has acquired a new disease pattern in new ecological conditions. The author argues that many human diseases are 'new' diseases, originally derived from animal sources, but which have become completely adapted to man as the sole host within the past 5,000 years.

Studies in Archaeology series

Analytical Archaeologist

Collected Papers of David L. Clarke

edited by his colleagues

February/March 1979, approx. 560pp., £13.60/\$26.60 0.12.175760.9

David Clarke was one of the leaders of what has been called the 'New Archaeology', and his first book, *Analytical Archaeology*, has been recognised as a seminal work since its publication in 1968. As both a prehistorian and an exponent of methodology in archaeology his influence extended far beyond Cambridge University, where he spent his entire professional career. Apart from his major books which included *Models in Archaeology*, *Beaker Pottery of Great Britain and Ireland* and *Spatial Archaeology*, as well as *Analytical Archaeology*, David Clarke published a number of papers in diverse journals. At the time of his death in 1976 he had begun to assemble a selection of them for collected publication. The present volume includes this selection, in the planned sections, with introductions written by four of his colleagues and friends and a prefatory biographical sketch and bibliography of David Clarke's writings.

Computer Methods in Geology

T. V. Loudon

March/April 1979, approx. 250pp., £15.60/\$32.75 0.12.456950.1

This book is designed to bring computer methods fully within the reach of the professional geologist, without imposing upon him unnecessary algebraic and statistical formulae. In a simple, readable and logical way, it combines up-to-date ideas from such different quarters as statistics, computer programming, systems analysis or mathematical geology, and presents the computer not as mystifying hardware but as a machine for sharing knowledge. The aim is to introduce the geologist to a range of systems concepts and computer techniques which are making a major impact on his science, and to give practical advice on how they can be implemented, and when they should be avoided.

Biochemistry of Insects

edited by Morris Rockstein

July 1978, 656pp., £19.15/\$29.50 0.12.591640.X

This book covers the biochemistry of insects from various aspects, ranging from the functional role of carbohydrates, fats and proteins to chemical genetics, protein synthesis, insect pigments, and the biochemistry of the insect cuticle. Of special note in this first book on this rapidly growing subject since Gilmour's 1961 volume is the coverage of the biologically important, relatively unique biochemical features of this highly diversified group of animals. These are covered under such subjects as chemical control of intraspecific and interspecific behaviour, pheromones and their use in the control of insects, as well as the chemical defence and detoxication mechanisms in insects, and the biochemistry of the toxic action on insects.

NMR and the Periodic Table

edited by Robin K. Harris and Brian E. Mann

February/March 1979, xvi + 460pp., £35.00/\$62.00 0.12.327650.0

NMR spectroscopy has become firmly established as one of the major methods available to the chemist for structure determination and for obtaining physico-chemical information about molecular dynamics. Recent years have seen several major developments of the technique. In particular multinuclear studies are now more feasible and are becoming popular. Consequently, it is an appropriate moment at which to summarise such work, since no overview of this type has yet been produced. This book is a comprehensive survey of NMR studies of all the elements of the Periodic Table for which such information exists. It will serve both as a source of information for the teaching of NMR and also as a reference work for research chemists, be they involved with organic, inorganic, physical, theoretical or biological chemistry.

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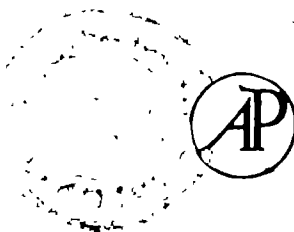
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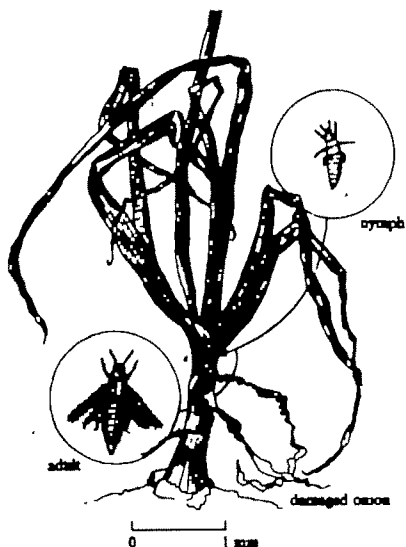
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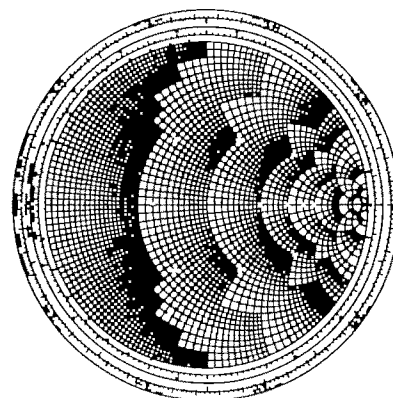
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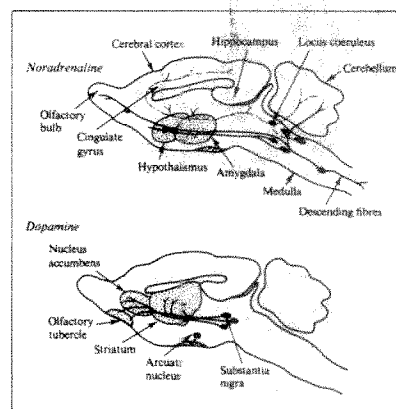
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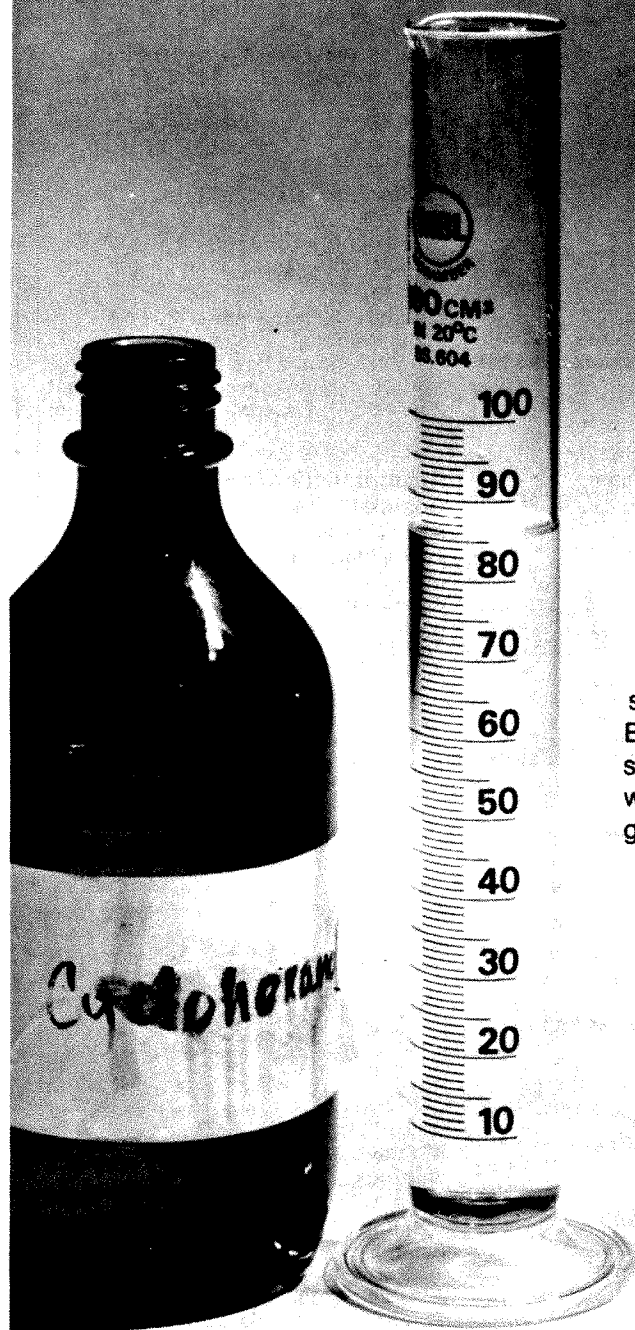
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Study of sedimentary rocks

Principles of Sedimentology. By G. M. Friedman and J. E. Sanders. Pp. 792. (Wiley: Chichester, UK, and New York, 1978.) £15.75.

THERE is no doubt that this book is a major benchmark in the study of sedimentary rocks similar to the publication of Pettijohn's *Sedimentary Rocks* in 1949.

Principles of Sedimentology is divided into six parts of fourteen chapters, five complements, a glossary, a bibliography and a subject index. Part I reviews the scope, evolution, techniques and philosophy of sedimentology. Part II describes sedimentary particles. The text has been divided into two courses, course 1 for innumerate, and course 2 for numerate students. As only six pages are considered too mathematical for many students this seems an unnecessary distinction which will increase the neuroses of innumerate readers. Part III deals with sedimentary processes, including deposition and diagenesis. Part IV is concerned with the classification and nomenclature of sedimentary rocks and Part V describes modern depositional environments and the environmental diagnosis of ancient sedimentary rocks. Part VI relates sedimentology to stratigraphy and includes a useful review of the relationship between sedimentation and plate tectonics and an introduction to wireline logs including the Dipmeter.

The next 90 pages of the book consist of sections titled "Complements" A to E. These are actually a series of chapters describing modern depositional processes and environments, including deltas, tidal flats and turbidites. This seems an odd arrangement, as most of this material logically belongs either in Part III (Sedimentary Processes) or Part V (Modern Sedimentary Environments).

The "Complements" are followed by a 22-page glossary which is totally unnecessary. One of the many good points of this book is that each new term is printed in bold face type followed by a definition in italic face. This arrangement, coupled with the excellent index, renders a glossary superfluous. Many of the terms included seem unnecessary—for example, "upwelling, viscosity, wadi, surface texture, stuffed basin (*sic*), very-shallow-water wave"; and does one really need to know that the bushmen of the Kalahari desert call a valley "omiribi"?

The glossary is followed by a biblio-

graphy which is nearly 200 pages long. In their preface the authors say that, in response to popular demand, they have not followed the custom of quoting authors and dates at appropriate places in the text coupled with full bibliographies at the conclusion of each chapter. Instead, sections of text are punctuated by lists of names and dates, but not paper titles. Thus the reader must refer to the terminal bibliography to discover the titles of recommended books and papers. Each chapter then concludes with a short reading list. Though the bibliography is very comprehensive the selected reading lists are less so. It seems strange that, for example, Allen's *Physical Processes of Sedimentation* and Wilson's *Carbonate Facies in Geologic History* are not suggested for further reading at the appropriate places in the text. The bibliography itself is excellent and includes citations up to 1977. An odd

feature of this is the frequent use of the term "*sic*" at an average rate of once a page throughout the bibliography.

Considered overall, the book is comprehensive, accurate, well written and excellently illustrated. Students' minds will not be overtaxed by monotonous slabs of text. Every page contains an illustration or heading to break the monotony. Wiley are to be congratulated for producing a book of this magnitude for a mere £15.75.

Despite the eccentric arrangement of its material there is no doubt that this book will be invaluable to sedimentologists, students, teachers, and industrial geologists working with sedimentary rocks.

R. C. Selley

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Elementary meteorology

Elementary Meteorology: A Course. By The Meteorological Office. Second edition. Pp. 208. (Her Majesty's Stationery Office: London, UK, 1978.) £4.95.

It is probably not too unfair to say that this book was intended for the education of entrants to the Meteorological Office and is being offered for sale to the general public as a bonus. Perhaps it helps to show that the Ministry of Defence is not only concerned with things secret.

Explicitly, the book is aimed at "weather observers with a background of physics equivalent to that of Ordinary level of the General Certificate of Education"; they will indeed find here much of use and interest.

Chapters on temperature, pressure, water vapour and clouds start from basic physical principles and go on to consider in some detail methods of observation and classification. Chapters on precipitation, thunderstorms and optical phenomena are more descriptive of the processes at work, but the chapter on synoptic meteorology owes much to the early origin of the book and concentrates on correct identification and classification. Forecasting, radar and satellite observation are mentioned briefly. It is a pity that the two rather nice satellite pictures were not dated and do not have an accompanying synoptic analysis.

For this observer, with ordinary level physics, wanting to familiarise himself with meteorological practice and jargon, it is good value for money.

Where else can you find Regnault's equation, the precise authoritative distinction between fog, mist and haze, the definition of Ci spi cgen (no kidding—p81) and much else, for less than £5.

Mind, HMSO does manage to produce an incredibly dull combination of typeface and layout that just shrieks 'educational manual', as you open any page. They do not do justice to Pedgley's nice pictures either.

The book is not suitable for the inquisitive, advanced, student who wants to find out what science there is in meteorology—it does not pretend to be and is not an introductory text. There are too many silly mistakes, like saying the pressure "*in pascals*" is given by weight/area, whereas the relationship is true whatever units it is measured in; and *that* is pretty fundamental physics.

It is ludicrously parochial for a general reader, referring, as nearly as it can, only to weather over the British Isles. Office publications and research are similarly oversold. There is a delightful blit in the Introduction that lists "research into the microphysics of clouds" as one of two "great advances in meteorological knowledge since 1938"—few meteorologists would agree with such an extravagant appraisal. It saddens me to see that the exciting new concepts that have been developed by modern science are going to find their way so slowly and capriciously into the meteorological services.

J. S. A. Green

J. S. A. Green is Head of the Atmospheric Physics Group at Imperial College, University of London, UK.

Fuel and its supply

Energy Resources. By J. T. McMullan, R. Morgan and R. B. Murray. (Edward Arnold: London, 1978.) Paperback £3.50.

Four years ago, in 1975, John McMullan, Roger Morgan and Robert Murray of the New University of Ulster at Coleraine completed a 500-page book entitled *Energy Resources and Supply*, which was published a year later by Wiley. It was a book for graduates and advanced undergraduates, one of the better examples of a genre then burgeoning in popularity. Shortly thereafter the three authors wrote a non-specialist version of the book, only about one-third the length, entitled *Energy Resources*. The paperback version of this second book has now been brought out by Edward Arnold, in their Resource and Environmental Sciences Series, aimed at late school and early university readers. The new book begins with a chapter on energy as an issue; there follow chapters on energy conversion, 'power from natural sources', fossil fuels, nuclear power, and 'inefficiencies'. There is also an appendix on units and an odd and idiosyncratic bibliography.

Energy Resources exhibits many of the virtues of its forerunner. It is readable, written with a light touch and generally good at explanations which convey the shape of a mathematical argument while eschewing the mathematics. The authors do now and then adopt a slightly sneering tone, doubtless intended as jocular but not quite coming off. Anyone who presumes to correct the misapprehensions of others would be better advised, in this fast-moving field, to manifest a certain humility. A great deal has happened since the authors first put together the thesis of their earlier book, and even since the text of their second was finalised in 1976. Their viewpoint, while still widely shared, particularly by official planners, has been overtaken by evolving developments, to such an extent that many of their unstated assumptions have since been scrutinised, analysed and found wanting: for instance their uncritical reiteration that the breeder reactor makes it "possible to use all the available uranium" as fuel, or their distinction between 'weapons-grade' plutonium and any other kind (all plutonium is possible bomb material). Chapter 3 on 'energy conservation', says virtually

nothing about the most important stage of energy conversion: that which takes place at the point of end-use of the energy. The book devotes ten pages to nuclear fusion—which does not yet even reliably exist as a controlled phenomenon, much less an energy source—while giving less than two pages to thermal insulation, undoubtedly the energy technology of highest current priority. Recent work, notably the report *A Low-Energy Strategy for the UK*, by Gerald Leach (Science Reviews Ltd and International Institute for Environment and Development: London, £7.50; for review, see *Nature*, 277, 162; 1979) demonstrates the startling potential for improvement in the

end-use energy-conversion infrastructure, to make far more use of both ambient and fuel energy. In retitling the slimline version of their book the authors would have been more accurate to refer neither to 'energy' nor to 'resources' in general, as they deal only with the narrow range categorised as 'fuel' and its 'supply'. As our understanding of the true dimensions of opportunity in energy policy deepens and broadens, 'fuel supply' seems likely to become less and less interesting.

Walt Patterson

Walt Patterson is Energy Consultant to Friends of the Earth (London) Ltd.

Global chemistry

THE consequences of the release of chemicals into the environment, including their chemical pathways and their effects on flora and fauna (including man), have attracted a great deal of attention in recent years, which shows no sign of abating. Much of the concern has followed in the wake of the development of methods of analysis which allow chemicals to be detected in ever smaller concentrations, and it is becoming clear that we live in a most complex chemical soup. Whether this necessarily places us at more risk than our immediate ancestors, however, is not at all certain, although this is one of the questions to which the two books reviewed here address themselves.

R. A. Horne, in *The Chemistry of our Environment* (Wiley: Chichester, UK; £19.40) has set himself a much more ambitious task than J. Lenihan and W. W. Fletcher, in *The Chemical Environment* (Blackie: Glasgow; hardback £8.90, paperback £4.50)—that is, to give an account of the chemistry of the total environment beginning with nothing less than the Solar System. On the whole he succeeds well, and there is a great deal to learn from a study of his book. The writing is as concise as can be expected in a tome of over 800 pages and the text is illustrated by many excellent figures and tables; each chapter also includes an extensive set of references for those with the stamina to explore the subject further.

What spoils the book for me were Horne's not infrequent incursions into polemic, although in his introduction he does warn that he writes both to instil and to provoke. When he does provoke, however, he often also shows the lack of deep understanding which

characterises the instructional parts of his book. Much of the controversial material appears in chapter 12 which deals with "Man's Perturbation of the Biosphere", which includes, in a section entitled "Internal Pollution", an outburst on the evils of drugs, drink and tobacco. Although no-one would suggest that any of these did not present serious dangers to health, I doubt that the present book is the right forum in which to deal with them.

The final section of the book considers whether or not the Earth will survive, at which point Horne finds himself overcome with a sense of futility. Although not convinced that we are all doomed, Horne nevertheless does consider that life on the planet will become very unpleasant indeed, because of man's propensity increasingly to pollute his environment. The pollution he is most concerned with is this context, however, is not chemical but human. Horne believes that the weight of population will finally produce stresses in the environment which are irresistible. This gloomy prophecy seems a long way from the book's original starting point.

The book which Lenihan and Fletcher have edited, in the series *Environment and Man*, is a much slighter work, and although called *The Chemical Environment*, is a highly selective account of some environmental pollutants preceded by an excellent, short survey of the natural cycles of the elements in the environment by Bowen. Those who read Horne's book would do well to use this chapter as their introduction to the subject.

The remaining chapters of this book vary in style and content, but are all interesting and informative and I enjoyed particularly Kipling's elegant chapter on arsenic.

The book does not give anything resembling a complete account of the hazards which chemicals in the environment present to man, nor does it pretend so to do. However, I am surprised that

the editors felt that they could leave cadmium out of their considerations; perhaps they will include it in a future edition.

Both these books contain much that will instruct and interest the student who wishes to know more about the chemistry

of the world in which he lives, and I recommend both to him.

Tony Waldron

Tony Waldron is Senior Lecturer in Occupational Medicine at the London School of Hygiene and Tropical Medicine, London, UK.

Environmental mixed bag

FOUR books on various aspects of the environment, each useful in its own way, are aimed at very different audiences. Sir Alan Cottrell's *Environmental Economics* (Edward Arnold: London; paperback £1.95) is a succinct, readable, but not very exciting, canter through conventional economics as applied to the environment. It contains all that the non-economist needs to know about these in one handy book, but it is a pity he has not covered the 'Materials Balance' approach of Ayres and Kneese, nor the recent work of Pearce, both of whom tackle the aspects of the environment which are not amenable to cost-benefit concepts.

Measuring and Monitoring the Environment (Blackie: Glasgow; hardback £8, paperback £4), by J. Lenihan and W. W. Fletcher, is the best and most interesting of the series *Environment and Man*, but like the others seems aimed simultaneously at diverse audiences, not all of which are equally well-served. In view of the detailed descriptions of methods for chemical analysis of water, and for analysis of hair, it is a pity that some account of biotic indices, as devised by Woodiwiss, Cairns, Chandler, Cowell and others and recently surveyed by Hellawell were not included as an additional chapter. It is a useful source of references for advanced level students and interested lay-readers and for others seeking an introduction to how the environment is monitored and by whom.

A *Dictionary of the Natural Environment* (Edward Arnold: London; hardback £8.95, paperback £2.95), by E. J. Monkhouse and J. Small, is attractive and easy to use, but is heavily oriented towards geographical aspects of environment. For example, 'species' occurs in connection with clouds, but not with organisms! 'Speciation' is not listed, nor are 'biocides';

and 'biomass' should not really be defined as 'an amount of energy'. These are, however, quibbles, and the book will be useful for ordinary and advanced level students and interested lay-readers. Professionals might find it frustrating.

Richard Fiennes' *The Environment of Man* (Croom Helm: London; £6.95) is more serious, and more ambitious than the others. It ranges over all the man-environment issues which have surfaced in recent years: nutrition and food resources; diseases of affluence; stress; genetics and inheritance; and carci-

nogens. For this it is a convenient point of departure for discussion and investigation. Like all such ventures, however, it has been overtaken by time. For instance, widespread undernourishment is no longer attributable to failure of food production to increase as fast as population; and protein deficiency *per se* is no longer identified as the cause of kwashiorkor and marasmus, nor animal protein as of necessarily better quality than a vegetarian diet. Some controversial claims, such as deterioration of the human race when most infants survive to adulthood, and that man is a product of adversity, as though other species were not, are made as though they have wide acceptance. With these reservations the book is interesting and stimulating.

Pauline K. Marstrand

Pauline K. Marstrand is a Senior Fellow at the Science Policy Research Unit, University of Sussex, Brighton, UK.

Shaping the Earth's surface

The Earth's Changing Surface. By M. J. Bradshaw, A. J. Abbott and A. P. Gelsthorpe. Pp. 336. (Hodder and Stoughton: London, 1978.) £5.95.

THIS is in many ways a remarkable book. The authors have undertaken the daunting task of examining the processes that shape the Earth's surface while at the same time stressing the complexity of landforms in terms of form and evolution. As a consequence the range of coverage is immense, extending from plate tectonics to meteorite impact structures (including a brief discussion of lunar surface morphological development) and from surface geomorphological processes by way of anthropogeomorphology to applied geomorphology. In fact almost every topic of relevance to the study of the Earth's surface is touched upon. Much of this information is conveyed visually, there being no less than 215 plates (many of which are in colour) and a similar number of line drawings, mostly taken from recent publications. The approach is thus refreshing and visually attractive.

However, coverage of such a range of topics in 324 pages of text, much of which is taken up with illustrative material, means that depth of analysis has had to be sacrificed. The text is often disappointing, reference-free and

full of generalities and in places seems merely to be providing continuity between the figures. There are, as must be expected, omissions (for example, no mention of the periglacial hypothesis of tor formation or dilatation jointing) and minor errors; and the use of 'boxed sections' for the examination of 'side issues' or 'background information' (for example, continental drift) may be found distasteful by certain readers. The bibliography is also rather disappointing and lacking in structure, being essentially a list of the more readily available geomorphological textbooks.

In spite of these criticisms this book clearly represents a reasonably priced, well presented and welcome addition to the recently much expanded literature about the Earth's surface. It is to be highly recommended to the interested non-specialist as an informative introduction to the subject. It will also certainly find its way into most school libraries and will provide a definite stimulus to both advanced school students and teachers. Its potential value to the university student and specialist is more difficult to define. There is almost certainly something here for everyone, for it provides pleasant browsing, but in itself it lacks sufficient depth for the average first-year undergraduate and must, therefore, be envisaged as a springboard to more advanced and specialised texts.

David K. C. Jones

David K. C. Jones is Lecturer in Physical Geography at the London School of Economics, London, UK.

● The UK edition of *Soviet Science*, by Zhores A. Medvedev, will be published in April by Oxford University Press. For review, see *Nature*, 276, 146; 1978.

● This year's Spring Books supplement will be published on 26 April.

Physical geology

Holmes' Principles of Physical Geology (Nelson: Sunbury-on-Thames, UK; hardback £14.95, paperback £8.50) is one of the classics of geological education and, now in its third edition, has been used by generations of advanced school students and undergraduates. The new edition is substantially the same as its predecessor, though the revolution in plate tectonics has caused it to have its final chapters reorganised and rewritten. There have also been modifications to the sections on batholiths, granite, granitization, ring dykes and migmatites. Some of the figures and plates have been changed. This does tend to highlight the antiquity of some of the material left over from earlier editions, for the years have not treated some of the illustrations kindly. Overall, however, the volume seems to be quite handsome without going in for some of the more flamboyant touches of its American competitors. The references have been updated for this edition though this is more evident in some chapters than in others.

It is not entirely evident that anyone who owns the second edition needs to go out for the third, for there are some major fields in which important recent developments are not reported. The geomorphologist, for example, will find that descriptive and evolutionary (cyclic) geomorphology predominates, whereas the student interested in the Pleistocene will find many discredited ideas (for example, glacial = pluvial) but far too little on ancient ergs or the deep-sea core record. The desert geomorphologist will find that siefs and barchans are still the basic framework for a study of desert dunes and that salt weathering is not considered.

In sum *Holmes' Principles* is now rather like an old sports coat. It is comfortable and familiar, has been patched up rather obviously, and is not such a good fit as it was, but the basic material is still good for a few more years. At £8.50 for 730 big pages the paperback remains something of a bargain.

Introduction to Geology: Physical and Historical (Prentice-Hall: Englewood Cliffs, New Jersey) by W. L. Stokes, S. Judson and M. D. Picard is also a new edition rather than a totally new book. It very much follows the standard format of basic college texts in physical geology and seems to be neither greatly better nor greatly worse than some of its competitors which I reviewed in *Nature* (266, page 100) this time last year. It has been updated by the addition of the usual material that is put into such new additions: something on plate tectonics, something on space, and something on hazards, environment, energy and resources.

The book covers a great deal of

ground in no great detail and by means of relentless subsectioning. The geomorphologist will find some of the geomorphology irritating: rock weathering rates are largely discussed with reference to grave stones, karst landscapes seem to consist of stalagmites, stalactites and water witches, there is virtually nothing on the Pleistocene sequence determined from ocean cores, and there is very little on modern process geomorphology. This is, however, not of course a textbook for geomorphologists but it is disturbing how poor the geomorphic content is. Overall, however, this book is adequate but a little lacking in character.

Students wishing to gain basic instruction in the geomorphological component of this book would do better to go to B. W. Sparks' *Geomorphology*; R. J. Small's *The Study of Landforms*; A. L. Bloom's *Late Cainozoic Landforms*; R. J. Rice's *Fundamentals of Geomorphology*; or A. F. Pitty's *Introduction to Geomorphology*.

The third volume in physical geology to be considered is the third edition of *Putnam's Geology* (revised by P. W. Birkeland and E. E. Lawson; Oxford University Press: Oxford; £12.25). This book proceeds in a logical way "from a discussion of the materials that make

up the visible earth to the various processes responsible for wearing down and shaping the landscape, concluding with a panorama of earthquakes, mountain building and plate tectonics—in general, the processes that continue to elevate portions of the Earth's Crust".

Of the three volumes this is the one that I liked best. The structure is logical; the illustrative material (including colour plates) is original, simple, clear and striking; there is some attempt at highlighting major concepts and important scientists; and the geomorphological component of the book shows signs of being written by somebody who knows about geomorphology.

By way of conclusion, all three volumes will to varying degrees be acceptable for good advanced school students in geography and geology and they will satisfy some first-year undergraduates in these subjects. Course texts, such as these, are not perhaps used as widely in British Universities as they are elsewhere, presumably because such books have to cover too much in too short a span.

Andrew Goudie

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Metamorphic petrology

Petrology of the Metamorphic Rocks. By Roger Mason. Pp. 254. (George Allen and Unwin; 1978). Hardback £10.95; paperback £5.95.

This book is Volume 3 of the *Textbook of Petrology*, which already includes *Petrology of the Igneous Rocks* (Hatch, Wells and Wells) and *Petrology of the Sedimentary Rocks* (Greensmith). It is a most welcome addition to this set and is aimed at about the level of the second-year undergraduate in a three-year geology course. Dr Mason's objectives are fairly modest and largely concerned with a description of what metamorphic rocks are like. For the most part this approach is very successful, aided considerably by the author's evident desire to be understood. The language used is concise but simple, and definitions are given careful attention. A particularly good feature of much of the descriptive writing is the constant supply of useful tips concerning the actual identification and distinguishing features of minerals under the microscope. From the above it should be clear that the affinities of this book lie more with Harker's *Metamorphism* than with more theoretical works, though it is less comprehensive in terms of rock types covered.

Part I of the book consists of three chapters dealing with definitions, meta-

morphism in general, field relations, structures and fabrics. The bulk of the descriptive work is in the nine chapters of Part II. Here some dozen areas are covered in detail to illustrate various types of metamorphism. Contact metamorphic rocks are illustrated by the Skiddaw, Comrie, and Beinn an Dubhaich aureoles, dynamic metamorphic rocks by, amongst others, the Lochseiten Mylonite, lunar breccias and the Ries crater. Regional metamorphic rocks of Precambrian shields are represented by the Lewisian, and metamorphism in Phanerozoic belts is illustrated by the descriptions of the Sulitjelma and Galway regions and by parts of the Alps (for example, blueschists). Most of the above sections are excellently done and form the most useful part of the book. Much of it is highly readable and the author's personal experience of and interest in the rocks comes through. Two short chapters respectively on ocean floor and upper mantle rocks seem less satisfactory, though credit is due for including these topics at all. This central descriptive section of the book will probably make it very popular, for apart from Harker there is no comparable source of good, meaty descriptive writing on metamorphic rocks (the section in the new *Petrology for Students* by Nockolds *et al* (Cambridge University Press: Cambridge, 1978; hardback £17.50, paperback £6.50) is similar in level but substantially shorter).

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Fundamentals of Sensory Physiology

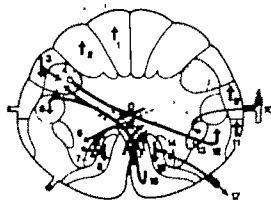
Edited by Robert F. Schmidt
With contributions by H. Altner, J. Dudel, O.J. Grusser,
U. Grusser-Cornehl, R. Klinke, R.F. Schmidt and
M. Zimmermann
Translated by M.A. Biderman-Thorson
Springer Study Edition
1978. 138 figures, 5 tables IX, 286 pages
DM 33,60, US \$ 18.50
ISBN 3-540-08801-6

These companion volumes present an integrated introduction to the neurosciences, accessible to students with only minimal background in the life sciences. The emphasis in both books is on clarity and each term is defined and explained as it is introduced in the text. Superior illustrations and suggested readings permit the student to easily grasp the subject. These books are of interest to everyone who wishes to, or must, delve into the potentialities and limitations of human sensory experience.

R. NIEUWENHUIJS, J. VOOGD, C. van HUIJZEN

The Human Central Nervous System

A Synopsis and Atlas
1978. 154 figures. IV, 253
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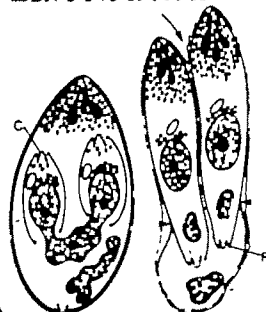
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E. SCHOLTYSECK

Fine Structure of Parasitic Protozoa

An Atlas of Micrographs, Drawings and Diagrams

1979. 186 figures. VIII, 206
pages. DM 80,-; US \$ 44 00
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This atlas utilizes a new method of analyzing electron micrographs allowing a more definitive characterization of protozoan fine structural organization. An electron micrograph and an analytic drawing are arranged, along with a descriptive text on pages facing each other to facilitate comparison. The atlas is designed for students, instructors, and researchers in the fields of protozoology, parasitology, veterinary or human medicine, and makes a valuable tool in the study of fine structure in general.

A. F. WILLIAMS

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1979. 144 figures, 17 tables. Approx. 330 pages
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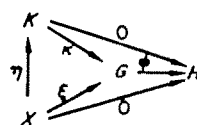
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A Contemporary Interpretation
1978. 94 figures. XXIX, 637
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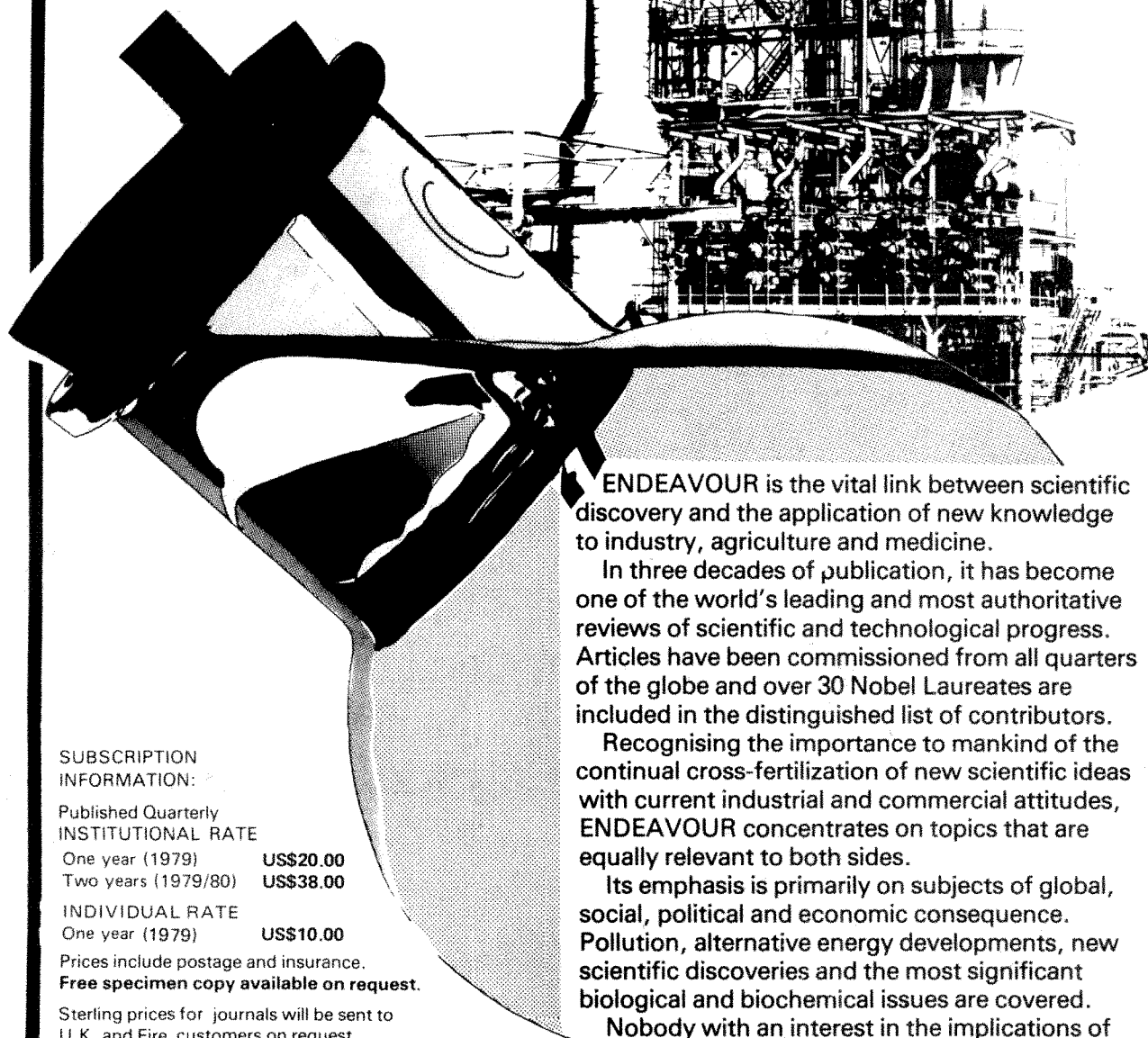
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The book also has chapters on metamorphic reactions (including a discussion of facies), isotope geology, the use of the electron-probe, and a final chapter on metamorphic rocks and the evolution of the Earth. The theoretical sections are on the whole somewhat condensed and the reader will probably only acquire a superficial familiarity with the topics discussed. For example, in the section on isotopes there is no mention of the fundamental equation of radioactive decay and its derivatives as used in isochron diagrams. Similarly, in discussing metamorphic reactions thermodynamics is more or less avoided. These are both cases where more detail would have enabled problems and exercises to be inserted, probably with a considerable benefit to the understanding gained by the reader.

In summary, the book is rather

disappointing on the topic of metamorphism itself: it is not particularly stimulating, at least partly because it gives very little coverage to some of the fundamental problems of metamorphism such as where the heat comes from and how the study of metamorphism is applied to the understanding of tectonic processes. On the other hand the accounts of metamorphic rocks are rather well done, and the book is worth it for these parts alone. One sympathises with the desire to keep the text short (and cheap) and it may perhaps be impossible to cover all aspects of the subject satisfactorily in a work of this length. None-the-less this book should provide useful background reading for most courses in metamorphic petrology. **K. G. Cox**

K. G. Cox is Lecturer in Petrology at the University of Oxford, UK.

Three branches of petrology

Petrology for Students. By S. R. Nockolds, R. W. O'B. Knox and G. A. Chinner. Pp. 435. (Cambridge University Press: Cambridge, 1978.) Hardback £17.50; paperback £6.50.

ALTHOUGH Harker's *Petrology for Students* is discontinued after more than 80 years, this book with the same title and from the same stable is obviously its natural successor. It now has three authors, one for each of the main divisions of petrology. With its emphasis on the description of rock thin sections under the microscope it is very much in the Harkerian mould. This impression is reinforced by the use of many of Harker's original 'microdrawings'. From the amount of detailed information presented, the book seems to be most suitable for the second year of a conventional three-year geology course, but as the three branches of petrology are usually taught separately at this level, its success depends on a consistent approach and level of achievement.

The section on igneous rocks (approximately half the book) provides a thorough and systematic account of the petrography of the main igneous rock groups, with useful supporting information on the major element chemistry. The introductory chapter on textures would be more effective if it was illustrated *in situ* (rather than referring to the appropriate figures in later chapters), and the chapter on classification is disappointingly brief and makes no concession to other schemes or to recent attempts to

standardise nomenclature. A more serious drawback is the persistent emphasis on specific examples, many of which come from obscure localities and represent material the average student is unlikely to meet in the flesh. This whole section has a distinctly old-fashioned flavour; the collection of facts is carefully curated, but the presentation is lifeless.

The treatment of sedimentary and metamorphic rocks is generally more effective in that it manages to combine the systematic petrography with some discussion of the rock-forming processes. The sedimentary section contains a useful introductory chapter on textures, followed by a logical and up-to-date treatment of the main groups of terrigenous and chemical sediments. The metamorphic section is less conventional in that a chapter on textures is followed by discussions of metasomatism and melting, facies classification, regional metamorphism, granulites and eclogites, blueschists, and low-grade metamorphism.

This book is quite good value in terms of the quantity of information provided, but it is not altogether successful as a coherent treatment of the three branches of petrology. It would seem more sensible to invest in separate books on igneous, sedimentary and metamorphic rocks, as these generally provide a more complete picture, combining the descriptive material with a discussion of processes and geological context. Without these petrography can be extremely arid.

W. J. Wadsworth

W. J. Wadsworth is Senior Lecturer in Geology at the University of Manchester UK.

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Organic chemistry

IN this section last year, four fat American textbooks were reviewed. This year there are two slim Americans, together with three British books (one large one): two new editions of established works, and a new book written by last year's reviewer, Stuart Warren.

Both of the American texts attempt to cover the main themes of modern organic chemistry, with some mention of biological chemistry, in about 500 pages. One of them, *The Essence of Organic Chemistry*, by J. M. Cram and D. J. Cram (Addison-Wesley: Reading, Massachusetts, and London) succeeds quite well in this endeavour; but the other, *Organic Chemistry: A Brief Survey*, by R. L. Baumgarten (Ronald: New York, £10.25, \$18.45) is rather disappointing. The first six chapters of Baumgarten cover the fundamentals (bonding, nomenclature, and so on), then the middle portion of the book is devoted to the chemistry of the various classes of organic compounds. Finally, more specialised topics are dealt with in eight chapters: these include stereoisomerism, synthetic polymers, primary and secondary metabolites and an overview of spectroscopy. There are an enormous number of problems, but only selected answers are given; and although there are some interesting half-tone illustrations, only rare attempts are made to represent realistic three dimensional structures of molecules. The chemistry throughout is fairly basic, and given the intended readership—students majoring in biology, home economics, nursing, and so on—this is probably all right; but the big disappointment is the lack of emphasis of the relevance of organic chemistry to the Life Sciences. Surely these students should know something about prostaglandins, insect pheromones, and biologically active peptides, to name but a few omissions.

In contrast, the relationships between organic chemistry and biochemistry are nicely handled in Cram and Cram. The first four chapters introduce bonding and structure, etc., through a discussion of hydrocarbon chemistry, and this sets the scene for subsequent coverage of the various classes of organic compounds. Mechanistic considerations are slotted in where relevant, and the illustrative examples are well chosen: the involvement of substitution reactions in terpene biosynthesis; a discussion of the Claisen ester condensation leads on to the reactions of acetyl-thioenzyme A; and many others

The final four chapters deal with aromatic chemistry, the formation of carbon-carbon bonds, carbohydrates and nucleic acids, and spectroscopy. One glaring omission from this last section is mass spectrometry—in fact, both books are rather weak on spectroscopic methods. Throughout the book discussion of fundamental chemistry is a prelude to coverage of applications to biochemistry. For example, after basic stereoisomerism we have the dynamic stereochemistry inherent in enzyme structure and catalysis, and a section on epoxides includes mention of the sex pheromone of the gypsy moth (an epoxide), and of 2,3-epoxy-squalene as progenitor of the steroids. At the end of each chapter there are summaries of reactions, new terms and topics, and also many useful problems (others are interspersed throughout the chapters) for which answers are supplied. The illustrations are good and include realistic structures for complex molecules.

In summary, although neither book is suitable for degree courses in chemistry, Cram and Cram can be recommended for service courses in chemistry for students of biochemistry, medicinal chemistry, and so on.

The large British book under review is Vogel's *Textbook of Practical Organic Chemistry* (fourth edition revised by B. S. Furniss, A. J. Hannaford, V. Rogers, P. W. G. Smith, and A. R. Tatchell, Longman: London and New York; £17.50). Twenty-two years have elapsed since the previous edition of this book was published, and many new experimental techniques have appeared in this period. This is reflected in the changed emphasis of the present edition. Thus, chromatography increases from seven to thirty-eight pages; and there is a big new section on the interpretation of spectra: infrared and NMR spectra are particularly well covered, and the coverage of mass spectrometry is fair. At the end of the book there are additional correlation tables for IR, NMR and mass spectra (as well as sections on solution characteristics, hazards, and first aid). The bulk of the book comprises a total of 460 primary experiments with many additional cognate preparations, for which full experimental details are given. This is followed by a chapter on qualitative organic analysis, which includes the discussion of spectroscopic techniques as well as all of the classical methods. There are many new experiments, which include: the use of sulphur ylides (one experiment), allene formation (2), cuprates (1), enzymic resolution (1), phase transfer catalysis (1), hydroboration (2), and so on. However, there are several omissions: no

examples of the Baeyer-Villiger oxidation, or the Simmons-Smith reaction, and no mention of the use of alkane dithiols and other reagents which can bring about a reversal of polarity of functional groups (*umpolung*). Perhaps most disturbing is the dearth of Wittig reactions—only one phosphorus ylid is prepared and used—yet there are 31 experiments involving Grignard reagents—surely this excess does not reflect the respective utilities of the two types of reagent. One other disappointment is the total absence of NMR or IR data from the experimental section. Had the chapter on spectroscopy been introduced earlier in the book, and data supplied for each of the compounds synthesised, this book would have been enormously enriched. As it stands the reaction products are characterised by melting and boiling points which, though useful, hardly reflects the modern reliance on spectroscopic proof of structure. The authors state that the book is a one-volume reference text for undergraduates and postgraduates, and with certain reservations this is true, though at £17.25 I doubt whether many individuals will buy it. Certainly all research laboratories should possess a copy, but the people who will find it most useful are those who must devise experiments for chemistry courses.

The remaining books—*Some Modern Methods of Organic Synthesis* (second edition, by W. Carruthers; Cambridge University Press: Cambridge; hardback £25, paperback £7.95); and *Designing Organic Syntheses: A Programmed Introduction to the Synthon Approach* (by S. Warren; Wiley: Chichester, UK, and New York; paperback £3.95)—cover the methodology of synthetic organic chemistry. They are complementary, as the former deals with synthetic methods, whereas the latter shows the reader how to analyse a synthetic problem, and choose the most expeditious synthetic route.

The first edition of Carruthers appeared in 1971, and this new edition has increased in size by about 130 pages. Much of this expansion is due to new sections on organo-sulphur, -selenium, and -silicon chemistry, *umpolung*, and the selective alkylation of ketones, but there are also about twice as many references and a larger index. The basic format remains the same with chapters one and two on the formation of carbon-carbon single bonds and carbon-carbon double bonds. There are much enlarged sections on α -thiocarbanions, and the Wittig reaction, which now includes useful and timely information about sulphur ylides; and new sections on *umpolung* and cuprates. Chapter three covers the Diels-

Alder reaction and includes a new section on the intramolecular version of this reaction, with extended coverage of catalysis by Lewis acids, and of the ene reaction. It also has a new section on the utility of allyl cations, but this is too short, and rather out of date to be of much use. Of the remaining four chapters, the one on organoboranes and organosilanes is particularly good. There are a few disappointments, such as the poor coverage of phase transfer catalysts and crown ethers, and the fact that none of the references are post-1976, and most are pre-1974. Otherwise, the book provides a very good introduction to the methodology of organic synthesis, and should be essential reading for advanced undergraduate courses, and for all postgraduates and others actively involved in organic synthesis.

Stuart Warren's book is a programmed introduction to the design of organic syntheses. It has all of the advantages of a programmed text in that problems are set, and then solved once the reader has had chance to attempt a solution; and the choice of problems is very good. This book represents the first serious attempt to teach undergraduates how to disconnect a target molecule, to provide fragments (synthons) for which syntheses may be discerned. Stephen Turner's book, *The Design of Organic Syntheses* (Elsevier, 1976) is pitched at a much higher level. One particularly appealing feature of the book is the way in which the analysis of many simple target molecules is tackled before the

same methods are applied to complex targets. It is often forgotten that undergraduates are primarily taught synthetic methods, and it is strange at first to think in a retro-synthetic sense. Initially, Stuart Warren takes the reader through the various types of disconnections: one-group, two-group, and illogical (that is, ones where a functional group interchange must be applied before the disconnection can be made). He then passes on to more complex disconnections, such as those involving 'retro-Diels-Alder' reactions, and those involving heteroatoms or small rings; and finally discusses strategy at some length. In fact this section could almost stand by itself as a sort of guide to 'gamesmanship' in organic synthesis. Throughout the book there are review problems, many difficult, which will be of particular utility for lecturers as a source of set work; and most problems have a key literature reference supplied. Any person working through this book will gain a fundamental and very useful understanding of 'synthetic analysis', and it should be essential reading for anyone involved in organic synthesis. My only small reservation is that it might be too difficult for undergraduates, except those taking advanced courses, although their understanding of organic chemistry will be much improved if they can work through it. And at £3.95 it has to be the bargain of the year

John Mann

John Mann is Lecturer in the Department of Chemistry at the University of Reading, UK.

Communicating chemistry

THE large void which separates scientists from non-scientists is initially due to the language barrier. Each particular branch of science has its own linguistic pattern, and communication between practitioners in each branch is often completely incomprehensible to non-scientists. Thus, it behoves the scientist who wishes to communicate with the non-scientist to teach the basic language thoroughly. It is well to recall that we all begin as non-scientists and it is undoubtedly true that many great minds have been lost to scientific endeavour because initial interest was destroyed by a failure of the teacher to lay down a basic framework for the subject. Conversely there are numerous working scientists whose enthusiasm for their subject was nurtured by excellent teachers who could not only teach well but also transmit their own love for, and excitement derived from, their

particular area. (Also, one must admit that many of us can recall teachers who clearly did not understand the principles they were employed to transmit to us.)

Because of the nature and requirements of US degree courses there are several hundred thousand students taking general chemistry courses every year, and probably the majority of these do not intend to eventually major in a science subject. The market for general chemistry textbooks is very large and the competition amongst authors is very keen. This has resulted in some excellent texts being published in the past five or so years. The requirements are often severe, for example, teaching thermodynamics to large mixed-ability classes, but many authors have managed to simplify quite esoteric chemical and physical concepts without loss of integrity, usually with the aid of excellent diagrams and artwork. Thus, a good general chemistry book can often represent the firm basis of an undergraduate's chemical knowledge. At the other end of the

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scale one finds, however, texts which contain little chemistry but much repetition. The best texts I have come across are those written by active researchers, for example, by L. Pauling, H. B. Gray, D. H. Busch; the worst are almost invariably written by 'chemical educationalists'.

The texts reviewed here represent a fair cross-section of the available books, although none are as good as the very best available. The second edition of Fine's *Chemistry* (Williams and Wilkins: Baltimore; \$16.50) admirably exemplifies the wide scope frequently offered.

Fine's book is the most attractive of the present collection, but having said that I shall use it to illustrate some of the inconsistencies which appear in it and some others reviewed here. For example, in a book which includes two chapters on quantum principles and discusses the Schrödinger wave equation why is it necessary to include a section in chapter 1 entitled "Using Powers of Ten" and includes the mind-stretching equation $1.5/10^4 = 1.5 \times 10^{-4}$. Furthermore, a feature of many of these books is contained here, inasmuch as in a book of almost 800 pages only one chapter (37 pages) is given over to organic chemistry. Also, many of the vignettes in the margins of the pages seem to have little to do with text generally and I think are unnecessary. Unlike most of these books Fine's book contains some bad art-work, e.g. the structures drawn on pages 738 and 743.

I enjoyed, too, perusing *Chemistry: A Modern Introduction* (W. B. Saunders, Philadelphia and London; £12) by Brescia *et al.* and similarly admire the wide scope, but am again dismayed to find that nuclear chemistry and organic chemistry warrant approximately equal treatment in terms of pages devoted to them. I was surprised to find in the first two paragraphs of the preface the statements: "This book is written for highly motivated students . . . the work is therefore, in part, intentionally repetitious and written in a language to stimulate students and help maintain their enthusiasm." Repetition will never appeal to the highly motivated student. Again, I suspect that the highly motivated student will wonder why Figure 1.3, which contains an illustration of a rule marked in inches alongside one marked in centimetres, is necessary.

I would find it difficult to recommend E. I. Peters' *Introduction to Chemical Principles* (W. B. Saunders: Philadelphia and London; £10.50). More than the previous two books I find it difficult to know at whom the book is aimed: at those students who can appreciate the quantum mechanical model of the atom (p70) or those

who need to be taught how to count to significant figures or to be told that meter sticks are calibrated in millimetres (p45).

W. L. Masterson and E. J. Slowinski's *Chemical Principles in Qualitative Analysis* (Holt-Saunders: Philadelphia and London; £10.50) is a "student-oriented book", but I can quite truthfully say that I have not in the past ten years come across a book which would in my opinion deaden any interest in chemistry a student had before being unfortunate enough to have to use this book. The fault essentially lies not in the chemistry, but in the attempts to introduce old-style wet methods of qualitative analyses wherever possible, quite apart from the large section of the book (153 pages) devoted to qualitative analysis of cations and anions. Despite the revolution which has occurred in analytical procedures during the past 15 years I am prepared to admit that wet-method qualitative analysis deserves some, essentially historic, treatment in modern books. But I find it depressing that this book makes no mention of NMR or atomic absorption spectroscopy (and little mention of mass spectroscopy), and even more depressing to think that there are Colleges in the US which must still be introducing practical

chemistry to Freshmen by way of qualitative analysis.

G. C. Hill and J. S. Holman's *Chemistry in Context* (Nelson: Sunbury-on-Thames, UK; £5.95) is aimed at students studying chemistry to advanced level lab school, and one can clearly see the influence of American style in the presentation. One might roughly equate the attainment of College Freshmen students in the US with advanced level students in the UK, and thus it is interesting to note that this book devotes 142 out of 518 pages to organic chemistry. I liked this book, but did occasionally find that the peripheries are allowed to intrude on, and confuse occasionally, the serious matter of communicating chemistry. Thus, for example, page 1 includes a picture and a biographical note on John Dalton, a reproduction of Dalton's symbols for the elements, an electron micrograph of copper phthalocyanine, and a short quiz on atoms. Incidentally, the thoughtful student will find their d-orbitals splitting diagram on p272 confusing.

C. A. McAuliffe

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Physical chemistry conceived

CHEMISTRY is a three-dimensional subject. Those incapable of visualising the shapes of molecules represented by two-dimensional structural formulae have to struggle with the topic. The first introduction to this aspect normally comes with tetrahedral carbon bonds and the topic of stereochemistry remains central, especially in organic and biochemistry. The fact that Kurt Mislow's *Introduction to Stereochemistry* (Benjamin/Cummings: Massachusetts; \$11.50) is now reprinted for the sixth time speaks for itself. It was one of the first undergraduate monographs used to supplement a major text and as such outstandingly successful. Emphasis is placed on structural stereochemistry and reactions are avoided. It has three sections; structure and symmetry; stereoisomerism and the separation of stereoisomers. Although explained, it rightly describes hybridisation as "no more than a convenient mathematical fiction".

In *The Chemical Bond* by J. N.

Murrell, S. F. A. Kettle and J. M. Tedder (Wiley: London and New York; £11, \$23.50) hybridisation again takes a back seat, despite its historical importance in the understanding of three-dimensional shapes. This new book, by the authors of the established *Valence Theory* (Wiley: London and New York) is written from the contemporary standpoint with the evidence of the thousands of published *ab initio* molecular orbital calculations in mind. Valence bond and molecular orbital theories are not presented as equal rivals. The fact is that computational ease has left the molecular orbital approach dominant over the alternative, just as the internal combustion engine eclipsed the steam car, despite theoretical objections. Computational techniques are not included and indeed the book demands minimal mathematical skills. Nonetheless any undergraduate, having read it would be much better able to make the transition to the research literature than one brought up on the traditional diet. The topics are introduced in what is perhaps an unfamiliar order (perturbations in chapter 11 and valence bond theory in chapter 13), but this is amply justified. Strong points are the inclusion in an undergraduate text of good accounts of band theory in solids, reactivities of molecules and of intermolecular forces.

The chemist's chief device for coping with the three-dimensional nature of his subject is to use molecular models, although these have limitations as Mislow points out. The models of the title in *Chemistry through Models* by C. J. Suckling, K. E. Suckling and C. S. Suckling (Cambridge University: Cambridge; £15), are interpreted in a much wider manner than mere mechanical analogies. Theories, hypotheses and laws all fall within the definition of a model, so that, for example, the ideal gas law, $PV=RT$, is in this sense a model. The book is subtitled *Concepts and Applications of Modelling in Chemical Science, Technology and Industry* and it thus attempts the rare feat of bridging academic chemistry and biochemistry and industrial application. It is very original, well written and thought provoking. Early chapters introduce models in the general sense and explain the modelling process. The main body of the text then applies models to the concepts of chemistry; quantum mechanics; molecular orbital and valence bond theories; spectroscopy and structure determination; synthesis and reactivity. A biochemical chapter emphasises enzymes but also considers simulations of the origins of life on Earth. Two final chapters treat the design of a chemical plant and innovation, including some account of economic models. The book is highly recommended both as a fresh look at some familiar topics and as an extension of chemistry beyond a student's normal confines. Modelling is a powerful tool which is prone to misuse especially, physical scientists often feel, in the realms of economics or sociology. Here the authors give clear warnings against unimaginative use and suggest how pitfalls can be avoided, all in a very readable fashion.

Physical Chemistry, by I. N. Levine (McGraw-Hill: New York and London; £21) is a late runner in the valuable stakes to find a successor to Moore's *Physical Chemistry* (Longman: London; Prentice-Hall: Englewood Cliffs, New Jersey), thought by some of the pundits to be ageing and capable of being overtaken. Earlier declared challengers were reviewed in this place twelve months ago (*Nature* 272, 101; 1978.). This new starter for the standard undergraduate course in physical chemistry comes from a successful stable and is in every way a thoroughbred. It is hard to find fault but looking at the field as a whole it is impossible not to choose the rival *Physical Chemistry* by P. W. Atkins (Oxford University Press: Oxford; Freeman: San Francisco) as favourite.

Graham Richards

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Biophysical chemistry

THE two books under review arrived in the middle of the usual self-examination that follows marking terminal examinations and it was with some relief that I found that the problem of catching and holding the interest in physicochemical problems of biologically orientated students must be unsolved as yet. The authors of *Biophysical Chemistry: Principles, Techniques and Applications* (A. B. Marshall; Wiley: New York and Chichester, UK; £13.25) and *Physical Chemistry: Principles and Applications in Biological Sciences* (I. Tinoco, K. Sauer and J. C. Wang; Prentice-Hall: Englewood Cliffs, New Jersey; hardback \$19.95, paperback \$6.78) appreciate that in recent times several textbooks have been produced which offer recipes for solving this problem, but they must feel (as I do) that these texts all have deficiencies. My overall view of these two new textbooks is that jointly they seem to have good mixes which go a long way to solving the problem.

The authors of both books obviously appreciate that the first requirement is to teach through examples taken from biochemistry and biology using wherever possible familiar problems which lead easily into the physicochemical concepts of molecular action. The descriptions inevitably lead to mathematical formalism, so both textbooks give appendices which summarise the essential components of the mathematics. In addition to the numerous solved examples in the texts, both books give extensive lists of testing problems at the end of each chapter, and, unlike some earlier textbooks, both give answers to the problems, although this means purchasing an additional volume to Marshall's book.

Despite their apparent overlap in the intended audience, Marshall's book is a more specialised text for students of biochemistry studying the wide range of subjects which are collectively called biophysics, whereas the second covers all aspects of physical chemistry for first-year students and omits a lot of the physics given by Marshall. In view of the differences in content, this review may serve a more useful purpose if their details are discussed separately.

Biophysical Chemistry is well written and uses many clearly explained examples from biology to illustrate the principles. Generally the approach has been to take a simple concept, develop this and use the models as a basis to discuss a range of topics throughout

the section. A good example here is the description of the motion of a weight on a spring as an illustration of the concepts of harmonic motion. These concepts are then used to discuss the underlying principles of absorption and dispersion of light, scattering phenomena and transient phenomena such as jump kinetics. This section is one of a total of six sections which cover the laws of thermodynamics, hydrodynamics, kinetics, quantum and statistical mechanics, and one must admire the extensive coverage given to each division.

It must be inevitable in a book of this wide range that the same symbols would be used in apparently unrelated proofs, but occasionally this does lead to confusion. For example, f is used to describe hydrodynamic friction as well as a damping constant for harmonic motion: although the two could have been related through polarisability of the molecules (which incidentally only receives a passing mention), this would not be apparent to undergraduates. One further minor criticism was the omission of transport numbers in the treatment of gel-electrophoresis. Surely this concept would have simplified the understanding of disc(ontinuous)electrophoresis and isotachophoresis.

My own personal sorrow was to see the passing of names associated with equations and concepts: the relationship between electromagnetic intensity and square of the amplitude is no longer called the Poynting theorem, nor are the names Lorenz-Lorenz associated with the relationship between polarisability and refractive index. It always seems more human to have these associations, although I realise that the modern trend is to reduce these connections.

Physical Chemistry is structured differently from *Biophysical Chemistry* and covers both the classical concepts of physical chemistry as well as giving a good coverage of quantum and statistical mechanics. As expected from authors of their reputation, the treatment of these two forms of mechanics is particularly good and could be recommended for all first-year science students. At the end of each chapter is a useful summary of the important relationships derived in the chapter. This makes it easy for students to cross-reference between chapters during revision. I was sorry to see that the Gibbs adsorption isotherm was not included, as it is an old friend which can form a sound basis for discussing hydrophobic effects: possibly this is a logical omission, as hydrophobicity was not discussed, nor were the properties of aqueous solutions given special mention.

Both books seem to avoid the thorny problem of concentration dependence of parameters. Experience shows that one stumbling block for most astute non-physical undergraduates studying physical chemistry is the apparent inability of its theories to deal with properties of solutions at finite concentrations; simply assigning the deviations to virial coefficients (which are not mentioned in *Physical Chemistry*) is not sufficient. I had hoped that the authors of these books may have found a satis-

enthusiastic, fying simple solution for explaining these important solute-solvent interactions. Despite this small disappointment, I feel I could strongly recommend my students to use these textbooks in the future, but I suspect that the cost of Marshall's will be the greatest barrier to all but the most S. P. Spragg

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Primary and secondary metabolism

PRIMARY metabolites or metabolites that are essential to organisms by providing materials and energy for their maintenance and growth have traditionally been studied by biochemists. *Primary Metabolism: A Mechanistic Approach*, by J. Staunton (Clarendon, Oxford University Press, Oxford; £6.50), takes a chemist's view of the reactions involved in primary metabolic pathways and, as the title suggests, looks at the steps from a mechanistic point of view.

The text commences with an excellent treatment of the main reactions involved in the pathways. The energetics and mechanisms are explained with particular clarity. The role of ATP and other energy-rich phosphates is considered in detail and this is followed by a study of thioesters including coenzyme A derivatives. Subsequent chapters deal with other important cofactors; thiamine pyrophosphate, lipoic acid, nicotinamides and the flavins are introduced and their catalytic roles explained. Current theories on the organisation of the electron transport system are presented in a clear and convincing manner. Here and throughout the book areas under active investigation are indicated.

Having set the scene and described the principal characters, the author describes some of the major metabolic pathways. The catabolism of glucose and its role as an energy source is first described, then photosynthesis and the anabolism of glucose is considered with emphasis on the energetics. The remaining chapters discuss the use of fatty acids as an alternative energy store, transformations of tetrahydrofollic acid derivatives and amino acid metabolism. Mechanisms are discussed for each reaction and the overall strategy and interlocking nature of the pathways are presented in an admirable way that leaves the reader marvelling at the beauty and intricacy of

the transformations.

The book can be confidently recommended to all students of chemistry and biochemistry as a well balanced and refreshing account of primary metabolism. Research workers will also find stimulating ideas for both biological research and organic synthesis within the book.

Secondary metabolites are those metabolites, often called natural products, that seem to be non-essential to the organism. *Secondary Metabolism*, by J. Mann (Clarendon/Oxford University Press, Oxford; £9.75) surveys our current knowledge of the biosynthesis of these metabolites and emphasises the ecological, toxicological and pharmacological properties of the compounds.

The text begins by introducing some aspects of primary metabolism essential to the understanding of secondary metabolism. The second chapter deals with compounds derived from acetate, including prostaglandins and polyketides. Chemical analogies are given for the more important biological reactions emphasising that any theory of biological transformations must be

based on sound mechanistic reasoning. The third chapter dealing with isoprenoids pays particular attention to the stereochemistry of the transformations. Well established results are used for most of the examples and when a pathway or mechanism is not substantiated this is clearly stated.

Aromatic compounds derived from shikimic acid and the alkaloids are discussed in the next two chapters. Metabolites of particular contemporary interest have been chosen to illustrate the biosynthetic pathways. The treatment of the major classes of secondary metabolites is completed with chapter six which concentrates on metabolites of mixed biosynthetic origin. The final chapter gives an enlightening view of the ecological aspects of secondary metabolites and throughout the book the functions of the compounds are stressed to give purpose to the biosynthetic study.

The text is well organised, covers a large area of natural product chemistry suitable for undergraduates and is a valuable introduction for those beginning research in this field. At the end of each chapter there is a small number of problems selected from the recent literature, and the student is referred to the original literature for solutions. The author stresses that it is not a reference text but there is a comprehensive list of such texts in the list of books and reviews suggested for further reading. Overall this is an excellent book and should be very popular.

R. A. HILL

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Physics applied to biology

BECAUSE of the complexity and number of variables involved in biological phenomena it is difficult to get precise quantitative data on which to base theories of structure, function and organisation. Yet as understanding progresses it is becoming more common to interpret biology in physical terms, and move towards the goal that "all science as it goes to perfection becomes mathematical in form". Physical and mathematical formulation are the inevitable direction in which the biological sciences must develop. There is a growing appreciation of the need to increase the quantitative and mathematical content of courses in the life sciences. To meet this educational requirement a number of books have been published over the past decade at the introductory, intermediate and specialised levels of physics and mathematics applied to biology.

The present text is at the first level, and although it is claimed to be written for degree and diploma students, who only have a knowledge of ordinary level school physics and mathematics, its content is just above that of an advanced level school syllabus. It broadly covers the field given in the first MB syllabus to Medical students, and, like the latter course, attempts to introduce basic physics by graphical methods without the use of calculus. There are a number of good textbooks available in this field and any new addition will as a consequence be subject to very critical appraisal. In curricula content it is well balanced, starting with a chapter on force, motion and energy, followed by chapters on waves, atomic and molecular structure, electricity, light, heat, and finishing with radioactivity and radiobiology. Most of the text is precise, and in respect of descriptive material well presented. It is with the problem of introducing new concepts and conveying an understanding of the principles of physics that the book

is weak. Particular areas that need revision are the meagre introduction to capacitance, inductance and AC theory; the description of phase contrast microscopy with terms such as "this is done by inserting a glass phase ring in plane P", with no explanation of phase rings, will leave students who have only the basics floundering; and in a similar manner the introduction to electron microscopy and the wave nature of moving electrons is badly skimmed. Throughout the text there are curious statements which suggest that the book was written to meet a deadline. On page 5 it is stated, "It is a common experience that when a stationary object is pushed sufficiently hard it moves".

Later (p 35), we have, "The eye is sensitive to light because the retina responds to the electric field". About ionic conduction through membranes it is stated (p 84), "The permeability of the membrane for Na^+ ions is less than that for K^+ and Cl^- ions so that when the fibre is in its normal state there are more Na^+ ions on the outside than inside". There are too many statements of this kind to unreservedly recommend it for reading by biologists as an introduction to physics.

John A. Sirs

John A. Sirs is Reader and Head of the Department of Biophysics at St Mary's Hospital Medical School, University of London, UK.

Insights into biological phenomena

Introductory Biophysics. By F. R. Hallett, P. A. Speight and R. H. Stinson. Pp 243. (Chapman and Hall: London 1978.) Paperback £5.95.

THERE is currently a need for a comprehensive modern textbook on biophysics suitable for university students. There are, of course, many books which are in effect physics primers for biologists, but

what is required is rather a book that illustrates the great insight into biological phenomena that can be gained from the application of physics. This book falls into the latter category. It not only describes the application of classical physics to biological phenomena but also covers the more modern areas such as the biological effects of electromagnetic and ionising radiations and the application of X-ray crystallography to biological molecules. Thus, physics is brought in as a tool to solve problems rather than as an end in itself. One can criticise the book for omission of some important topics—for example, thermodynamics and respiratory processes—but for a book of its size it covers a reasonable range. Furthermore, it is good to find a book aimed at the life science student which does not attempt verbal gymnastics in order to avoid a mathematical description of a process, and one that lays considerable emphasis on problem solving to consolidate concepts in the mind of the student.

Unfortunately, although the book has many good points, it is nevertheless something of a disappointment on two counts. The first is a lack of balance apparent in some of the chapters. It is, for example, difficult to understand how a chapter labelled 'molecular biophysics' can contain a discussion of protein structure and yet not show even one diagram of a protein model, and contain only one paragraph on how the modern understanding of the architecture of such molecules allows an interpretation of their function at a molecular level. Similarly, in the chapter on radiation biophysics, a good section on target theory is followed by a trivial discussion of the actual damage that ionising radiations can cause; furthermore, in such a chapter surely the biological and medical applications of ionising radiations deserve mention.

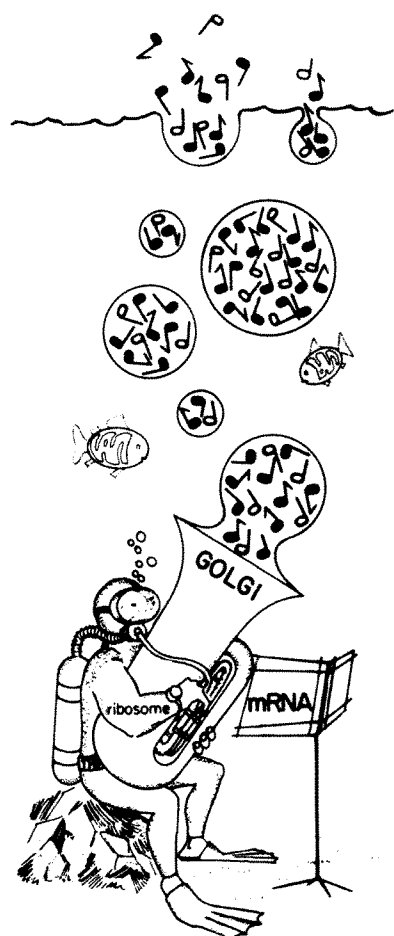
A second area of disappointment lies in the terse manner in which many crucial concepts are covered. This may be acceptable in a revision book, but this is clearly meant to be a primary text

and as such these concepts deserved a fuller treatment. To give only two examples: if, as occurs in chapter 7, it is necessary to have a section explaining the physical meaning of the terms work and energy, then more than the two paragraphs given are needed for adequate coverage. Similarly, in the chapter on heat flow in biological systems the phenomena of convection and radiation are explained at some length but conduction is not explained at all except in so far as the relevant equation is given. Indeed this particular chapter is rather poor because, though many of the ways in which heat may be lost by an animal are explained the control of heat production and loss in animals is described in so disjointed and incomplete a manner that it would be difficult for the student to obtain a coherent picture.

So we are still in need of a really good textbook on Biophysics, for, although this book does contain some very good material, it is frequently marred by omission and inadequate presentation.

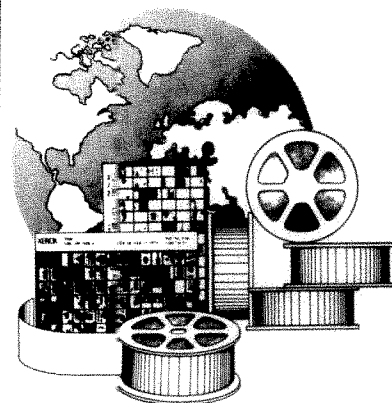
M. W. Rampling

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Taken from Membranes and their Cellular Functions, reviewed on page 88

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Bioenergetics

THE main effort of research in bioenergetics, as shown by a glance through a journal such as *Biochimica et Biophysica Acta Bioenergetics*, is at present devoted to the biochemical details of energy transfer in mitochondria, chloroplasts and bacteria. However, underlying these processes are the basic physical principles embodied in the laws of thermodynamics (from which the concept of energy is derived), and quantum mechanics. These principles are the subject of *Foundations of Bioenergetics* (Academic: New York and London; \$26.50; £17.20), by Harold J. Morowitz.

The teaching (or rather the learning) of thermodynamics always seems to entail special difficulty. It is as if a deep gulf divides the majority of humankind who are baffled by, or ignorant of the subject, and those who feel they understand it. Morowitz leads the way across this gulf with confidence, carefully explaining where all the postulates of this strange discipline come from. Statements are punctuated by (definition), (empirical generalisation), (postulate) or (conclusion). The introduction of the method follows the conventional way of physical chemistry; steam engines and information theory both feature in the description of entropy. The treatment is quite rigorous. Biologists may be daunted by the variety of mathematical methods that are used, from calculus to Boolean algebra, but these are all given some introduction.

The book then continues into statistical mechanics, approached from a quantum mechanical viewpoint. Frequent references are made to the relevance to biological systems, from cells to ecosystems. The power of thermodynamics and statistical mechanics lies in their ability to draw wide-ranging and sometimes unexpected conclusions on topics such as the origin of life. In a section, "Life at absolute zero", he notes that "structure is the result of where the molecules [in a living system] are located, and function is a description of where they are going". From this, and the observation that simple life forms can survive freezing to very low temperatures, we are forced to the conclusion that the process of originating living material reduces to a problem of synthetic chemistry. Finally, it must be admitted, however, that there are severe limitations to the applicability of equilibrium thermodynamics (or 'thermostatics') to biological systems which are in a state of flux, and the later chapters cover fluctuating systems and the approaches to irreversible thermodynamics. These too have their limitations, caused by the complexity and non-ideal behaviour

of living systems, but the book provides a good introduction to these potentially useful methods.

In the 15 years since the publication of Lehninger's classic monograph *The Mitochondrion* (W. A. Benjamin: New York and Amsterdam), there have been considerable advances in our understanding of this organelle. These are documented in many books for the specialist and symposium proceedings, but it is surprisingly difficult for undergraduate students to obtain a straightforward overview of the present state of the subject. *Mitochondria: Structure, Function and Assembly* (Longman: London and New York; £3.75), by Peter A. Whittaker and Susan M. Danks, is designed to fill this gap. In price and length it is the sort of book that students of biology and biochemistry are likely to buy and read through. In condensing a large amount of research data, however, inevitably some of the excitement is lost. In reading the section on the mechanism of coupling between respiration and phosphorylation, there is only a faint echo of the spirited debate that has enlivened the Biochemical Congresses of the past ten years. The three major hypotheses are described and their merits are discussed; and it is con-

cluded that Mitchell's chemiosmotic theory is the most acceptable explanation of the available data (a conclusion recently endorsed by the Nobel committee). Oxidative phosphorylation may be the keystone of mitochondrial function; but despite the attention devoted to it recently we should not forget that many metabolic processes take place within the organelle, and here these are accorded their due weight.

The book is divided into five chapters: structure and isolation of mitochondria; tricarboxylic acid cycle and fatty acid oxidation; oxidative phosphorylation; transport across membranes; and mitochondrial assembly. The descriptions are punctuated by brief details of experimental methods, and general references for further reading are given at the end of each chapter. Advanced students may find that they need a more comprehensive set of references and will need to plunge into the specialist literature. However, this is a well balanced account, up-to-date and without frills. It should be a bestseller.

R. Cammack

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Membrane functions

Membranes and their Cellular Functions. Second edition. By J. B. Finean, R. Coleman and R. H. Michell. Pp. 157. (Blackwell Scientific: Oxford and London, 1978.) Paperback £4.80.

OPENING the first edition of *Membranes and their Cellular Functions* was a great surprise as one found the excellent cartoons by T. A. Bramley. The authors are doing a valuable service in showing the reader that scientists are not deadly serious data-processing robots. We should also remember that students need some light relief during the work of absorbing the great mass of information presented to them. The sympathetic attitude of the authors also comes over in the text; they are not trying to impress with their sophistication but are simply and clearly telling the reader about biological membranes.

The first edition was excellent for supplementary reading but I felt that some of the more molecular aspects received rather scant treatment. In this new edition, although the original material and style has been retained, the subject matter has been re-arranged and not only brought up to date but considerably expanded, particularly in the molecular aspects. For example,

membrane structure and composition, mechanisms of solute movement across membranes—including the ion-translocating ATPases, energy coupling by ion gradients, neuro- and hormonal transmitters, and recognition responses—are now fully described. The result of this revision is the ideal book to cover the membrane section of a first- or second-year course in biochemistry, physiology or cell biology. It fills a gap at this particular level; Harrison and Lunt's *Biological Membranes* (Blackie: Glasgow and London, 1975) and Quinn's *The Molecular Biology of Cell Membranes* (Macmillan: London, 1976) being more detailed and suited to final-year courses, whereas Davies's *Functions of Biological Membranes* (Chapman and Hall: London, 1973) is rather elementary and now somewhat dated.

Our second-year students will be left in no doubt that they should read this book. Any students who have not encountered a course on membranes should read it in the long vacation before a final-year course in biochemistry, physiology or microbiology. Excellence does not mean perfection and there are, of course, some faults. What is called the Nernst equation (p86) is really the Henderson equation for diffusion potentials; the Nernst equation for equilibrium potentials is not given. This omission may explain the misconception on p75 where it is stated that the distribution of Rb^+ ions

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Transport by Proteins

Proceedings of a Symposium, held at the University of Konstanz, West Germany, July 9-15, 1978.
 FEBS Symposium No. 58

1978. 17 cm x 24 cm. XV, 420 pages.
 Hardcover DM 145,- ISBN 3 11 007694 2

This book presents the proceedings (lectures and discussions) of a Symposium on "Transport by Proteins", held at the University of Konstanz. Well-known scientists discuss general problems, selected transport proteins, membranes and transport by proteins, metal transport by proteins and the molecular aspects of transport by proteins in physiology and pharmacology.

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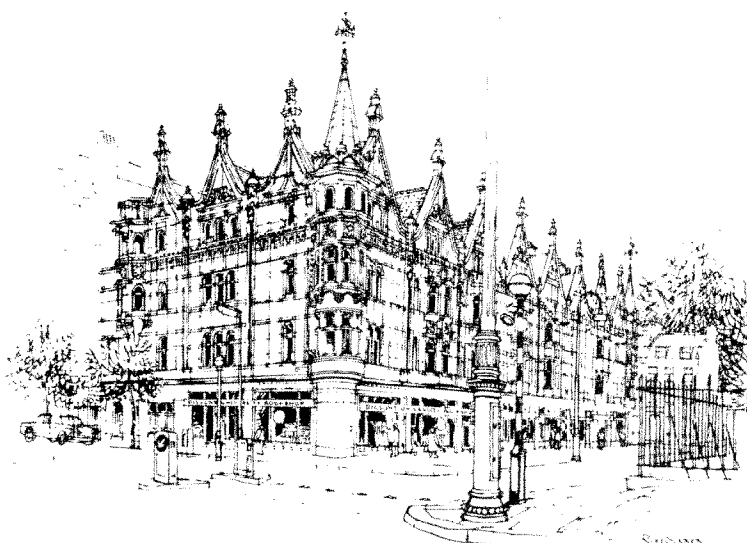
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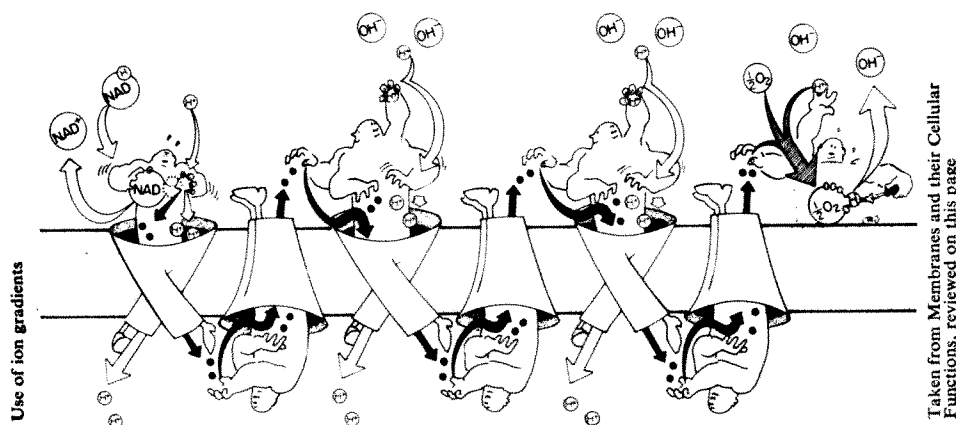
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Use of ion gradients

Taken from Membranes and their Cellular Functions, reviewed on this page

"at equilibrium neutralises the $\Delta\psi$ (membrane potential)"—if it did it could not be used to measure the membrane potential. On p44 the unfortunate Nernst suffers again, $\frac{d\psi}{dx}$ being described as the charge, instead of the electrical potential, gradient. In my own research field, mitochondrial bioenergetics, I would quibble with the authors' selection and acceptance of some of the very recent data. I am sure others will find the same with their own speciality but this will not seriously affect the student or non-specialist reader: if it stimulates discussion with lecturers so much the better.

A more general criticism is that abbreviations tend to be thrown into the text leaving the reader to search

figures or tables to find their meaning. Also, although the lists of further reading at the end of each chapter are suitably short and carefully chosen, I felt that more help should be given to students in relating articles to particular topics. Further use of the wide margins could be made for both this and explanation of abbreviations.

These are all relatively minor criticisms and do not alter my view that this is now a really excellent book. It is accurately printed in attractive typeface on good paper making it, with the authors' style and the illustrations, a real pleasure to read.

M. J. Selwyn

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Biochemistry texts

THREE recent biochemistry texts reflect different approaches to the student market.

The second edition of *An Introduction to Practical Biochemistry* (McGraw-Hill: New York and London, £6) is wide-ranging in its scope, describing in all some 140 simple experiments that can be carried out in any reasonably equipped biochemistry teaching laboratory. It is simply and clearly written. Descriptions of experimental procedures are interspersed with accounts of the appropriate underlying principles. In some cases I feel that this aspect of the book is rather overdone and would best be left to a general textbook. Experiments are adequately described together with lists of quantities of reagents and equipment needed for a given number of students. The book leans heavily to the physical and chemical rather than the physiological side of the subject. There are sections dealing with pH and buffers, separation methods (dialysis, gel filtration, column chromatography, partition chromatography, electrophoresis), colorimetry and spectrophotometry, protein isolation and N-

and C-terminal determination, artificial membranes, nucleic acids (base-composition, isolation and estimation), simple enzyme kinetics and also rather extensive sections dealing with qualitative and quantitative tests for sugars, amino acids and lipids. The final chapter, entitled "Metabolism", contains experiments of the more physiological type including isolation of mitochondria and chloroplasts, adipocyte isolation and metabolism, intestinal transport of amino acids, glucose fermentation by yeast, bacterial growth and β -galactosidase induction and a few whole-animal experiments. In general, quite a good book that is suitable for use in the first two years of a biochemistry degree course.

Basic Biochemistry: A Visual approach for College and University Students (Heinemann Educational: London, £2.90), by J. Edelman and J. M. Chapman, might appeal to some who like a smattering of biochemistry presented in 'comic-strip' form. Personally, I hated it. The book is in three sections. The first deals with the components of the cell, proteins, carbohydrates, lipids and nucleic acids, and in my view is the best part of the book. From this an elementary student should gain some feel for the basic structures in the cell, without gaining

such idea about their chemical properties and reactions or the chemical principles underlying these. The second section deals with enzymes and attempts to explain enzyme kinetics and action with the aid of a multitude of diagrams (which in many cases are rather scrappy) and no mathematics at all. I think a student is more likely to be confused rather than informed by this section. The third and largest section deals with metabolism. Here again, the use of a plethora of rather scrappy diagrams seems more likely to sow confusion than understanding. The treatment of much of intermediary metabolism is scimpy and there is no attempt to set it in a physiological framework. There is also a heavy use of terms such as 'high-energy bond', 'energy release' and 'energy trapping' which often are not appropriate. This book might be suitable for a student with little previous chemistry who is required for some reason to acquire a veneer of biochemistry, otherwise, it is not suitable as a serious biochemistry book.

Amino Acids, Peptides and Proteins: An Introduction (Macmillan: London; £15), by E-D. Jakubke and H. Jeschkeit is really a specialist book which might be of use as a reference text for final-year undergraduates taking an Honours degree in biochemistry or chemistry. Visually, the book is rather unattractive, having plain diagrams and a small type-face. On the other hand, the amount of information it contains is considerable. The book falls into three parts. The first deals with the amino acids; their classification, physical properties, synthesis, analysis and reactions. The second part covers peptides—in particular, synthesis (problems encountered in the latter, general strategy, together with detailed methods). In fact one third of the entire book is given over to peptide synthesis. There is also an interesting section dealing with an extensive range of important naturally occurring peptides. The third part deals with proteins and covers classification, physicochemical properties, isolation and purification, sequence analysis and structure. The text is quite readable and does not seem to have suffered in the course of translation from the German version. The book is interspersed with frequent reference sections which by now are not very up-to-date (1972–74 are the latest references). In conclusion, a useful book, more to be used for reference than as a student's reading book.

E. D. Saggerson

E. D. Saggerson is Lecturer in Biochemistry at University College, London, UK.

Insect biochemistry

Biochemistry of Insects. Edited by M. Rockstein. Pp. 649. (Academic: New York, San Francisco and London, 1978.) \$29.50; £19.15.

IN 1961 Darcy Gilmour's book *The Biochemistry of Insects* (Academic: London) was published and in a text of under 300 pages it covered the available information on insect biochemistry, and included a generous amount of background information on general biochemical principles. The growth of the subject in the intervening years has been prodigious and to effectively review the field in a book that is little more than twice the size of Gilmour's is no mean achievement. The book that Morris Rockstein has edited covers the ground admirably. Its production and format closely resembles the six-volume revision of *The Physiology of the Insecta* produced by the same editor, and publisher in 1974, and these two works will stand as a major source for entomology students for many years to come.

The majority of undergraduates who undertake specialist entomology courses these days will have received some basic biochemical training and this is essential if maximum benefit is to be derived from this book. However, it is not simply an exposition of the biochemical specialisations to be found in the *Insecta*. The authors have each taken the trouble to fit the biochemical facts into the wider biological picture and no matter what aspect of entomology interests the reader, he or she will find valuable facts and ideas within this book.

Inevitably, in any work which is compiled from several contributing authors there is some lack of uniformity in approach. To quote one example, the chapters on carbohydrates and lipids deal with the nutritional requirements for these compounds, and with their digestion and absorption, but no corresponding information is available in the chapters on proteins and amino acids. A lack of uniformity is also evident in the references to original literature, the chapter on insect biochromes having a bibliography of more than 30 general references and about 140 specialist references, whereas that on the toxic action of insecticides has only five general references. The separate listing of general works in the bibliographies is an admirable idea for the undergraduate reader interested solely in reviewing a particular topic. On the other hand the postgraduate reader who consults those chapters where

specialist references are lacking, will require access to several other reference works before the original papers can be tracked down.

This very readable book is a valuable and significant contribution to the entomological literature. It provides important background information for

undergraduate courses and will be a standard reference work for a wide range of professional entomologists.

John B. Ford

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Biochemistry of viruses

The Biochemistry of Viruses. By S. J. Martin. Pp. 145. (Cambridge University Press: Cambridge, 1978.) Hardback £10.50; paperback £3.95.

KNOWLEDGE of the nature and biosynthesis of viruses has progressed to a stage where it is clearly impossible to encompass all aspects of the biochemistry of viruses in the space of 145 pages. The author of this latest volume in the series *Cambridge Texts in Chemistry and Biochemistry* has adopted a sensible compromise presenting a coherent survey of the subject based largely on his own experience and interests. The text progresses from a brief but adequate review of the history of virology through chapters on classification, assay, purification, structure, strategy of infection (that is, transcription, replication, maturation, defectiveness), therapeutic and prophylactic agents, and evolution. The book begins and ends on a philosophical note contrasting the virus as the invader of the genosphere with man as the invader of the biosphere.

The best and most comprehensive portion of the book is the chapter on virus architecture. The chapter on strategy of infection, which takes up 54 of the 145 pages, is well written but is less satisfactory, as it fails to deal adequately with the animal DNA viruses and the RNA tumour viruses. The text also suffers from having been prepared before the application of restriction endonucleases to analysis of

the structure of the genomes of DNA viruses. In my view these deficiencies, together with the virtual exclusion of genetics, will limit the usefulness of this book as a course textbook. It can be recommended, however, as supplementary reading because Dr Martin has a lucid and simple style of writing which provides the hard-pressed student with an excellent example of the organisation and presentation of information in essay form.

Over-simplification occasionally generates inaccuracies such as "all RNA tumour viruses . . . appear to have similar base sequences" (p123); "most DNA viruses contain circular DNA molecules" (p44); and the like. Also a number of errors have slipped through. For instance, part of Figure 5.26 is missing; two eminent virologists have their names mis-spelt; the molecular weight of herpes simplex virus DNA is given variously as 54.92 (Fig. 2.4), 75 (p92) and 80-100 (Appendix) million and that of oncornavirus RNA as 10 million (Fig. 2.5). The retroviruses do not appear in the virus classification in the Appendix, apart from Rous Sarcoma virus which is listed as the prototype of the genus coronavirus of the family rhabdoviridae.

These imperfections will be sufficiently obvious to most readers not to detract from the basic worth of the book as a simple introduction to the biochemistry of viruses.

C. R. Pringle

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Molecular genetics

THERE have been three exceptional textbooks written about microbial genetics. These are *The Genetics of Bacteria and their Viruses* by W. Hayes first published in 1964, *The Molecular Biology of the Gene* by J. Watson first published in 1968, and *Molecular Genetics* by G. Stent which was first published in 1971. These books cover a lot of similar territory, but each has a distinctive flavour. In Hayes' treatment the development of the subject

seems a trifle disorganised in places, but the book is written with a sense of enthusiasm and wonder which is quite infectious. Watson's approach is autocratic, or "sovereign didactic" to quote the felicitous if somewhat ambiguous description from Stent. One has the feeling that no matter how cells originally functioned that after reading Watson's book they would have considered it advisable to conform to the royal dogma. Stent's book is undoubtedly the most literary of the three. He set out to write an introductory narrative and the book tells the story superlatively well.

Of these books Hayes came out in a

second edition in 1968 so is now in need of revision, Watson had a third edition published in 1976, and a second edition of *Molecular Genetics* (Freeman: San Francisco and Reading; hardback £14, paperback £9.70) has been prepared by Stent in collaboration with R. Calendar. The new edition retains the greater part of the original text (605 pages) and adds to it about 150 pages of new material either in the form of extra paragraphs or plates inserted at strategic places in the narrative, or by completely new sections added to relevant chapters. An examination of the small modifications which have occurred to the old text gives a fascinating insight into some alterations which have happened over the years to scientific language interpretation and technique. For example, the heading "The sex factor as an episome" has become "The fertility factor as a plasmid" and in the same vein "The conjugal tube and mobilisation" is now "The conjugal bridge and mobilisation". Also, the classic autoradiograph of a bacterial chromosome by Cairns has been re-interpreted to allow for bidirectional replication, and some unspecified development has introduced substantial changes to the curve depicting the kinetics of ONPG hydrolysis by β -galactosidase resulting in a threefold drop in affinity constant. One sad loss, no doubt inevitable because of cost considerations, is the four colour plates of the original edition, particularly that by I. M. Wallace of eye-coloured mutants in *Drosophila*, which is a work of art in its own right. A less comprehensible omission is that of the Newcombe resspreading experiment. This is the example I would propose to students if asked to name a single experiment as an ideal they should aim for in their research. It is, ingenious, simple in principle and practice, and unambiguously proves its point. When this edition is reprinted perhaps the missing two pages could be added as a supplement by way of expiation.

The more extensive new sections are written in the same style as the original and have up-dated the text on a selection of topics including DNA replication, gene expression, RNA phages, genetic engineering, transcription and translation. However, the limited scale of the revision couldn't hope to do justice to all the advances that have occurred in the past 8 years, so there is little discussion of recent developments in a number of other areas—for instance, in DNA repair, plasmids or transposons. Nevertheless this edition is the most useful text available at present for teaching introductory courses in molecular biology. It is not, however, a contemporary classic like its progenitor was in its time. There is still a real need for a completely new and comprehensive treatment of the subject.

Two books by D. Freifelder are more specialised aids to teaching. *Molecular*

Biology and Biochemistry: Problems and Applications (Freeman: San Francisco and London; paperback £4.10) is a useful text in the manner of *Biochemistry: A Problems Approach* by Wood, Wilson, Benbow and Hood (W. A. Benjamin: New York). Each of 17 chapters starts with a concise introduction to a particular topic, and this is followed by some references and a collection of relevant problems. Answers to the 550 problems are supplied at the back. The book will be of considerable use to both elementary and advanced students.

The DNA Molecule: (Freeman: hard-

back £13.40, paperback £7.40) contains a collection of 44 original articles arranged in sections, each of which has a short preface and is concluded with some pertinent questions and references. The collection has a structural rather than genetic bias and although it will be a useful reference book for microbial geneticists its role in teaching is more likely to be in courses of biophysics or structural molecular biology.

N. Symonds

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Textbooks of genetics

Most textbooks of genetics seem to fall into three main categories: those designed for an educated general readership, those designed for university students taking courses in which genetics is not a major topic (for example, social sciences and medicine) and those designed for students with a specific interest in genetics. However, Sam Singer's slim (139 pages) *Human Genetics: An Introduction to the Principles of Heredity* (Freeman: San Francisco; paperback £3.10) does not fit easily into any of these categories. It is a very nicely presented summary of some of the essentials of human genetics, but it is difficult to know for whom it might be of value. It is too technical for the general reader or advanced school student and yet would be inadequate for an undergraduate course in human genetics. This is a pity because the author is clearly equipped to write a fuller text which undoubtedly would be very appealing to undergraduates. On the credit side I would rank it highly in being remarkably up-to-date, accurate and very readable. The illustrations are simple but of a particularly high quality. This is presumably a reflection of the publishers' association with *Scientific American* which features prominently among the suggested readings at the end of each chapter each of which also concludes with a summary. There is an Appendix of 25 problems (with answers) mainly concerned with what is often loosely referred to as "pedigree genetics".

Two recently published books on population genetics illustrate very well the differing approaches to the subject. One is designed for the relatively non-specialist undergraduate (Bryan Shorrocks; *The Genesis of Diversity*; Hodder and Stoughton: London; hardback £5.75, paperback £2.95), the other for the specialist undergraduate or postgraduate (Eliot B. Spiess; *Genes in Populations*; Wiley: Chichester, UK, and New York; £13.95). I would warmly recommend both of these books to the readership to which

they are directed. Both deal with factors affecting gene frequencies in populations, natural selection, polymorphisms and the underlying genetic basis of individual variations. But whereas Shorrocks concentrates more on the biological aspects of variation and requires no more than a basic knowledge of simple algebra, Spiess' approach is largely based on mathematical reasoning. Regarding the *Genesis of Diversity* most examples are chosen from *Drosophila*, a field in which the author has made his own contributions. Industrial melanism in moths (*Biston betularia*) is also given special attention. It seems odd that there is no mention of Clarke's work on frequency-dependent selection (though the subject itself is discussed), and there is very little about the population genetics of man. The latter is particularly unfortunate because this is an aspect of the subject which is rapidly growing in interest and importance. The presentation is clear and is well illustrated with line drawings and graphs. There is a list of well chosen references and an index. In its field it will have a number of competitors but as a straightforward and well written introduction to the subject it will no doubt be welcomed by many students and their teachers.

Whereas Shorrocks' book can be read from cover to cover in a couple of evenings, Spiess' book would defy any such approach. It is, at least to me, a book to dip into. A knowledge of differential calculus and matrix algebra, though not essential, would certainly make for a fuller appreciation and understanding of the text. There is, however, a very helpful appendix of various mathematical and statistical formulae used in the text. This too is a very attractive book and the way the material is clearly divided into well defined sections is very helpful to the reader. Also, examples are chosen from a wide range of organisms including man. Unfortunately, though each chapter concludes with a number of questions, no answers are provided, which is unhelpful to students and a possible source of embarrassment to teachers.

Alan E. H. Emery

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Contemporary microbiology

Two recently published microbiological textbooks are aimed at opposite ends of the student market. One comes close to the bull's-eye of its target. The other discharges its material with all the accuracy of a sawn-off shotgun. *Essays in Microbiology* (Wiley: Chichester, UK; £13.50), edited by J. R. Norris and M. H. Richmond, is a set of sixteen chapters (not, in my opinion, essays) of exactly equal length. The readership is expected to be "the early stage undergraduate student with some topics of relevance to the senior school pupil". *Companion to Microbiology* (Longman: London and New York; £12), edited by A. T. Bull and P. M. Meadow, consists of nineteen chapters of varying length and format covering a wide range of topics chosen for a readership of "advanced undergraduate and postgraduate students".

The editors of *Essays* have declared as one of their objectives a need to inform the undergraduate embarking on a microbiology degree course just what he is letting himself in for and they wisely achieve a balance between the descriptive and molecular aspects of the subject. The editors of *Companion* have solicited articles from experts in some of the growth areas of microbiology and have included topic neglected in recent textbooks "for discussion in broader perspective than is usual in review articles and in more depth than is possible in a general textbook". The latter formula sounds very much like that of an essay and, indeed, the chapters in *Companion* are very much more like essays than are the sections in *Essays*.

With new texts for the student market appearing with a frequency approaching that of new bacterial species in the hey-day of descriptive microbiology, the reviewer must clearly advise the potential consumer as to whether the various objectives of the editors and authors have been achieved and whether the publishers have been able to offer value for money. It is difficult to avoid a comparative approach when reading two books such as these and, at the risk of being unfair to *Essays*, I feel obliged to comment that *Companion* is more successful in achieving its stated objectives and much better value for money than *Essays*. Having made this judgement, and because the articles in *Essays* are on sale separately I shall devote more space to this volume than to the other.

Professor Stanier's contribution, "What is Microbiology?", maintains the high standard we have come to expect from this source and the article by one of the editors, Professor Rich-

mond, on "Genetic organisation in bacteria and its expression" is equally successful. Four chapters on form and function in bacteria, fungi, viruses and protozoa are of variable quality. Dr Murray's article on bacteria adopts a cytological and ultrastructural approach but there is some careless proof-reading which detracts from its intelligibility in places. The chapter on fungi (Dr Booth) is a dull, detailed descriptive survey which will not generate much enthusiasm for mycology amongst younger readers. The viruses are better served. Professor Watson succeeds admirably in presenting the intricacies of virus structure, particularly that of icosahedral viruses, in a lucid manner. Herpes may not be so simplex these days but the author manages to express the most complex geometrical concepts in a manner which would be understandable to an advanced school student and stimulating to a final-year undergraduate. Alas the protozoa suffer much the same fate as the fungi, although the authors (C. R. Curds and C. G. Ogden) are mercifully less obsessed with taxonomy than Dr Booth. The chapter begins rather well with the taxonomy compressed into a page of suitably small print, but one soon encounters phrases like "In tectinous allogromiid foraminifera . . ." without either warning or explanation. Such experiences tend to have a somewhat anaesthetising effect on the non-specialist.

One curious feature of these two books is that one of the co-editors of each has an article not in his/her own book but in the other, which all goes to emphasise what a small universe we microbiologists inhabit. Thus Professor Norris writes for *Companion* and Dr Meadows, a co-editor of *Companion* writes on "Chemistry and composition of micro-organisms" in *Essays*. Her contribution gets to grips with the structure/function relationship very well and a reader with a good background in structural biochemistry could gain much by reading it. However, readers with ordinary level German will be surprised to learn that the nomenclature of O and H antigens derives from "the German *ohne Hauch*, without whip"! I think that the intended readership would find Professor Tempest's approach to "Dynamics of microbial growth" rather dull and theoretical. He considers the ecological implications of substrate-limited growth only very briefly and some mention of the growth rates of organisms in their natural environments or the implications for industrial microbiology would have made the chapter more interesting. "Intermediary metabolism" is covered by Professor Clarke in a clear and interesting account which is well-directed at the target set up by the editors.

Professor Sneath has been permitted two bites of the cherry with separate chapters on "Classification of Microorganisms" and "Identification of Microorganisms". Both these articles are at an advanced level inappropriate for this collection. They would in fact have fitted in much more comfortably in *Companion*. Professor H. Smith's chapter on "Determinants of Microbial Pathogenicity" is a good summary of the field and its future prospects but is necessarily rather superficial. Some line drawings, diagrams or photographs would have added to its impact. The rather more specialised topics, "Interactions between phage and bacteria" (Kay), "Resistant forms" (Slepecky), "Symbiosis in the microbial world" (Smith) and "Bacterial nutrition" (Whittenbury) will find interested readers at several levels.

As the individual contributions to *Essays* were written to be available separately some duplication of material was inevitable and even desirable. Nevertheless there is too much of it. Some authors show an awareness of the content of the contributions of others, but in other cases there is needless repetition. In contrast there is little duplication in *Companion* and some useful cross-referencing between chapters. The editors of *Companion* did not impose fixed-length or other formats upon their contributors but the book achieves a remarkable cohesion. The print is small but very clear and the quality of the illustrations and photographs excellent. The nineteen topics chosen reflect to some extent the idiosyncracies of the editors (or those of their friends) but the book is none the worse for that. The topics range wide and most are of direct relevance to contemporary microbiology courses. It is a pity therefore, that they too cannot be purchased as separate offprints by students. However, I feel much more confident in recommending the whole of this book as a cohesive collection than I do about *Essays*.

At £13.50 *Essays* is rather poor value as the articles are of variable quality and aimed at too many targets, but I do recommend teachers and students to look it over and purchase the offprints which interest them. Best buys are Stanier, Richmond, Clarke, Watson and Slepecky. At the lower price of £12 *Companion* is excellent value. The size and format allow for the text to be approximately twice the length of that of *Essays* and the editors and publishers have turned out an attractive authoritative book.

G. E. Mathison

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Microbiological dictionary

Dictionary of Microbiology. By P. Singleton and D. Sainsbury. Pp. 481. (Wiley: Chichester, UK, and New York, 1978.) £17.50.

It seems to me that two sorts of useful dictionary could be compiled for microbiology. The truly comprehensive dictionary would be a large scholarly work and probably could only be written by five or six people. But to have a single reference work for the whole terminology of this subject (stretching from phytoplankton ecology to the molecular biology of animal viruses) would be of enormous value. The second sort of dictionary is not comprehensive and is of little use to the professional microbiologist but can be of great value to those who lack a microbiological background yet who need to know about some aspects of microbiology in association with another main interest.

The authors' preface indicates that this dictionary is intended for undergraduates and postgraduates in microbiology but I think it is only likely to find a place in my second category. Microbiology students would find little

in the dictionary which was not covered at least as well by their textbooks, even if they have only a very small collection. The complete absence of any references to source of information or to more detailed literature would also reduce the use which could be made of the contents.

The authors have set out to cover "pure and applied microbiology, biochemistry, immunology, genetics and . . . microbiological aspects of allied subjects such as medicine, veterinary science and plant pathology". This is probably too wide a range of material to encompass in less than 500 pages and has led to omissions. Although I was not surprised that there is no entry for *Sterigmatomyces*, it is unfortunate that for instance *Trichoderma* and *Dunaliella* are not included; that is, the choice of organisms has been rather arbitrary, with some very obscure genera included and some important or commonly used ones left out. Comparable examples are the absence of bacteriophages μ and P1, which have an important place in microbial genetics.

The coverage of biochemical terms may equally be criticised for the lack of entries concerning microbial products that are of interest in a wider context, such as pronase, subtilisin and taka diastase. A much fuller account of restriction endonucleases would also have been a timely improvement.

The cross reference of one entry to another is not thorough. For instance, although the entry "red tides" attributes this phenomenon to the alga *Gonyaulax*, the entry "*Gonyaulax*"

makes no mention of red tides. Similarly, although the Helmstetter-Cooper model of the bacterial chromosome replication cycle is described at some length under 'growth (bacterial)', the entry "Cell cycle" does not refer to it. Indeed, the entry for "Cell cycle" gave me the clear impression that this term is only used with reference to eukaryotic (usually mammalian) cells, which is misleading.

Coverage of growth physiology and fermentation is poor, with a wide variety of omissions. There is no mention of substrate-accelerated death, maintenance energy or fed-batch culture, which are the kind of terms which non-microbiologists are most likely to seek for definition in a subject dictionary of this sort. The description of continuous culture is very sketchy.

An appendix of metabolic pathway schemes is included, which is a laudable idea, but again the selection is not beyond criticism. Why the tricarboxylic acid cycle is in this appendix whereas the reductive pentose phosphate cycle is in the main body of the text (under "Photosynthesis") is not obvious.

Recognising the limitations of space I feel that many will share the view that too much space has been devoted to material which can easily be found in the literature of biochemistry, genetics and general biology at the expense of many microbiological terms and ideas which could so usefully have been included.

Christopher F. Thurston

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Basic phycology

In the introduction to *Introductory Phycology* (Wiley: Chichester, UK; £12.25), F. R. Trainor stresses the importance of consulting more specific works in order to learn how new information is added (to the science) and older facts and interpretations are modified or discarded. Such an outlook was clearly foremost in the author's mind when writing this book for throughout the book the different subjects are suitably placed in their historical perspective and questions are raised in areas deserving of attention in a way that a student might identify with the specialist researcher. The book is perhaps suitable not so much as a reference text but to be read straight through as an introduction to the subject. As such it is well conceived. The author succeeds in conveying his interest and enthusiasm with style and the layout has obviously been

given a good deal of consideration.

Following two introductory sections each of the algal groups is considered separately in twelve chapters, which should give the student a fairly comprehensive account of algal forms. In each of these Trainor deals with the morphological organisation, the characterisation and the reproduction of the class before considering representative genera and other features of special interest. The classification of each group has been deliberately kept to a minimum. Although this is laudable in many respects it might serve to confuse a student particularly in a group like the Chlorophyceae.

The succeeding chapters deal with algal habitats and distribution, classification and morphological variability of benthic organisms, the plankton and their environment, algal control, their usefulness and harmful effects, and finally their culture. In the course of these a good deal of interest is generated and many aspects are treated in

some detail, for example, the section concerning algal products. In the ecological sections the remarks on freshwater and marine environments are nicely balanced and indeed the algae discussed will be familiar to or soon encountered by students in the UK. *Introductory Phycology* is nicely produced and surely a well worthwhile investment for the would-be phycologist.

Illustrations are plentiful both as line drawings and half-tones, a few of which have their limitation. In this respect the book suffers a little by comparison with H. C. Bold and M. J. Wynne's *Introduction to the Algae* (Prentice-Hall: Hemel Hempstead, UK; £16.80), a work which is also a little more detailed in the taxonomic section but which lacks the very interesting general chapters of Trainor. Bold and Wynne's book is subtitled *Structure and Reproduction* and in the preface they declare that the reason for this emphasis is the existence of suitable treatments of algal physiology, biochemistry and genetics elsewhere. It is surprising therefore that the book lacks much ecological information, considering the fact that no equivalent text on algal ecology has been published recently. As an introduction it may for this reason fail to present the algae in their proper biological perspective.

Following a detailed introduction there are nine chapters devoted to the different algal taxa, followed by an appendix dealing with algal cultivation in the laboratory. Sadly Bold and Wynne do not consider whether the prokaryotic 'Cyanochloronta' should be included among the otherwise totally eukaryotic algae, although it would take courage to exclude the group from an algal textbook. However, this approach is consistent with the rest of the book in which the authors have lumped together taxa which would be kept apart by many other workers. For example the inclusion of the prasinoflagellates within the Volvocales may surprise some students and the grouping of the Chrysophyceae, Prymnesiophyceae, Xanthophyceae, Eustigmatophyceae, Bacillariophyceae and Chloromonadophyceae in the Chrysophycophyta on the grounds of similarities in storage products and general pigment composition will be regarded as a retrograde step by many phycologists.

Each chapter consists of general information at and above the ordinal level and a more detailed treatment of representative genera. Regular inclusion of dichotomous keys will help the student to recognise characters of diagnostic (and taxonomic) importance provided it is appreciated that the keys

are not comprehensive. It is a pity, however, that this format is not adhered to in all the sections, for example, those concerning the Cryptophycophyta and the Bacillariophyceae. The treatment of the diatoms is particularly disappointing and only about 20 pages are devoted to this group compared with 33 pages for the dinoflagellates and 186 for the Chlorophycophyta. Admittedly there is not a great deal of variation in basic diatom morphology but no genera are dealt with in detail and there is not one good illustration of a raphe.

Introduction to the Algae has as

Ecology textbooks revived

SINCE the population explosion of ecology textbooks some five or six years ago, there has been something of a lull in the field. Presumably the market has been saturated and the plethora of books then produced have entered the cut and thrust struggle for existence. During this time, book prices have risen sharply, and many of the texts by such authors as Ricklefs, Colinvaux, Krebs, McNaughton and Wolf have passed beyond a level where the undergraduate is likely to be able to afford them. As a result, my impression is that such authors as Odum and Komondy have risen like a phoenix from the ashes to re-establish themselves, as a consequence of their low price. Some new invaders in the field are reviewed here.

One of the current releases in Britain is R. E. Ricklefs' *The Economy of Nature* (Cheron, Oregon: £6.75). This, in fact, made an earlier appearance in 1976, but with Blackwells taking over its British distribution, it is more likely to make an impact on its second invasion. It was designed to bridge the gulf between the lengthy ecological tomes and the brief, introductory paperbacks. It is lucidly written, well produced and illustrated, and covers a very broad field from soils and plant geography to environmental physiology, population dynamics and community energetics. Refreshingly, it steers clear of the 'environmental crisis' syndrome. It is essentially very basic and descriptive in its aims. It avoids numerical data; even population dynamics are discussed without reference to mathematical formulae. In my opinion this is a disadvantage, but it may prove a useful way into ecology for those without a scientific background such as some students in the

social sciences, and some of those taking such courses as environmental studies may appreciate its simple approach.

Serious, potential ecologists I would direct elsewhere, possibly to Ricklefs' larger book *Ecology* (Nelson: Nashville, 1973).

J. L. Richardson's *Dimensions of Ecology* (Williams and Wilkins: Baltimore) also starts from a very basic level and avoids numbers wherever possible. Rather than attempting the very broad coverage of Ricklefs, Richardson picks out certain habitats and deals with these in some detail—namely, deciduous forest, fresh water habitats and deserts. Unfortunately, from a European point of view, these chapters have a very parochial, North American flavour. No doubt they will be far more valuable on that side of the Atlantic. The production quality of this book is not what it might be; many figures are very poorly produced. The style is extremely informal, even chatty, to which I have no objection, except where it leads to inaccuracy.

For example, when discussing the influence of succession upon biogeochemical cycling in forests, the author states that "we see ecosystems performing excellent plumbing jobs on themselves, repairing leaks in an efficient and seemingly co-ordinated fashion". Apart from the garish anthropomorphism of this statement, it implies (as does its full context) that the "leaks" are fully "repaired" at the climax. This, of course, is not the case, for nutrient discharge must increase as ecosystem growth (and nutrient demand) diminishes.

R. S. De Santo's *Concepts of Applied Ecology* (Springer: Berlin and New York: DM28, \$14) is another attempt at a basic, introductory text. It has certain unique features some of which could have been more fully developed with profit, such as the section on animal and plant physiology. A large portion of the book is given over to terrestrial, freshwater and marine

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THE PHLEBOTOMINE SANDFLIES (Diptera: Psychodidae) of the ORIENTAL REGION

By D. J. Lewis

*Bulletin of the B.M. (N.H.)
Entomology series Vol 37 No 6. 1978.
128 pp including 345 references,
273 text figures and 13 maps, paper
covers.*

£17.85

This first survey of the known Oriental sandflies includes a key for use as an aid to identification in conjunction with original descriptions which are indicated by numerous citations. Descriptions include much information on the skin-piercing stylets which are related to kinds of host animals and thus link taxonomy with functional morphology. Study of the cibarium or buccal cavity of several species sheds new light on the structure of this organ which is important in the classification and blood-feeding process of sandflies. Special attention is paid to the vector of visceral leishmaniasis or kala-azar in India, *Phlebotomus argentipes*, which shows regional variation. This paves the way for cytotaxonomic work in relation to localisation of man-biting and disease transmission by this species. The known Oriental distribution of all species is shown by detailed lists and maps. There are sections on zoogeography, host-animal records and seasonal prevalence. A review of medical importance includes a summary of the relation of *P. argentipes* to kala-azar which is now causing an alarming epidemic in India.

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P. W. Atkins

'It is a book of its time not only in content and outlook but in format and style too . . . could well become a milestone in the development of textbooks of physical chemistry.' *Chemistry in Britain*. 'An important and original work.' *Education in Chemistry*. 'The author is to be congratulated on the production of such a good text and the publishers for its presentation at a price within the reach of students.' *British Book News*. £15 paper covers £7.95

Primary Metabolism

A Mechanistic Approach

J. Staunton

The traditional line of demarcation between biochemistry and chemistry has dissolved in recent years as chemists and biochemists have joined forces in investigating the mechanisms of reactions which take place in living systems. This book introduces this area of common interest to students of both chemistry and biochemistry. £6.50 *Oxford Chemistry Series 29*

Secondary Metabolism

J. Mann

'It is encouraging to see published a book such as Dr Mann's, which is designed for the undergraduate and beginning researcher. His book is very well put together and should facilitate and encourage the preparation of undergraduate courses on modern natural products chemistry.' *Chemistry in Britain*. £9.75 *Oxford Chemistry Series 27*

Numerical Solution of Partial Differential Equations

G. D. Smith

This substantially revised second edition covers linear and non-linear parabolic equations, the analysis of errors, first- and second-order hyperbolic equations, elliptic boundary-value problems, systematic iterative methods, consistent orderings of matrices, and recent methods for large linear systems. Second edition illustrated £9 paper covers £4.95 *Oxford Applied Mathematics and Computing Science Series*

The Bowels of the Earth

John Elder

This paperback reprint has replaced the boards edition which was published in 1976. 'Original, persuasive, intensely individual . . . the book is plainly a *tour de force*, and it may set a new model for scientific exposition . . . The prose . . . is direct and evocative.' *Scientific American*. Paper covers £2.95

Human Growth and Development

Wolfson College Lectures 1976

Edited by Jerome S. Bruner and Alison Garton

These papers explore some of the biological, social, and political factors involved in growing up in modern society. They indicate the strong admixture of biological and social determinants that influence behaviour during child development, and two main themes run through the book: the importance of achieving social connection, and the active nature of childhood. £4.25 paper covers £2.25

Mechanics and Motion

L. Mackinnon

This book, based on the first-year course given by the author at the University of Essex, provides a survey of those topics that are an essential background to modern physics and advanced engineering. Throughout, the emphasis is on the basic physical concepts necessary to a study of the more advanced courses that will follow. £6 paper covers £3.25 *Oxford Physics Series 17*

The Properties of Nuclei

G. A. Jones

This book describes the properties of nuclear levels, starting with stable states and progressing, via quasi-stable states (radio-active ground states and nuclear-stable excited states), to unstable (nucleon-emitting) states. The shell model, the collective models, and the optical model of the nucleus are described, and nuclear reactions are introduced in terms of the compound nucleus and direct processes. £5.95 *Oxford Physics Series 12*



Oxford University Press

habitats, but the most distinctive chapters are those dealing with the construction of an Environmental Impact Statement and with toxic compounds in the environment. The former, in particular has an emphasis on the practical issues of ecological survey, which undoubtedly stem from the author's personal experience. The other two unique features consist of a table of conversion factors 28 pages long (it even contains leagues to miles, and kilderkins to metres), and a glossary of 89 pages, which could be regarded as a dictionary of ecology. The latter is reasonably extensive in some areas, but others (for example geomorphology and soils) are not adequately covered. Some definitions are far too vague, for example marsh, bog and fen appear synonymous ('swamp', it seems, is dominated by trees).

Finally, we have two new plant geography texts. The bulk of R. Daubenmire's *Plant Geography* (Academic: New York and London: \$21.50, £15.25) consists of descriptions of plant communities in relation to climate and soils, with an almost complete concentration on North America. Only in the tropical section does Daubenmire step beyond the United States to any significant degree, and here the emphasis is mainly upon Mexico. Within these geographical constraints, however, the author has produced an interesting and informative book. It is obviously aimed at giving the North American student an understanding of his country's natural vegetation. Introductory chapters set the scene in terms of plant dispersal and limits and also provide a condensed account of the history of North American vegetation. It fills a gap in the literature and will undoubtedly be welcome in the USA.

A. S. Collinson's *Introduction to World Vegetation* (Allen and Unwin: London; £6.50) has a somewhat misleading title, for the bulk of it is concerned with general plant ecology, and only the last one third is devoted to the subject of the title, *sensu stricto*. All of the topics dealt with in the first part are treated more fully and more effectively in a variety of other texts, but the second part makes a real and useful contribution to the undergraduate literature. It deals with the world's major vegetation formations, but instead of listing plants, as so many texts do, it collates much interesting ecological information derived from current literature. Such features as soils, variations in vegetation with topography, productivity, and nutrient cycling processes, receive some prominence in Collinson's treatment. It is a shame that more space in the book was not given to this section and less

to the rather superficial account of plant ecology.

It is probably a semantic impossibility to define a vacant niche (in the Hutchinsonian sense), but there does seem still to be a vacancy in the ecology textbook scene for a low-priced, brief, and up-to-date introduction to the subject from a biological point of

view. Odum's *Ecology* (Holt, Rinehart and Winston) and Kormondy's *Concepts of Ecology* (Prentice-Hall) still hold their own in this sphere, but this may in part be due to lack of competitive challenge.

Peter D. Moore

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Population ecology

ECOLOGISTS, in seeking to explain the distribution and abundance of organisms, must be versed in a wide range of skills that embrace both theory and observations made in the laboratory and field. As in most other branches of science, they must understand the principles upon which their subject is founded and must know how to collect and analyse useful information. Sometimes this information is further used in the development of mathematical models, and all ecologists should be at least aware of the aims and limitations of modelling in ecology. The complete ecologist's library thus includes works on the principles of population and community ecology, on sampling, statistics and the techniques of analysis. The quartet of books to be reviewed here conveniently spans this range.

There is now an abundance of general textbooks of ecology, which usually encompass much the same material treated in similar ways. *An Introduction to Population Ecology*, by G. Evelyn Hutchinson (Yale University Press: New Haven and London; £12.60) does not fall into this category. It is a personal view of the subject that will delight and stimulate ecologists from the undergraduate level onwards. The principles underlying population growth, mortality, natality, competition and the niche are all presented with masterful clarity as befits one of the founders of modern ecology. But this is no abstract discussion of ecological theory. The wealth of examples from both animals and plants place the emphasis firmly in the real world, and at the same time serve to show how valuable can be the basic tenets of ecology when properly wielded. The flaws lie in what is not included. Predation is as important an ecological process as competition and yet is quite inadequately treated in five pages. As disturbing in an 'introductory' text, is the complete absence of any discussion of dispersal, migration and the general importance of spatial heterogeneity. But what singles this book out more than anything else for me, and will I think endear it most to its readers, is

the mine of historical information that runs parallel with the main text in 388 footnotes. Many of these are just bibliographical references, but others are essays in themselves—such as that on the origins of ideas on territoriality that occupies over two full pages of text, and the delightful cameo portraits of William of Ockham, Malthus, Verhulst and many others. The material in these footnotes is wisely included in the main index which leads to such unlikely entries as "Hortense, Queen", "Josephine, Empress" and "Mussolini, B."

The second book in this quartet, *Guide to the Study of Animal Populations*, by James T. Tanner (University of Tennessee Press: Knoxville; \$8.95), makes a good companion to the first. It deals with the methodology underpinning the study of populations, with chapters on sampling and estimating density, sex and age composition, population growth, mortality and survival. For the first time since 1966 when T. R. E. Southwood's book on *Ecological Methods* first appeared (Methuen; and now extensively revised in a second edition by Chapman and Hall), there is a companion on the same general subject. At about one quarter the length (but only about half the price) and with a very much smaller bibliography, it can be no substitute for Southwood's classic work. It is, however, very clearly written and, by the use of carefully worked examples, presents the analytical techniques such that they may be easily understood and applied. There is for instance a very clear treatment on the derivation of instantaneous birth and death rates and how they may be estimated from data. The book concludes with a useful chapter on the mathematical relationship between population parameters, followed by a short compendium of some different kinds of population model.

A more detailed treatment of mathematical models is to be found in *An Introduction to Systems Analysis: With Ecological Applications*, by John N. R. Jeffers (Edward Arnold: London; hardback £10; paperback £5). This book explains well the objectives of systems analysis and guides the reader through the tangle of terms for

different kinds of model. There are deterministic and stochastic models, descriptive and predictive models, dynamic models, compartment models, matrix models, multivariate models, optimisation models, game theory and catastrophe theory models, all illustrated with particular examples. For a brief flavour of these, I can think of no better text. My main criticisms are aimed at the ten tables with computer listings. These convey little useful information and the space could have been more profitably used.

The final book in this selection, *Statistics*, by D. Freedman, R. Pisani and R. Purves (W. W. Norton: New York; \$13.95, including an *Instructor's Manual*), is a general statistics text, and not aimed especially at biologists. It fits into this quartet only in that a good grounding in statistics is important for any ecologist. The book is intended for "nonstatisticians who want to learn some statistics in order to go about their affairs", and relies on the plentiful use of exercises and a smooth narrative. The authors' have made no

concessions at all to what they call "pluginski", the rather mindless application of a statistical technique to a problem, and have whenever possible banished equations from the text. The result approaches 600 pages of text and notes with an almost complete absence of the different computational recipes needed in the application of everyday statistics. This over-reaction, together with the neglect of any treatment of the Poisson and negative binomial distributions that are so important in ecology, leaves a book ill-suited to the ecologically minded student.

For a concluding remark, I return to Hutchinson's *An Introduction to Population Ecology*. It stands apart from the rest as a work of distinction, that should be studied for its scientific content and savoured for its recondite footnotes.

M. P. Hassell

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Sociobiological literature reviewed

THE three books reviewed here all reflect the recent growth in sociobiology. They are, nevertheless, very different in character. *Readings in Sociobiology* and *The Sociobiology Debate*. *Readings on Ethical and Scientific Issues* are collections of reprinted papers (there also are some new articles in the latter volume), while *Sex, Evolution and Behavior* is a new textbook written at a level suitable for first- and second-year undergraduates.

The contrast between the two sets of readings is graphically demonstrated by the fact that only two out of 60 articles in the two are identical: Wynne-Edwards on group selection and Trivers on reciprocal altruism. *Readings in Sociobiology* (Freeman: San Francisco and London; hardback \$18, £9.50; paperback \$9, £4.50), edited by T. H. Clutton-Brock and P. H. Harvey, is aimed at undergraduate or graduate student audiences, and it covers the subject of sociobiology as understood by most academics working in the field. Concepts such as inclusive fitness, evolutionarily stable strategy, parental investment, and cost-benefit ratio crop up on every other page. *The Sociobiology Debate* (Harper and Row: New York and London; \$12.95, £7.95), edited by A. L. Caplan, is largely about the subject as

seen through the eyes of the popular press, politically motivated scientists, and some philosophers of science. Biological determinism, sociobiological theory, pluralism, and Science for the People are phrases which give an idea of its contents.

Both books have their place. The collection edited by Clutton-Brock and Harvey is well chosen and a fair representation of the current state of the field. Important subjects such as altruism, sexual selection, game theory and comparative socioecology are included; and Trivers, Hamilton and Maynard Smith between them wrote half the reprinted papers. There are brief and pithy introductions by the editors to each of the four sections. The collection reflects the bias in the sociobiological literature as a whole towards theory at the expense of data. Only three out of 18 papers are specifically concerned with testing ideas (Bertram on llons, Packer on reciprocal altruism, Clutton-Brock and Harvey on primate socioecology); and no experimental work is included. The book should be useful as an adjunct student text in places where the original journals are not available.

Caplan's book is, I assume, aimed at a different audience, the nature of which is not clear. The papers are in five groups: older writings of T. H. Huxley, Kropotkin and others; 1960s 'human ethology' (for example, Lorenz); philosophers' comments; the biology of sociobiology (Hamilton, and others); and two long sections on the polemics arising out of E. O. Wilson's book. These last two

sections are the core of the collection. There is probably some value in a number of book reviews, short comments, and the Wilson versus Science for the People exchanges collected together; however, the overall result has little coherence. Editorial summaries might have helped, but Caplan's own contributions are sometimes confusing in themselves. This is illustrated by an amusing juxtaposition at the start of the book. E. O. Wilson writes in his Preface "Sociobiology is a discipline, not a particular theory". Two pages later, Caplan starts his introduction by saying of sociobiology "The very boldness of the theory . . .".

M. Daly and M. Wilson's clearly written book (*Sex Evolution and Behavior*; Duxbury Press; North Scituate, Massachusetts) does more to disperse the clouds of confusion, both deliberate and unintentional, about what sociobiologists have said, in particular the little they have said about human behaviour. After a brief introduction to modern ideas about natural selection, the book races through the cost of sex, sexual selection, parental investment, mating systems, life-history strategies, the physiology and ontogeny of sex differences and finally human sexual behaviour. The style of writing is direct and easy to follow; the contents accurate within the constraints of an elementary treatment. A wide range of literature including evolutionary ecology, anthropology, physiology, psychology and sociology is discussed.

Daly and Wilson are strong advocates of the view that sociobiology has a lot to say about human behaviour. Those who think that this is an overenthusiastic standpoint will at least find that Daly and Wilson summarise their ideas with clarity. I quote from their last chapter. In discussing a statement of the Science for the People Sociobiology Study Group they say "Consider the following excerpt from a statement recently signed by thirty-five authors, some of them well-known scientists:

... determinists assert that the possibility of change in social institutions is limited by biological constraints on individuals. But we know of no relevant constraints placed on social processes by human biology. There is no evidence from ethnography, archaeology or history that would enable us to circumscribe the limits of possible human social organisation."

What can this mean? If human gestation, infantile helplessness, language capacity, sensory systems, cognitive abilities, maturational rates, senescence, and so on, are not "relevant constraints placed on social processes by human biology" whatever could be?"

John R. Krebs

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Marine science

An Introduction to Marine Science. By P. S. Meadows and J. I. Campbell. Pp. 176. (Blackie: Glasgow and London, 1978.) Paperback £5.25.

THIS is the latest addition to Blackie's *Tertiary Level Biology* series of textbooks designed for course options at advanced undergraduate level in universities and polytechnics. The word *Introduction* in the title should be interpreted in this context: the book assumes that its readers possess a good biological knowledge and a reasonable background in physical science. Advanced school students, first-year undergraduates and interested non-specialists would find R. V. Tait's *Elements of Marine Ecology* (Butterworths: London, second edition, 1972) an easier and more congenial first book on marine science.

Meadows and Campbell's book covers a wide field, both geographically and scientifically. Consideration of the marine environment ranges from the poles to the tropics and from abyssal depths to mangrove swamps. There is a very short introduction indicating the extent and characteristics of the oceans and of the ecological zones (with their associated biotic communities) that are described in later chapters. This is followed by a description of water circulation and movement, and the more important physical and chemical processes that occur in the sea. Intertidal regions, estuaries, the sea floor and tropical inshore regions are then treated in some detail with respect to geomorphology, ecological significance and interactions between the plants and animals that live there. Collecting gear and methods of investigation are described and illustrated. There is a special chapter on "Productivity, Plankton and the Pelagic Environment" and another on fisheries and farming. A good bibliography has been classified into reference books, popular books, marine review journals, abstracts, and research journals concerned with marine science. There is a comprehensive index with useful cross references.

The style of the book is very terse. A great deal of information has been packed into a short space by making the text and illustrations interdependent rather than complementary; imaginatively conceived diagrammatic presentation of facts and concepts is used to save more lengthy explanations. In consequence both text and illustrations require close attention from the student. Some unwelcome

effects of compression are evident: the book is rather uneven, physiological considerations receive scant attention and some sections—for example, that on fisheries—are too short to give a representative view.

I believe that this textbook will be a valuable adjunct to taught courses in marine science; it will give serious

students a good follow-up to lectures, and guide and encourage further reading. It deserves a place in libraries and on student bookshelves in many countries.

A. B. Bowers

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Freshwater biology

Biology of Fresh Waters. By P. S. Maitland. (Blackie: Glasgow and London, 1978.) Hardback £11.50; paperback £5.75.

Biology of Fresh Waters is one of a number of titles in a series called *Tertiary Level Biology*, which covers selected areas of biology at advanced undergraduate level. Although designed specifically for course options at this level within universities and polytechnics, the series will be of great value to specialists and research workers in other fields who require a knowledge of the essentials of a subject.

There are few, if any, books dealing with this subject in a comprehensive manner for undergraduates; this book is therefore a welcome addition to the list of available textbooks for biology students. The author has had considerable experience in both teaching and research in this field and has put this to good use in compiling this work.

After dealing with the physical, chemical and biological characteristics of the freshwater environment he introduces the reader to the plant and animal kingdom in a most thorough and concise manner, which is of great value to the student of both biology and ecology. In these days of early specialisation few students get the necessary basic grounding in plant and animal taxonomy and morphology, and the author has appreciated this in providing a 35-page summary on this topic.

Having provided the foundation to the subject Dr Maitland goes on to consider standing and running waters; here, there is inevitable repetition from

chapter 1. In this respect it is difficult to know whether or not the book has necessarily been produced with the chapters in a sequence to avoid repetition and produce a smooth continuity. The remaining chapters are: field studies; adaptation to environment; communities and energy flow; and fresh water and man—all admirable topics but not necessarily introduced to the reader in the right order.

Although freshwater biology has been the subject of many books designed for various levels of readership, quite often the same illustrations appear with a monotonous regularity. It is therefore refreshing to see new, and in some cases original, figures, diagrams and tables, and the author has used some of the results of his own research on the River Endrick and Loch Leven with good effect. The drawings of plants and animals are particularly good but those illustrating various types of equipment are poor (Fig. 5.3, 5.4, and 8.2) and in some cases naive (Fig. 5.15) or unnecessary (Fig. 8.5).

Man's influence on the freshwater environment has been given a wide, though uneven, coverage. Although this subject must necessarily be brief, a few more references to further reading could have been given to the reader who had an interest in any of the subjects covered, which include fish-farming, hydro-electricity, navigation, pollution, recreation and agriculture.

Dr Maitland should be congratulated on producing a very valuable student handbook on freshwater biology which will be of use to students both in the British Isles and abroad.

Derek Mills

Derek Mills is Senior Lecturer in freshwater ecology and fisheries management at the University of Edinburgh, UK.

The complete biologist

EVEN within a course the best teachers do not rigidly adhere to a recommended textbook. Although such bibliolatry would be an anathema, the choice of student texts is an important, even crucial process. What are the criteria?

Factual content, conceptual approach, readability, layout, production and cost rank among the more important. And for the general biology college teacher, the balance is particularly difficult to find. The three books reviewed here cover much of the same ground at a similar academic level; they are all well written; and they are all intended for a primarily American audience (although Hardin

and Bajema tend to use less idiosyncratic field examples, and might therefore be a more acceptable text in the UK). The contents are ordered slightly differently, but the major topics of modern biology (molecular biology, cellular biology, physiology, ethology, ecology, evolution and genetics) form the basis for each text.

The books reflect different underlying philosophies. G. B. Moment and H. B. Habermann in *Mainstreams of Biology* (Williams and Wilkins: Baltimore) believe that an historical perspective gives "a lasting sense of biology as an ongoing human enterprise", but they've gone overboard. No student should be expected to wade through the names of eight or ten scientists (and their institutions) on a page of text. Similarly, the book is liberally illustrated by photographs of the illustrious. (It's true, I didn't know that Lynn Margulis wearing dark glasses and a headscarf bore an uncanny resemblance to Greta Garbo.) B. T. Scheer, in *Patterns of Life* (Harper's College Press: New York and London) is, if anything, prone to the other extreme. He is clearly a good, experienced teacher who knows how to communicate to his students, but his book lacks lustre in both conception and production. The dedicated student might do well with the somewhat stodgy approach. The average student would not. In Scheer's defence, however, goal questions are effectively set at the beginning of each chapter and brief introductions at the start of each of the six sections aid in a sense of achievement. G. Hardin and C. Bajema chart a good middle ground in *Biology: Its Principles and Implications* (Freeman: San Francisco). Their book is excitingly written, clearly and liberally illustrated and beautifully produced. They have thought hard and well about this new edition, and Bajema's academic qualities (of which more later) make a welcome complement to Hardin's hard-hitting evangelism.

But what of content? Perhaps this is best treated at two levels. First, the balance that the authors give to the various components of biology. Second, the extent to which they are in touch with new ideas and research, and have the ability to integrate them into a student text. Ignoring the books' excesses for the moment, I found Scheer extremely weak on molecular biology. It is hardly integrated into the text, but almost totally confined to an Appendix entitled "The Chemical Explanation". That's not good enough and, as a consequence, topics such as photosynthesis, hormone action and nutrition are very poorly dealt with. Similarly, Hardin and Bajema are relatively weak on molecular and hormonal biology. Perhaps because they want to excite their audience, they gloss over some of the things students ought to learn—straight ethology, for example. If

students are going to progress beyond the level at which these books are aimed, or if they are going to develop an amateur interest in Natural History, then taxonomy will be of importance. It is here that two of the three texts are inadequate. Moment and Habermann barely mention the topic, whereas I found Hardin and Bajema's treatment over-technical and unexciting. I know that taxonomy is not usually exciting, but Scheer makes a valiant (90-page) attempt with a clear phylogenetic exposition. He deserves credit. The books each had so much ground to cover that they rarely deal with topics in excess, though Hardin, not surprisingly, is one for the eco-crisis.

These books are generally up-to-date, using new material which has been largely condensed from specialist sources. It is here that Hardin and Bajema come right to the fore. Not only have they assimilated current research from each area that they cover, but (within my own specialities at least) they have also managed to describe the more exciting and potentially important findings. At the

end of each chapter, they refer to *Scientific American* offprints and other easily conceptually accessible readings. And they haven't missed the human angle. In "boxes" scattered through the text are reprints of short, classic papers from Darwin to Watson and Crick (including, of course, *The Tragedy of the Commons*—but that's excusable), asides on concept and method, and even political analyses. This text, alone among the three, is integrated at almost every level, has set problems (some of which are tersely answered at the back) and is a mine of accurate well-presented information. With the few reservations outlined above, I commend it for serious consideration even by those who already have a favourite text. Everyone, like me, will learn something new from the book.

Paul Harvey

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Comparative physiology

In *The Vertebrates: Their Forms and Functions* (Charles C Thomas: Springfield, Illinois; \$15), C. G. Crispin attempts to survey the whole of vertebrate zoology. Unfortunately, by the time he has covered histology and phylogeny in outline, and named and subdivided the skeletal, muscular, lymphoid, nervous, integumentary, respiratory, digestive, excretory, reproductive and endocrine systems he has virtually no space left actually to describe them, let alone discuss their functions. There is little to be said in favour of this relatively expensive book and it is hard to see for whom it could be useful. The blurb on the cover refers to it as "an alternative approach to contemporary vertebrate zoology" and so it is, by being about 50 years out of date. It is the worst sort of students' cramming book, giving more emphasis to artificial subdivisions and their names than to functional significance, and one can imagine them learning by heart the three names of the methods of tooth attachment to the jaw in order to pass a multiple-choice test. In spite of lip-service paid to function it is essentially morphological in outlook and leaves the reader with a false sense of what should be a living and exciting

subject. It is out of tune with modern ideas and contains just the sort of factual information best calculated to put advanced school biologists off their subject (and it is too elementary for any later course).

Older British readers should not be put off by the slangy title of *Mammalogy* (W. B. Saunders. Philadelphia and London; £12) by T. A. Vaughan, as it can be thoroughly recommended as a very satisfactory reference book for second- or third-year undergraduate zoologists onwards. Probably nowadays few university courses are so specialised on mammals that it could be a major required textbook, but for any course specialising in vertebrates it would be one of the first books in the library to refer a student to when a question arose concerning mammals.

After a brief description of mammalian characteristics, the evolution of mammals from the mammal-like reptiles is surveyed. The whole complex story of the therapsid radiation cannot be told, but sufficient description (including diagrams) of key types such as *Cynognathus* is provided to give a fair account. The remainder of the first half of the book deals with the various orders in turn, giving detail to family level, and stressing ways of life as well as standard morphological detail. On the whole, the less well known groups are described more fully.



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and in particular that miscellaneous collection of animals masquerading as the order Insectivora is introduced in masterly fashion. There are photographs of members of nearly every family and clear drawings of skulls, teeth, limbs and other skeletal details as appropriate, all portrayed in a clear, uniform style similar to that first adopted in Romer's *Vertebrate Palaeontology*.

The second half of the book is devoted to a number of topics of general biological interest which have special relevance to mammals: ecology, zoogeography, behaviour (especially social behaviour), reproduction, metabolism and temperature regulation and echolocation, with a final brief comment on the effects of man on mammalian wild life. From this list it can be seen that only certain subjects are included, but many of them are of obvious importance like the section on zoogeography. This covers a summary of types found in the various regions, a description of continental drift and its effects including a very useful discussion of the "unusual" faunas, past and present, of South America, Australia and Madagascar. Other sections like that on echolocation in bats and cetaceans provide concise summaries of detailed topics which presumably reflect the author's interests.

It is very easy to read large sections of the book straight through, as well as to use it as a reference book for facts, ideas and as a lead in to further reading. It is well produced and relatively cheap—in fact a good buy.

At first sight one might suppose that *Animal Physiology*, by Roger Eckert and David Randall (Freeman: San Francisco and London; \$19.95, £12.95), is merely another in the series of comparative physiology texts which started with Prosser's and which has been added to at the rate of about one every four years ever since. And so in many ways it is, for much of the content is in common with the latest editions of its rivals. But this attempt does the standard job well, and has many advantages and few disadvantages when its special emphasis is identified. The book starts with basic concepts of physics and chemistry (for example, Ohm's Law and osmosis), works through cellular processes such as the electron transport chain, active transport and membrane potentials, and then covers individual nerve and synaptic functioning. This leads on to sense organs and the principles of central nervous integration, then to the other systems: muscle, cilia and flagella, chemical messengers, osmoregulation and excretion, blood circulation and gas exchange. There are appen-

dices containing useful formulae (though kinetic energy should not be printed as $1/2mv^2$) and a glossary.

The main theme of the book is the explanation of physiological processes and their control; it is not a survey of comparative physiology. The choice of which processes are to be described and explained is rather selective, but mammalian functions are in general fully covered (apart from digestion and temperature regulation) and so are the major variants and additions that occur in other vertebrates and in some invertebrates, for example, osmoregulation and circulatory systems.

In spite of the complexity of the subject the book is readable. Within its chosen limits, it is an excellent textbook, clearly most appropriate for second- and third-year undergraduates taking courses in comparative or in mammalian physiology. It highlights the fact that so many processes in physiology now have their explanations pushed back to more molecular levels than they did 10 years ago. The reader

of this book might be forgiven for thinking that there is little more to be found out, so complete and satisfying are so many of the descriptions given. Rightly, in the cause of clarity, the text contains only sparing use of "probably" or "as far as is known at present" and the topics described tend to be those for which an explanation has been suggested in the literature. So it is the sort of textbook which reinforces and extends a lecture course, rather than taking its place, and it is essentially aimed at helping students understand how animals work. It is intentionally not a reference book, but the references which are given should be quite enough to allow a student (or a lecturer for that matter) to use it as the start of a more detailed library search on any specific topic.

R. W. Murray

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Fundamental nutrition

Fundamentals of Nutrition. Second edition. By L. E. Lloyd, B. E. McDonald and E. W. Crampton. Pp. 466. (Freeman: San Francisco, 1978.) \$19.50.

THE title of this book gives a very good indication of its contents and the intended readership. Each chapter is short so as not to overface the first-year undergraduate or college student, and at appropriate points boxes are inserted with topical information such as breeding cereals for high lysine. Potted biographies of eminent nutritionists also add to the readability of the main text.

Introductory chapters describing the composition of foods and animal tissues are followed by sections on energy and protein, vitamins and minerals; the latter, sensibly, not illustrated by well-used photographs of animals suffering from various deficiencies or excesses. Photographs could, however, have been usefully added to illustrate some of the more complex methods which are well described in the text.

A further group of chapters includes, refreshingly, discussions on experimental design and analysis and descriptions of the major areas of animal experimentation in nutrition, although

many students would presumably be taking a biometry course in parallel with nutrition and would be referred to a more detailed and comprehensive text in that subject. The same might be said of some of the biochemical coverage; surely no modern nutritionist can escape without at least one full course in biochemistry which would render superfluous those areas dealt with in this book.

The last five chapters, occupying almost 100 pages, deal admirably with quantitative aspects of nutrition—how much of each of the previously defined nutrients are required daily by various species, including man, in various physiological states. This is not to imply that the book is a practical feeding manual, and those examples of farm animal feeding that are given are based on American standards which are not relevant to a large part of the world. The use of the calorie rather than the internationally agreed joule as the unit of energy adds further possible confusion for the majority of students who are now familiar with the latter. There is a useful index.

This is a comprehensive coverage of the whole subject of nutrition and is written in a manner that will be appreciated by all who are tackling this subject for the first time.

J. Michael Forbes

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Plant pathology

THE approach of *Fundamentals of Plant-Pest Control*, by D. A. Roberts (Freeman, San Francisco and London; \$17.50; £10.20) is to try to unify all aspects of plant pest control, pointing out common features as much as possible, and culminating in the necessity for the systems approach in order to achieve integrated pest control. This is a theme of much recent thought on the subject and it is evidently right that undergraduates should study and evaluate the approach.

After a general introduction to the plant-pest ecosystem, a series of chapters reviews the structure and functioning of crop plants, followed by the dynamics of plant pest populations and the basic elements of plant pest control. The second main section reviews different types of pests, in the wide sense: microbial pathogens, nematodes, arthropods, weeds and vertebrates. Finally, the systems approach is outlined and the author concludes with his reflections on pest control in the future.

Desirable though the ultimate objective may be, one is left somewhat dissatisfied with the book as a student text and not altogether convinced by the arguments as they stand. There is an unsatisfactory discrepancy between the potential readership of the book and the background knowledge one would expect of the reader. If it is intended for the naive reader, who has not studied plant pests in the more traditional way, then it is hard to see how he can properly evaluate the whole of the argument or cope with the use of terms like mycoplasma-like organisms or /2-chloro-4-(ethyl-amino)-6-(isopropylamino)-s-triazine/. If, on the other hand, the reader has the background, then why does he have to be given a diagram showing an elementary view of plant structure, be told that organic compounds contain carbon, or study a table setting out among more appropriate things the type of metamorphosis in stoneflies, book-lice and fleas. Such a book must surely aim to refine the understanding of a student with an adequate background, and in this sense more than a third of the text is rather unnecessary.

Leaving this aside, the sections on each type of plant pest are well structured accounts of the application of basic principles to each case. These chapters, contributed by different specialists working consistently to the author's theme, are, with the exposition of population dynamics and the

basic elements of control in the first section, the strongest section of the book. The plant pathology, entomology or weed biology student, in a context where such people are still separate, as is largely the case in the UK, will most profitably read the chapters which do not relate directly to him. The unifying approach cannot, however, always accommodate the real situation altogether. There is ultimately not so much in common between vertebrate pest control and that of nematodes as there is between the latter and plant disease control. The concepts of vertical and horizontal resistance arising from van der Plank's view of plant pathology and applying to races of host-specific pathogens and varieties of their hosts transpose rather superficially to the interactions between plant and insect species. Nevertheless, detailed differences should not submerge the most important fundamental similarities.

The systems approach of the last section is illustrated by the system "undergraduate education in crop-plant-pest control". Why, one wonders, is it not possible to illustrate it by a real plant protection system? The latter only appears in a highly condensed account of computer models such as EPIDEM. One can accept the validity of computer modelling or integrated pest control as such, but this text does not altogether convince one of the real, productive, value of the "higher systems level", or systems approach itself. Undergraduates may profitably

read this text but should keep their feet firmly on the ground and their critical faculties well in trim.

G. N. Agrios' *Plant Pathology* (Academic: New York and London, \$22.95) was in its first edition a textbook one was only too glad to recommend to students. It was invaluable for its thorough and clearly expressed accounts of selected diseases, for its abundant and excellent illustrations and life-cycle diagrams, and for the confidence with which one could advise students to refer to it as a supplement to lecture courses. It had its faults: the introductory chapters on general concepts were rather solid catalogues of information, without much feel for the essentially research orientation of subjects such as biochemical defence mechanisms; the rather limited number of diseases treated was often a problem, especially for the European user.

This new edition triumphantly corrects the latter fault, and is generally revised and brought up to date. The introductory chapters have been somewhat polished and some superfluous material has been removed. There is no doubt that this new edition is even better than the first and it can be thoroughly recommended as an invaluable teaching aid with very few reservations.

I. M. Smith

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Plant anatomy

THE first edition of *Plant Anatomy* (Part I: Cells and Tissues) by E. G. Cutter, was published in 1969 and contained 168 pages. Now, almost ten years later (Edward Arnold: London; hardback £13.50; paperback £6.75), the 315 pages contain considerably revised and updated information while retaining the essential character of the original volume. Considerable use has been made of both scanning and transmission electron micrographs and, in general, the reproduction of these is quite respectable considering the quality of paper and method of printing. It is good to see such comprehensive revision—to the extent that transfer cells warrant a whole new chapter of their own—but it is a pity to find that some other cell types (for example, endodermis and hypodermis) are relatively very neglected. To some

extent this arises because this book is a companion to the second part (Organs) in which the endodermis, for example, is more fully described. Nevertheless, it is regrettable that while, as written in the review of the first edition (*Nature*, 222, 1100; 1969), "No cell types are omitted . . .", the degree of coverage is so variable.

This is very much a plant anatomy book with a difference: constantly probing and questioning and never very far away from the functions of the cells and tissues being described. Thus, the author is not satisfied simply to classify or to catalogue but attempts to show how the specific structures serve their different roles. Glands, and other cells and tissues, are no longer viewed simply as interesting structural aggregates but also as morphological entities that are uniquely adapted to serve their (described) function. Moreover, mature cells are not assumed to appear *de novo* and Dr Cutter takes pains to stress the subject of differentiation, both in a specific chapter where genetic control, totipotency,

polarity, pattern formation and nuclear-cytoplasmic interactions are briefly reviewed and also at appropriate times in the other chapters. This style leads to a book that is very readable and well suited to elementary students as well as to the more experienced botanists who will undoubtedly benefit from it.

Cell contents and cell walls are despatched briefly but efficiently in consecutive chapters. All the major organelles are reviewed in up-to-date fashion. It is good to see the most recent views on, for example, C_4 chloroplasts and plasmodesmata, although we might have hoped for a more comprehensive assessment of the structure and role of microtubules than the dozen lines allowed for them. Indeed, the whole area of membrane structure and techniques such as freeze-etching is ignored, as is the work on cell wall formation by, for example, Willison and Brown. This is surprising and disappointing in a book that tries to relate structure to function as membranes are of such paramount importance.

It would have been much more useful if the subject coverage in this book had been more evenly distributed. However, taken as a whole, this is an attractive volume that should encourage botanists to think more about how the structure and function of plants are intimately inter-related. Anything that does this is to be welcomed and Dr Cutter has made a notable step in the right direction.

Applied Plant Anatomy (Longman: London and New York, £4.95), by D. F. Cutler, seeks to illustrate the way that a sound knowledge of plant anatomy can be applied to the solution of many important every day problems. It is good, in a textbook of plant anatomy, to see pictures such as that of a papyrus sandal and to read about "wood in archaeology": this does help the subject to come alive. This novel approach leads to a book that is different from most others on elementary plant anatomy and one that is both readable and enjoyable for even the newest student of botany.

The introductory chapter contains brief notes on techniques for both light and electron microscopy. This is followed by chapters on the basic morphology of plants; a good, simple, illustrated glossary, the histology of plant organs; meristems; vascular tissues; adaptive features; flower and fruit; and economic aspects of applied plant anatomy. Each chapter is well illustrated by clear line diagrams and micrographs. The distribution of micrographs is a little uneven and, indeed, there might have been more of them altogether. At the end of each chapter there is a small list of suggested further

reading and, where appropriate, lists indicating where to look to find particular anatomical characters. This is a most valuable addition and covers plants worldwide. The further reading lists are rather too short and often quite dated. Having commented on the usefulness of electron microscopy to the study of plant anatomy, the author then fails to cite a single further reference in this area. Indeed, the four books suggested at the end of chapter one serve as a rather limited coverage of the subject area.

The introductory chapter on techniques is useful but it is a pity that

some of the figures concerning the resolution of microscopes seem to have been muddled: both TEM and SEM resolution are out by a factor of 100! The index is concise and helpful and, indeed, reflects the whole tone of the book, which is to convey a very considerable amount of information in a palatable and easily understandable style.

A. W. Robards

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Principles of plant nutrition

Principles of Plant Nutrition. By K. Mengel and E. A. Kirkby. Pp. 593. (International Potash Institute: PO Box 41, CH-3048 Worblaufen-Bern, Switzerland, 1978.)

This book is broadly based in accordance with the implications of its title and achieves a commendable integration of soil and plant aspects of nutrition. The partnership of geographically separated authors, who are both practically orientated university teachers, provides a background of wide experience and as shown by the principal bibliography (about 1,400 references) an extensive familiarity with the subject.

Chapters 1 to 6 (totalling 290 pages) describe some general principles. Considerable attention is given to plant-soil relationships; nutrient uptake, translocation and storage; nutrient availability in relation to crop needs; general effects of nutrient deficiencies; and methods of nutrient application and correction. A number of major physiological activities—photosynthesis, nitrogen fixation, nitrogen assimilation, water relations, active transport, nutrient absorption—are described in sufficient depth to provide the necessary background for understanding the role of various elements, although respiration seems less adequately considered.

Chapters 11 to 18 deal separately with each of the familiar essential elements—except chlorine, which is reviewed in three pages in a chapter confined to some possibly beneficial or indirectly essential elements: cobalt, silicon and vanadium. Reviews on separate elements cover soil aspects of their availability, their metabolic roles and deficiency and some toxicity effects on growth. These chapters provide a good general idea of the importance of the elements. These and the other chapters are each concluded with a general reading list to supplement

the numerous specific citations in the text.

Chapter 19 concludes the treatise with examples of several toxic or less familiar elements which are discussed in more general terms and it includes several interesting miscellaneous reports. There are a few minor omissions—for example, regarding the production by certain plants of apparently specific citrate and oxalate metal complexes especially with nickel and chromium; the quite extensive work on tungsten relegated here to a few words on page 149 on tungsten inactivation of nitrate reductase by unspecified means; the original, rigorous and model investigations of Brownell on the absolute need for sodium by *Atriplex vesicaria* (though his later discovery of its possibly general importance in C_4 -type photosynthesis plants is nevertheless noted); the differentiation of apparently two types of malate enzyme; the exciting conclusions of Meish *et al.* regarding the essential role of vanadium in green algae, thought to involve the formation of delta aminolaevulinic acid (though this news may have 'broken' as the book went to press); and finally (though possibly again just too late), the further work of Polacco on the need for nickel in urea metabolism since the first report on urease by Dixon *et al.* briefly cited on page 514.

This quite comprehensive and well documented book will be of value to students and lecturers alike in faculties of agricultural science, botany and biological sciences and should also be welcome in the libraries of agricultural institutes, advisory workers and consultants. It is especially commended for its relevance to practical aspects of crop nutrition and for proper appreciation of the general principles on which practice should be based.

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Plant taxonomy

Essays in Plant Taxonomy. Edited by H. E. Street. Pp. 304. (Academic: London, New York and San Francisco, 1978.) £12.50; \$24.50.

PROFESSOR Tom Tutin has no rival for the title of *doyen* of British plant taxonomists, and few would question the appropriateness of this *Festschrift* on the occasion of his seventieth birthday, which so nearly coincided with the completion of *Flora Europaea*, his second *magnum opus*. We must here, however, examine the book, not from the standpoint of an admiring friend, but of a student asking what use it will be to him. Symposia are at present, perhaps, an over-popular form of publication: in many of them the thread of supposed common interest which binds the essays together is far too tenuous, and indeed, in the case of some complimentary volumes such as this, completely non-existent. The present work, however, is free from this reproach, for all but one of the essays are relevant to taxonomy on the strictest definition. The editor, who unfortunately did not live to see his work in print, planned the volume well, but part of the credit for its efficient and speedy production must go to Dr C. A. Stace, whose share was greater than his modesty permits him to disclose.

Four of the essays are wide-ranging reviews with no special claim to an original interpretation, and these are the parts of the book which will be of most value alike to the general student and for those who are specialising in taxonomy. Valentine on ecology, Smith on chemistry and Moore on chromosomes, all considered in relation to taxonomy, give judicious summaries of their themes, with well chosen and up-to-date bibliographies; and Richards on the taxonomy of bryophytes gives an excellent account of a field to which most angiosperm taxonomists are complete strangers. Stace on breeding systems and their taxonomic implications also covers a wide field, but with a more individual approach; he ends with a justified *cri de coeur* for a less Procrustean system of infraspecific categories than that laid down by the International Code, but the genius who can produce a system which is at once flexible, unambiguous and reasonably simple has not yet been found.

Somewhat more specialised, but still in the nature of reviews and thus of value to students whose interests lie in the fields concerned, are the essays by

Hawkesworth, who introduces the reader to the peculiar and complex problems posed by the taxonomy of lichens and their fungal constituents; Parker on taxonomic problems in crop plants; and Hawkes on the related theme of conservation of genetic diversity, especially in plants of economic interest.

Cook on the *Hippuris* syndrome (the frequency in aquatic plants of very diverse families of a vegetative structure superficially similar to that of *Hippuris*) belongs rather to the realms of morphogenesis and evolution than taxonomy, but it should generate some constructive head-scratching of benefit even to the elementary student.

Yeo attempts to explain why *Euphrasia*, in spite of a normal breeding system, presents taxonomic problems as intractable as those of many apomictic or autogamous genera. To the reviewer his effort must be classed

as a gallant failure, but the essay is essential reading for anybody working on semiparasitic Scrophulariaceae. The remaining essays (Walters and Richardson on different aspects of endemism, Sneath and Chater on a rather esoteric analysis of dichotomous keys, and Heywood on past and future Flora-writing) are also of interest mainly to the research-worker or teacher.

Almost without exception the essays have a lucid and readable quality which sets them apart from the contents of most biological journals. The book should be dipped into by all honours students of botany. Those whose interests lie mainly in fields close to taxonomy should read most of it carefully and chase up some of the clues given in the excellent bibliographies.

D. A. Webb

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Pollen analysis illustrated

An Illustrated Guide to Pollen Analysis. By P. D. Moore and J. A. Webb. Pp. 133. (Hodder and Stoughton: London, 1978.) Hardback £8.50; paperback £4.95.

THE authors have produced this book, intended for undergraduates and advanced school students, as a laboratory manual. They hope that it will be used as a supplement to Pennington's *History of British Vegetation*. The book is a guide to pollen-bearing Quaternary deposits, the collection and treatment of samples, pollen morphology, the identification of pollen, the practice of pollen counting and interpretation of results.

Chapter 5 (Pollen and Spore Key with Glossary) will, I am sure, be much used by pollen analysts whether beginners (from school to postgraduate) or established workers. The authors rightly stress more than once the need for a reference collection to corroborate identification made from the key and illustrations. In its liberal supply of plates, including stereoscans, of pollen grains and spores the chapter contrasts strongly and favourably with Faegri and Iversen's famous textbook (*Textbook of Pollen Analysis*) in which the keys are discouraging in their lack of

illustration. The beautifully illustrated, two volume *An Introduction to a Scandinavian Pollen Flora* by Erdtman *et al.* is not easy to come by and is expensive, as are other pollen atlases.

The accounts of important modern developments such as absolute counting, statistical treatments, computing and zonation, are lucid and level-headed, with the right balance of the need for greater objectivity and credit for the many successes of the more traditional methods.

The plates and figures are well executed and appropriate but a reader with little archaeological knowledge could be misled, if temporarily, by Fig. 1.1 which shows the sequence of archaeological periods but gives no indication of timespans. Also I wonder what a beginner will make of the description and drawings (Fig. 3.1 (b)) of the Russian borer, redrawn inaccurately from West's *Pleistocene Geology and Biology*? He may well be puzzled. Perhaps the Mackereth corer, an important though elaborate tool in palaeolimnology, deserves a little more detailed treatment, though few readers will have the chance to use or even see one.

However, these are small points, detracting little or nothing from this valuable, readable book which will be used freely and successfully in the ways the authors intend.

J. H. Dickson

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Present state of academic psychology

Psychology Survey. No. 1. Edited by B. M. Foss. Pp. 220. (George Allen and Unwin: London, 1978.) Hardback £5.50; paperback £2.50.

WHAT is the perfect introductory text for psychology students? The selection pressures of the market should by now have weeded out the unfit, and we ought to be approaching a form well adapted to the British students' needs. The American form, with its pictures and snippets of information, seems fairly stable, but the comprehensiveness of such products at a shallow level is unsuitable for most British introductory courses. Usually our lectures and tutorials are not part of a single planned course, and tutors, left to devise their own essay titles, naturally look out for up-to-date surveys of currently fashionable topics. The *Annual Review of Psychology* is too heavy and unselective, and the professional review articles, such as those published in *Psychological Bulletin*, involve too drastic a plunge in at the deep end.

It is not surprising, therefore, that there is a market for books of introductory reviews. Early examples were the present editor's *New Horizons in Psychology* (at slightly too elementary a level) and the *British Medical Bulletin's Experimental Psychology* of 1964, followed in 1971 by *Cognitive Psychology*. Recently several publishers have issued series of short paperbacks, each on a special topic within psychology, but these have been uneven in standard; and buying even half a set would be expensive. In the book under review, we have the beginning of what may prove an ideal solution. It is the first of an annual volume of reviews, designed, judging from the contents, to throw the student in just out of his depth. It is, therefore, for the beginner who can rely on personal guidance if necessary, and not for someone setting out on his or her own. Each year, to keep the series fresh, there will be a new editor; and providing we can be kept up to date by suitable recycling of topics, the enterprise deserves to be very successful.

In this volume there are 14 chapters of about 15 pages, each by a different author who is a well known specialist in his field. The titles, in order, are, Visual Selective Attention, Verbal Remembering, Conditioning, Mental Imagery, Psychophysiology, Cognitive Effects of Cortical Lesions, Experimental Child Psychology, Sex Differences, Behaviour Modification, Human

Learning, Cross-Cultural Studies, The Bases of Fluent Reading, Occupational Psychology, and Environmental Psychology. There is thus a gradual shift from the 'core' subjects, highly organised areas currently under tough minded experimental scrutiny, to applied areas of research. In core subjects the reviewer's problem (generally solved remarkably well) is to get across, in a brief and simple form, the involved theories that make sense of the experimental work and that mark the boundaries of the area. Applied areas, on the other hand, are bounded by the set of practical problems that define them, and within such an area different approaches are possible; the result is diffuse, and short reviews inevitably seem selective and sketchy. In all cases,

however, the student is given a start, and a useful list of references.

The reader is left to work out the thread that holds the chapters together. Michael Eysenck, author of *Human Memory*, finds encouragement in "the emergence of general theories that have considerable application outside the limitation of a specific experimental task", but there is not much sign of this in the book and one feels by the end that the areas of psychology, like games, share no more than a family resemblance. That, no doubt, accurately reflects the present state of academic psychology.

A. W. Still

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Physiological psychology

A NOTABLE feature of this set of books on the central nervous system and behaviour is that each is written by one author, an unusual and welcome event now that so many books consist of collections of previously published papers, accounts of conference proceedings, or chapters by writers whose only common link is an editor. A single author can avoid the well known problems of multi-author works—repetition, abrupt change of style, and the difficulty in developing a point of view by sustained argument. The advantages of going it alone certainly show in the present selection, albeit to different extents. In *A Primer of Psychophysiology* (Freeman: San Francisco and Reading; hardback £8.90, paperback £4.40), James Hasset presents a brief, clear and bouncy account of an approach to behaviour that is neglected in most British psychology departments. What is psychophysiology? After rejecting definitions that simply refer to the methods used by psychophysicists, for example, recording the EEG, he settles for "a strategy for studying human behaviour and experience". Now many will argue that the strategy is no more than a reflection of the methods used, and the bulk of the book is certainly devoted to a straightforward description—whose usefulness should not be underestimated—of such things as the electrical properties of the skin, respiration, cardiovascular states, pupillary changes, muscle tone, eye movements, and the gross electrical activity of the brain, together with detailed accounts of how to measure

them. It is undeniable that the measurements correlate well with psychological states such as fear, tension, anger, curiosity, embarrassment, arousal, and habituation, to name only a few, but the reader who is looking for some mechanistic or conceptual explanation of our behaviour will be dissatisfied. Psychophysiology may have useful applications, for example, in the therapeutic use of biofeedback or revealing individual differences and psychiatric conditions, but the book does not show whether it has explained anything important.

On the Texture of Brains (Springer: Berlin; DM16.30, \$7.50), by V. Braitenberg, is equally slim, but there the resemblance ends. It is a collection of eight short essays whose collective message is that "the structure of brains is information about the world", by which the author means not that we can understand the world from brain structure but that the structure of the brain has to reflect certain features of the world in order for the organism to deal with its spatial environment. It is an attempt to understand behaviour in terms of neuroanatomy. A crucial point is that not all levels of anatomical analysis are equally informative. To understand a brain by gross dissection is like trying to understand a text by dividing it into chapters; to analyse it by studying the structure of individual cells is about as helpful as examining prose in terms of the letters of the alphabet. The right approach, it is maintained, is to study how cells are connected. By so doing we find that the basic design is remarkably similar from insects to man. Complexity and variety of behaviour are therefore a product of the total number of cells and their interconnections, rather like the dif-

ference between a short poem and a long novel. It also follows that the general principles of how nerve cells control behaviour may be studied in organisms much simpler than ourselves, which explains why so much of the book is devoted to the author's own leading role in successfully demonstrating how visually guided navigation in flies can be explained by the fibre connections in the eyes and visual ganglia. Now this approach has undoubtedly been fruitful with insects, and promises to be so with the mammalian cerebellum, but major problems arise in considering the cerebral cortex, where input-output relationships fail to reveal what lies between them. It is unclear how learning, memory and prediction are to be dealt with in general terms except by old and loosely formulated notions like cell assemblies, changing patterns of excitation, and internally activated representations that are matched against sensory patterns. How these arise is not explained. And it is easy to see how a slight mistake can lead to serious misconceptions. To take one of many examples, it is stated that the principle of equipotentiality, by which it matters not a jot which part of the cerebral cortex is removed, demonstrates that regional specialisation does not occur. This results in a needless struggle to explain something, for we now know that the principle does not follow from the experiments on which it was based. Nevertheless, Braitenberg has written a joyous, learned and provocative book that makes the reader think at every turn.

Developmental Neurobiology (second edition, Plenum: New York and London), by M. Jacobson, and *Developmental Plasticity of the Brain* (Oxford University Press: Oxford, £4.95), by R. D. Lund, cover similar ground. Both deal with the ontogeny of the nervous system, the differentiation of nerve cells and interactions between them, and the mechanism of neuronal specificity. Apart from price there is not a lot to choose between them, but Jacobson is slightly more detailed and comprehensive, deals more effectively with the neuromuscular system, provides a succinct but excellent account of the extensive work on the retinotectal map in amphibia, and is more lavishly illustrated. Lund is more thorough on environmental influences on central connections and his method of imparting information by asking a question then answering it, instead of simply presenting facts, is helpful, as are his concise summaries at the end of each chapter. If either book lacks anything it is an account of work with invertebrates and some recognition of the importance of behavioural experi-

ments done in conjunction with developmental experiments. But these are slight criticisms of two scholarly works that will be deservedly popular. Their main rival is *The Formation of Nerve Connections* (Academic: London) by R. M. Gaze, which is now several years out of date.

Brain Systems and Psychological Concepts (Wiley: Chichester, UK; £12.50), by J. Boddy, is an undergraduate textbook of physiological psychology, and it is useful to know how it differs from those of similar length and subject matter by Milner, by Thompson, and by Grossman. The overlap with all of them is huge, particularly in describing the sensory pathways, hunger, thirst, sex, emotion, brain stimulation, sleep, memory, and research techniques. However, there is much more emphasis in Boddy's book on the relevance of experiments on human subjects, for example, in considering information processing, and the chapters on mental illness and the effects of drugs are novel. It is much easier to read than either of the two books by Grossman; the arguments are not as thoroughly developed as by Milner; and it is not as detailed as Thompson's book. The overall level of presentation is very similar to the earlier text by Morgan but of course

it is much more up to date. My major reservation is whether we need another textbook of physiological psychology, for the group published between 1967 and 1973 seem adequate as an introduction before the student moves on to more specialised books. But for the student whose reading has to be confined to one book Boddy has provided a sound account with a welcome cognitive flavour.

Who will benefit most from the books? Hassett writes for the undergraduate and his book is unashamedly introductory. Braitenberg will appeal to the widest audience, from student to colleague, because he is such fun and is trying to say something simple and fundamental about the brain; but the reader has to take a lot for granted. The books by Jacobson and Lund are most suitable for advanced students who already know a good deal about the nervous system, and they can hardly fail to become standard reference works for any developmental neurobiologist. Boddy's is the only book that is a student text covering a broad range of topics. It is good, but not clearly better than a number of rivals.

A. Cowey

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Psychology texts

ONE of the joys of working in psychology is the discipline's apparently unlimited ability to borrow from other areas. At times the joy can turn to anguish when the transplant seems to submerge the host but a new development for "grafting" always seem to come along.

Complications for teaching come from the diverse ways in which departments of psychology seem to split up the subject matter—for example, should there be separate courses on thinking and on psycholinguistics; should both be subsumed within a conglomerate memory, language and thinking; should there be a separate course on artificial intelligence (AI); or should all the preceding courses be collected within one course on cognitive psychology? Possibly a solution lies in the emerging discipline of 'cognitive science'—forming a linkage between linguistics, AI and cognitive psychology. It is against this background of shifting boundaries that course texts for psychology have to be assessed.

Artificial Intelligence (Edinburgh University Press: Edinburgh; £5), edited by

Bundy *et al.*, has more claim to be a course-text than many so-called textbooks. The book derives from lecture notes for the 'Artificial Intelligence 2' course given at the University of Edinburgh (one of the few UK centres where full AI courses are available to psychologists). The '2' is perhaps cautionary—not a course for first-year students; and also it represents a very full year's work.

For those planning their own courses programming exercises through the text, and appendices with examination papers and student response to the course should prove invaluable. Collection of the references throughout the text into a bibliography would have been useful. Readers likely to be disappointed with the book would be those working from a cybernetics: brain-theory: engineering background—the six pages on Perceptrons do not sufficiently reflect work within their area.

Comparison texts would include those by Winston, Boden and the Open University Cognitive Psychology course team. Winston's *Artificial Intelligence* (Addison-Wesley, 1977) is a more technical work with few compromises to non-LISPers. It is really for advanced undergraduates and postgraduates, and is perhaps an AI/computer science text rather than a psychology text. The OU

text relies heavily on access to a terminal tied to the SOLO system (specially developed for this course) and seems a difficult text to grasp in isolation. For many humanities students (including linguists, philosophers, and some psychologists) Margaret Boden's *Artificial Intelligence and Natural Man* (Harvester, 1977) provides a good introduction to the area of AI without becoming too embroiled in techniques. But if your psychology department is lucky enough to have access to a suitable computing system then the Edinburgh text is a good introduction to trying out techniques in AI.

Psycholinguistics (Prentice-Hall: Englewood Cliffs, New Jersey; £10.90) by Foss and Hakes lives up to its subtitle—*An Introduction to the Psychology of Language*. Concentration of psychology rather than linguistic theories or developmental processes produces an excellent overview of the chequered area of psycholinguistics. From a murky mixture of mechanical translation, S-R psychology and information theory, through the revolution of Chomskyan grammar, psycholinguistics has reached its present stage of uncertainty and division. Claims to form a separate discipline seem to be fading; merging into more traditional areas of psychology and supplying part of the backbone of cognitive psychology seems more likely.

The introductory chapters provide a good discussion concerning the nature of language, and anchor the book against a linguistic as well as psychological background. Three chapters then cover 'input operations'. An excellent exposition and discussion of speech perception is followed by a comprehensive treatment of comprehension—indicating other sources of information than pure syntax. The section concludes with an integration of sentence understanding with various models of memory. The next section deals with 'output operations' and is divided

into the planning of sentence production and the production mechanisms involved. There is good treatment of the information that speech errors can give to the psychologist interested in underlying mechanisms, and a thorough presentation of rhythmic processes in speech. This leads neatly into three chapters outlining the development of language. There is emphasis on communication skills, understanding and semantics.

The last three chapters of the book are perhaps the most interesting. Building on earlier discussion, they cover reading, language and the brain, and language and thought. The chapter on reading provides a nice summary of experimental work integrated with an interesting model. Language and brain links together a number of rather disparate areas including aphasia, localisation and lateralisation, and species-specificity in a comprehensive manner. The final chapter seems a little unhappy—considering linguistic diversity with an emphasis on Black English and linguistic determinism: language and thought.

Each chapter is well summarised and contains suggestions for additional reading. Good indices and a bibliography complete the book. With the exception of coverage of 'sociolinguistics' (which seems to be social psychologists using language variables as indices of social processes rather than psycholinguistics proper), the book is an excellent text for specialised psycholinguistics courses. Underplaying of relevant AI work and omission of much European work—notably the work on thinking and language by investigators such as Wason and Johnson-Laird—suggest that the integration that the umbrella of 'cognitive science' offers should not be ignored.

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Animal behaviour

HERE are reviewed two new textbooks in animal behaviour aimed at the American undergraduate market. Both assume little or no previous knowledge of anything, and both are written in a chatty, mildly condescending manner, eschewing complexity and encouraging a belief in simple solutions. Both also, as befits the type, are full of absurd and redundant illustrations, although the prize here is unquestionably taken by Seay and Gottfried—with a picture of a supermarket; of numerous smiling infants, some busily taking in nourishment; of adolescents standing on street corners or, if of different sexes, sitting

in one another's laps; of a teacher looking at a blackboard, visitors to a science museum looking at an exhibit, and Jean Piaget looking at children playing. The differences between the two books, however, are greater than the similarities.

D. A. Dewsbury's *Comparative Animal Behavior* (McGraw-Hill: New York and London; £14.95) is the more conventional of the two, essaying a relatively straightforward account of psychological, ethological and (to show we move with the times) sociobiological approaches to the study of animal behaviour. The book has many virtues. It covers a great deal of ground (some of it quite out of the way), seems to be reasonably accurate, and does not always settle for received opinion. Although extremely diffident about it, for example, Dewsbury is willing to

Soon to be available as a Student Paperback.

Plant Virology: The Principles

Adrian Gibbs and Bryan Harrison

This modern exposition of plant virology aims to describe the framework of knowledge about plant viruses and to outline the methods used in obtaining this knowledge. The numerous illustrations include superb electron micrographs—many of them original—and other photographs as well as numerous line diagrams. Thus the framework of the subject is provided and much practical information on how plant viruses can be studied is given.

'The production is excellent, with clear type and illustrations. . . .

Unquestionably this book is a valuable addition to the few on plant viruses and will be welcomed especially by the small band who teach the subject at English-speaking universities.' —*Nature* (of the cloth edition)

Paper £9.75

Publication May

Recent 'Studies in Biology'

104 Biological Clocks

John Brady

An introduction to these physiological processes that measure time by some means and do so in relation to environmental time-cues, such as high tide or sunset. This is the first work of its kind intended for the biologically informed but non-specialist reader.

Paper £1.60

105 Liver

W. H. Horner Andrews

The book brings together widely scattered knowledge on the structure and function of the liver and also shows how these functions—of man and other organisms—are interrelated.

Paper £1.70

106 Aquaculture

P. J. Reay

This book brings together some of the more recent advances in aquaculture in order to explore the biological basis of this increasingly important industry and the role of the biologist in its future development.

Paper £1.75

Publication March 23rd.



Edward Arnold
41 Bedford Square,
London WC1B 3DQ

challenge the conventional assessment of the nature-nurture issue as a pseudo-question. But, for my taste at least, these virtues were outweighed by one pervasive vice: Dewsbury talks down to his readers. The book is permeated with an attitude of 'not in front of the students'. Whenever a topic shows signs of becoming interesting, Dewsbury backs off as if such complex matters were far beyond our capacity to understand. Thus conclusions are proclaimed, but we are never told why they are reasonable. Most biologists, no doubt, reject the theories of Lamarck and of Wynne-Edwards, but it should surely be possible to explain why they do so. Theories must be taken on trust, as they are rarely explicated, certainly not if they contain a hint of mathematics. The concept of an Evolutionary Stable Strategy, however, as Richard Dawkins has shown, can be explained, with numerical examples, in a manner intelligible to a wide range of readers. Dewsbury makes no attempt to do so, and the student must therefore take his word for it that 'the approach appears to have great potential'. Sometimes Dewsbury's reluctance to enter into complicated detail will surely cause much worse confusion than that which he was trying to avoid. Unless already familiar with the problems of navigation, for example, the reader will be quite unable to understand from his account the distinction between Kramer's map and compass theory and Matthew's sun-arc hypothesis of bird navigation; and the sense in which the former is addressed to a simpler question than the latter. Once again, however, it is a reasonably simple matter to illustrate the distinction by reference to the experimental evidence which suggests that Matthews is probably wrong.

Authors are apt to feel hard done by when reviewers complain about the omissions in their books. Dewsbury would perhaps insist that he had no room to go into the necessary detail, but this is largely because he has used his space unwisely. He seems to be obsessed by the need to cover the field, without stopping to ask what should be included in the comparative study of animal behaviour. It is surely reasonable to restrict it to genuinely comparative work, or that which emphasises adaptive function and other evolutionary concepts. It does not need to include the standard facts of sensory and perceptual psychology, conditioning and learning, and physiological psychology. These, as Dewsbury reminds us, are taught in other courses. But this does not deter him from devoting half-a-dozen of his 17 chapters to a necessarily rather cursory survey of these topics. He recounts

some intrinsically interesting facts, but there is little attempt to set them in any theoretical context. His book may be quite useful, but only if students are required to read other matter which will make them think harder.

B. Seay and N. Gottfried, in *The Development of Behavior: A Synthesis of Developmental and Comparative Psychology* (Houghton Mifflin: Boston and London; \$15.50), do not at least waste space or time in the provision of potted information on such standard topics. On reaching the end of their book, however, I found myself at a loss to recall what they had told me. Isolated examples came to mind: an analysis of shopping in a supermarket (hence the picture) in terms of 'super-schemas'; an assurance that love is not a mere epiphenomenon of sex, but a basic need in its own right; and, in case I did not know it, the information that 'lovers also engage in precopulatory behaviour, with participation in sexual intercourse dependent upon local custom and the stage of development of their relationship'. These stood out from an otherwise rather humdrum assortment of standard topics, some superficially recounted facts, and many more uncritically accepted theories. Bowlby's views on attachment, Whorf's on linguistic relativity, and Seligman's on preparedness in learning are all presented to us without any serious suggestion that they might not stand

up to critical examination. The book starts with a series of chapters on 'Phylogenetic Set', 'Ontogenetic Set' and several more sets, defined as 'pre-disposing influences on behavior'; while Part II, just to be different, is organised around various behavioural systems, such as affection and social organisation, which, the reader is asked to remember, are all affected by an interaction of all these sets.

As its subtitle suggests, the book is a curious mixture of comparative psychology and ethology on the one hand and human developmental and social psychology on the other, with a dash of social anthropology thrown in for good measure. But it is not, alas, a synthesis. Throughout the book, we are switched back and forth from studies of animals to the study of man, with little discussion of the relevance of one to the other. It is not so much that the authors are rash or glib in their willingness to generalise, as that they do not involve the student in any serious consideration of the issues involved. Unlike Dewsbury, they have not chosen so to fill their book with facts that there is no room left for discussion: the space is there, but it is largely filled with platitudes.

N. J. Mackintosh

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Sensory physiology

Two recent books on sensory physiology have a similar aim and are therefore reviewed here together. The publishers have to be congratulated for producing two compact, well printed and well illustrated books.

Jacqueline Ludel's *Introduction to Sensory Processes* (Freeman: San Francisco and London; hardback \$17, paperback \$9) has the advantage that it is written by one author and this gives uniformity of style. She goes to great efforts to define accurately every new term, often illustrating the point with a clearly drawn figure. The book has a considerable number of colour illustrations which do help understanding. She has mastered the art of a good teacher—tell the student everything three times without boring him. This is a book to be read slowly and thoughtfully. It is reasonably up to date and certainly adequate for an introduction to the subject of sensory physiology.

Fundamentals of Sensory Physiology (Springer: New York and Berlin; paper-

back OM 33.60, \$16.80) is very well edited by Robert Schmidt so that, although there are seven different contributors, the style is uniform. As each section is written by an expert in the field it is accurate and fairly up to date. Again, each new scientific word or definition is carefully defined and printed in bold type. At the end of each chapter there are a number of thought provoking questions which ensures that the reader has thoroughly understood the contents.

Both books give excellent references, readily obtainable, for further advanced reading. Teachers of the subject will wish to have both books to hand as there are different teaching points and illustrations in each of them. Individual readers will have a hard task deciding which one to purchase as both are so good. Libraries will wish to have a copy of both. Both will appeal to physiologists, psychologists and scientists in industry dealing with man-machine interactions—for example, photography and sound recording.

F. W. Campbell

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Recent scientific and technical books

Physics

- CALVERT, J. M., and MC CAUSLAND, M. A. H. *Electronics*. (The Manchester Physics Series) Pp.xii+615 ISBN-0-471-99640-8. (Chichester and New York: John Wiley and Sons, 1978.) £20.
- CAPPELLINI, V., CONSTANTINIDES, A. G., and EMILIANI, P. *Digital Filters and Their Applications*. (Techniques of Physics, No. 4.) Pp.xii+393. ISBN-0-12-159250-2. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) £20.50, \$42.
- CHU, C. W., and WOOLLAM, J. A. (edited by). *High-Pressure and Low-Temperature Physics*. Pp.xii+602. ISBN-0-306-40014-6. (New York and London: Plenum Press, 1978.) \$31.18.
- DIXON, W. G. *Special Relativity. The Foundation of Macroscopic Physics*. Pp.x+261. ISBN-0-521-21871-3. (Cambridge, London and New York: Cambridge University Press, 1978.) £14.50.
- FUJITA, Shuji (edited by). *The Ta-Yu Wu Postscript: Science of Matter*. Pp.xii+334. ISBN-0-677-13650-1. (New York, Paris and London: Gordon and Breach Science Publishers, 1978.) £30.60.
- HANLE, W., and KLEINPOPPEN, H. *Progress in Atomic Spectroscopy, Part A (Physics of Atoms and Molecules)*. Pp.xvii+712. ISBN-0-306-31115-1 (Part A). (New York and London: Plenum Press, 1978.) \$31.18.
- HASS, Georg, and FRANCOMBE, Maurice H. (edited by). *Physics of Thin Films: Advances in Research and Development, Vol. 10*. Pp.xi+324. ISBN-0-12-533010-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) \$35; £22.75.
- KURSUNOGLU, Behram (chairman), PERLMUTTER, Arnold, and SCOTT, Linda F. (editors). *New Frontiers in High-Energy Physics (Orbis Scientiae)*. (Studies in the Natural Sciences, Vol. 14.) Pp.x+670. ISBN-0-306-40037-5. (New York and London: Plenum Press, 1978.) \$37.48.
- LONNGREN, Karl, and SCOTT, Alwyn (edited by). *Solitons in Action*. (Proceedings of a Workshop sponsored by the Mathematics Division, Army Research Office, held at Redstone Arsenal, October 26-27, 1977.) Pp.xiii+300. ISBN-0-12-455580-2. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) \$15; £9.75.
- MANDEL, Leonard, and WOLF, Emil (edited by). *Coherence and Quantum Optics IV*. (Proceedings of the Fourth Rochester Conference held at the University of Rochester, June 8-10, 1977.) Pp.xv+1011. ISBN-0-306-40038-3. (New York and London: Plenum Press, 1978.) \$40.95.
- MARTON, L. (edited by). In association with MARTON, Claire. *Advances in Electronics and Electron Physics*. Vol. 46. Pp.xii+404. ISBN-0-12-014646-0. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) \$37; £24.35.
- OEUVRES SCIENTIFIQUES DE LOUIS NÉEL. Pp.xvii+586. ISBN-2-222-02127-8. (Paris: Éditions du Centre National de la Recherche Scientifique, 1978.) F.290.
- PAPADOPOULOS, George J., and DEVREESE, J. T. (edited by). *Path Integrals and their Applications in Quantum, Statistical, and Solid State Physics*. (NATO Advanced Study Institutes Series, Series B: Physics, Vol. 34.) Pp.x+515. ISBN-0-306-40017-0. (New York and London: Plenum Press, 1978. Published in Co-operation with NATO Scientific Affairs Division.) \$31.18.
- POHL, Herbert A. *Dielectrophoresis: The Behaviour of Natural Matter in Nonuniform Electric Fields*. (Cambridge Monographs on Physics.) Pp.xii+579. ISBN-0-521-21657-5. (Cambridge, London and New York: Cambridge University Press, 1978.) £48.
- SQUIRE, G. L. *Introduction to the Theory of Thermal Neutron Scattering*. Pp.vii+260. ISBN-0-521-21884-5. (Cambridge, London and New York: Cambridge University Press, 1978.) £16.
- UBBELOHDE, A. R. *The Molten State of Matter. Melting and Crystal Structure*. Pp.xiv+454. ISBN-0-471-99626-2. (Chichester and New York: Wiley-Interscience, John Wiley and Sons, 1978.) £23.50.
- ZEWAIL, A. H. (edited by). *Advances in Laser Chemistry*. (Proceedings of the Conference on Advances in Laser Chemistry, California Institute of Technology, Pasadena, U.S.A., March 20-22, 1978.) (Springer Series in Chemical Physics, Vol. 3.) Pp.x+463. ISBN-3-540-08997-7. (Berlin and New York: Springer-Verlag, 1978.) np.

Chemistry

- BALLARD, R. E. *Photoelectron Spectroscopy and Molecular Orbital Theory*. Pp.ix+192. ISBN-0-457-341-6. (Bristol: Adam Hilger Ltd., 1978.) £18.
- BARLTROP, J. A., and COYLE, J. D. *Principles of Photochemistry*. Pp.x+213. ISBN-0-471-09667-4. (Chichester and New York: John Wiley and Sons, 1978.) £4.95.
- COULSON, J. M., RICHARDSON, J. F., BACKHURST, J. R., and HARKER, J. H. *Chemical Engineering, Vol. 2: Unit Operations*. Third edition. (SI Units). Pp.xv+807. ISBN-0-08-022919-0. (Oxford and New York: Pergamon Press, 1978.) Hard £20; Flexi £12.50.
- FLYGARE, W. H. *Molecular Structure and Dynamics*. Pp.xviii+696. ISBN-0-13-599733-4. (Englewood Cliffs, NJ: Prentice-Hall, Inc., 1978.) np.
- GREENLAND, D. J., and HAYES, M. H. B. (edited by). *The Chemistry of Soil Constituents*. Pp.469. ISBN-0-471-99619-X. (Chichester and New York: Wiley-Interscience, John Wiley and Sons, 1978.) £26.
- HAGENMULLER, Paul, and VAN GOOL, W. (edited by). *Solid Electrolytes: General Principles, Characterization, Materials, Applications*. (Materials Science and Technology) Pp.xvii+549. ISBN-0-12-313360-2. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) \$52; £33.80.
- HITCHMAN, Michael L. *Measurement of Dissolved Oxygen*. (Chemical Analysis: a Series of Monographs on Analytical Chemistry and its Applications, Vol. 49.) Pp.xvi+253. ISBN-0-471-03885-7. (New York and Chichester: Wiley-Interscience, John Wiley and Sons, 1978.) £16.50.
- HULANICKI, Adam, and GLAB, S. *Redox Indicators: Characteristics and Applications*. (International Union of Pure and Applied Chemistry.) Pp.465-498. ISBN-0-08-022383-4. (Oxford and New York: Pergamon Press, 1978.) £3.30.
- LAPANUE, Savo. *Physicochemical Aspects of Protein Denaturation*. Pp.x+331. ISBN-0-471-03409-6. (New York and Chichester: Wiley-Interscience, John Wiley and Sons, 1978.) £19.50.
- LAUB, Richard J., and PECOSK, Robert L. *Physicochemical Applications of Gas Chromatography*. Pp.xii+399. ISBN-0-471-51838-7. (New York and Chichester: Wiley-Interscience, John Wiley and Sons, 1978.) \$33; £16.60.
- MEHROTRA, R. C., BOHRA, R., and GAUR, D. P. *Metal Biotransformations and Allied Derivatives*. Pp.viii+382. ISBN-0-12-488150-5. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) £20.50.
- SEABORG, Glenn T. (edited by). *Transuranium Elements: Products of Modern Alchemy*. (Benchmark Papers in Physical Chemistry and Chemical Physics I.) Pp.xvii+488. ISBN-0-87933-326-X. (Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross, Inc., 1978. Distributed world wide by Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers.) \$36; £23.40.
- YATES, Keith. *Hückel Molecular Orbital Theory*. Pp.xii+371. ISBN-0-12-768450-1. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) \$32; £20.80.

Technology

- ACKERS, P., WHITE, W. R., and HARRISON, A. J. M. *Weirs and Flumes for Flow Measurement*. Pp.xix+327+39 plates. ISBN-0-471-99637-8. (Chichester and New York: Wiley-Interscience, John Wiley and Sons, 1978.) £19.50.
- AHMED, H., and SPREADBURY, P. J. (edited by). *Transmission and Propagation of Electromagnetic Waves*. (Electronics Texts for Engineers and Scientists.) Pp.x+410. ISBN-0-521-21924-8. (Cambridge, London and New York: Cambridge University Press, 1978.) Hard cover £24.50; Paperback £5.95.
- ANDRESEN, A. F., and MAELAND, A. J. (edited by). *Hydrides for Energy Storage*. (Proceedings of an International Symposium held in Oslo, Norway, 14-19 August 1977.) Pp.xvi+599. ISBN-0-08-022715-5. (Oxford and New York: Pergamon Press, 1978. Published on behalf of the International Association for Hydrogen Energy.) £30.
- BAILIE, Richard C. *Energy Conversion Engineering*. (Energy Science and Technology: a Series of Graduate Textbooks, Monographs, Research Papers.) Pp.xvii+337. ISBN-0-201-00840-8. (Reading, Mass. and London: Addison-Wesley Publishing Company, 1978.) \$19.50.
- CHEREMISINOFF, Paul N., and HELLERBUSCH, Fred (edited by). *Carbon Adsorption Handbook*. Pp.ix+1054. ISBN-0-250-40236-X. (Ann Arbor, Michigan: Ann Arbor Science Publishers, Inc., 1978. Distributed in UK by John Wiley and Sons, Ltd., Chichester.) £25.15.
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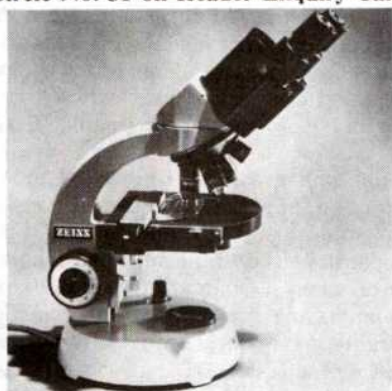
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Oximeter. British American Optical have recently introduced the AO Unistat oximeter into their range of medical equipment. This machine gives a value of oxygen saturation in blood in under 20 s. To obtain this information, the samples of blood (only 0.4 ml of whole blood is required) are placed in one of the disposable cuvettes and the per cent of oxygen saturation is read off on the large LED display.

Circle No. 35 on Reader Enquiry Card.

Gas-tight syringes with removable needles. Hamilton. Available from V. A. Howe are the new range of Hamilton 1000 and 0700 series gas-tight syringes with removable needles. There are six models, from 10 μl to 500 μl , with advantages including that the plunger, glass barrel and needles are field replaceable, that the needle plugs straight into the Teflon ferrule and is securely held in place by a locking nut, and the replaceable Teflon plunger tips allow injections at up to 7 atms.

Circle No. 36 on Reader Enquiry Card.

Microcellcounter. TOA Medical Electronics. A microcellcounter capable of performing RBC, WBC and platelet counting without changing transducers has been introduced by TOA Medical Electronics. The Sysmex PL-110 is a fully automatic system following sample introduction, including data printout with the Sysmex DP-411 printer option. Other options include the Sysmex auto dilutor Ad-220, which reduces pipetting errors to well under clinically acceptable levels. The counter allows fingertip control and visual inspection of the aspiration and ejection phases.

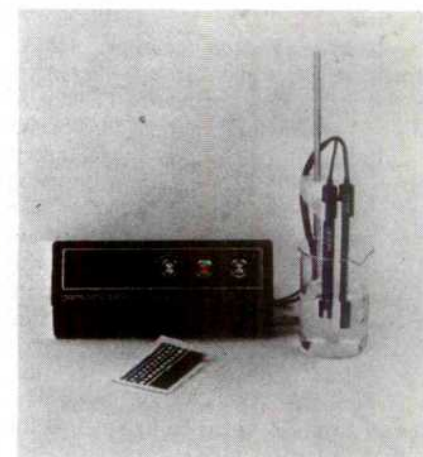
Circle No. 37 on Reader Enquiry Card.

Temperature linearisation amplifiers. Philips. Two temperature linearisation amplifiers have been introduced by Philips. The units, nominated PM9873/01 for Fe-Const, type J, and PM9874/01 for NiChr-NiAl, type K, provide an output of 1 mV per $^{\circ}\text{C}$ over the full temperature range of the thermocouple and include automatic cold junction-compensation, within a linearity of $\pm 1^{\circ}\text{C}$. Both units are basically designed for use with Philips modular compact recorders PM 8202 one line and PM 8222 two line.

Circle No. 38 on Reader Enquiry Card.

Digital pH meter. Orion. The 501 digital pH temperature system from Orion displays pH readings from 0.00 to 14.00 with precision to ± 0.01 pH (the system also reads out in mV). Temperature measurements can be made over -5° – 100°C with $\pm 0.1^{\circ}\text{C}$ accuracy. Also supplied with the 501 are: an unbreakable, platinum resistance probe which provides accurate automatic temperature compensation and digitalised temperature readings; and an unbreakable 91-05 gel-filled GX electrode that never requires refilling with KCl.

Circle No. 39 on Reader Enquiry Card.



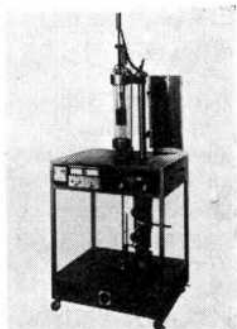
The Orion 501 digital pH system

Radiometer/photometer. Glen Creston now offer the model 730A radiometer/photometer for measurement of both continuous and pulsed light sources. Readout is direct in any one of 14 optical and electrical units. Both BCD and 0–1 V analogue outputs are standard. The wavelength range covered is 200–1,100 nm. The sensor consists of a stable, UV enhanced silicon photodetector with an area 1 cm^2 . Radiometric and photometric filters are provided.

Circle No. 40 on Reader Enquiry Card.

Float zone crystal growth systems. Metals Research. These modular systems, which can incorporate Czochralski, Bridgeman, RF float zone and Kyropoulos crystal growth facilities, allow continuous visual assessment of crystal growth. They have a choice of two chamber sizes and a wide range of accessories. The system avoids the need for temperature control equipment because the twin-walled, water-cooled reactor chambers are transparent, the outer walls being made of Pyrex and the inner of fused quartz. Using the standard RF induction heating system, vacuum, inert gas, oxidising or reducing atmospheres may be used at temperatures up to 1,800 °C. The system is modular so that the basic equipment can be added to at any stage. The basic unit consists of the main frame plinth, the selected heating system, furnace support column and Bridgeman lowering mechanism for the crucible or mould.

Circle No. 41 on Reader Enquiry Card.



Metals Research float zone system

Direct-drive vacuum pumps. GCA/Precision Scientific Group has introduced seven new models to its line of direct-drive vacuum pumps. This brings the Precision line of two-stage direct-drive pumps to 10, ranging in pumping capacity from 20 to 475 l m⁻¹. The new pumps range in size from 90 to 475 l m⁻¹. They are capable of an ultimate vacuum of 0.1 μm, and can pump at 50% maximum throughput at 1 μm. Extremely low operating noise levels are a key feature of the new units. Exceptionally low vibration and an internal exhaust baffle contribute to the pumps' quiet operation.

Circle No. 42 on Reader Enquiry Card.

Reagent grade water for elemental analysis. Millipore. To meet the growing demand for purer water, Millipore has developed the Milli-Q system, which produces reagent grade water of 18 MΩ resistivity free of organic, inorganic and particulate contaminants. Some typical applications are preparation of standards and sample make-up in AA and other elemental analysis, and organic determinations with GLC and HPLC.

Circle No. 43 on Reader Enquiry Card.

Aerosol and particulate monitoring systems. Phoenix Precision Instruments. A range of aerosol systems for detecting and monitoring airborne contaminants, indoors or out, is available from Phoenix Precision Instruments. The systems comprise models JM-5000, featuring logarithmic output, JM-6000, with 5-range selectable linear amplification, and JM-7000, a combination log linear instrument. Designed for testing and monitoring HEPA filters, these compact and completely self-contained photometers give direct readings in mass concentrations to eliminate statistical calculations.

Circle No. 44 on Reader Enquiry Card.

Edax system for Philips microscopes. Pye Unicam. An energy dispersive X-ray analysis system can now be ordered from Pye Unicam for use in conjunction with Philips scanning and transmission microscopes or columns. Features of the Edax 9100 series analytical system include: a 4000-channel data memory with 10⁶ capacity per channel; automatic vertical size normalisation of compared spectra; unique function keys that eliminate the typing of mnemonic codes; expansion for WD, EELS and other multi-task function.

Circle No. 45 on Reader Enquiry Card.

Computer system. Control Data. The Control Data Cyber 203 is a large-scale, high-speed logical and arithmetic computer system. It uses integrated circuits and offers advanced design features such as vector stream processing (up to 10⁸ results per s), virtual addressing, hardware macro instructions and a 2 × 10⁶ word semiconductor memory. It also offers separate scalar and array processing units, and functional units specifically designed for sequential and parallel operations on single bits, 8-bit bytes, and 32- or 64-bit floating-point operands and vectors. Virtual addressing uses a high-speed mapping technique for converting a logical address to an absolute storage address.

Circle No. 46 on Reader Enquiry Card.

FMOC amino acids. Chemical Dynamics Corporation announce the availability of a new protecting group for amino acids, the 9-fluorenylmethyloxy carbonyl (FMOC) group. This protecting group is a base-labile and acid-stable protecting group, easily removed by amine bases such as ammonia or piperidine. The FMOC group is ideally suited to the synthesis of peptides with special side-chain protecting problems, as it is completely compatible with side-chain BOC groups and *t*-butyl esters and ethers.

Circle No. 47 on Reader Enquiry Card.



Epitaxial system. Metals Research. New from Metals Research is an RF heated, epitaxial and chemical vapour deposition system—the system 100—designed as a low cost equipment for research and pilot plant production applications. The system 100 is based on two separate consoles, one for the gas control system and one for the quartz reaction cell. The consoles are made from polyurethane resin enamelled sheet steel and are 1,750 mm high × 1,070 mm wide. Consoles are bolted together to form a single unit. System features include interlocking for operator safety, air or water cooled cells and horizontal laminar flow.

Circle No. 48 on Reader Enquiry Card.

Lipid panel assays. Bio-Rad Laboratories are now offering a comprehensive range of assays in the field of lipid chemistry, the Bio-Rad HDL cholesterol assay, Quantazyme cholesterol, Quantazyme triglycerides and the Bio-Gram A lipoprotein electrophoresis test system. The HDL cholesterol system comprises a one vial, predispensed, lyophilised separation system and eliminates refrigeration steps. Both the cholesterol and triglycerides assay systems are single vial, fully enzymatic, easily automated and end-point assays.

Circle No. 49 on Reader Enquiry Card.

Cell disintegrator. Biox. The Biox X-Press cell disintegration system operates at -20 °C, taking advantage of the shearing stresses generated when ice is forced through a narrow orifice at 28,000 p.s.i. The method is rapid, easy to use and the low temperature, maintained throughout the process, prevents destruction of heat-labile components. The disintegration unit itself is autoclavable and corrosion resistant.

Circle No. 50 on Reader Enquiry Card.

Control sera. Bio-Rad Laboratories announce the availability of an extended range of "Lyphochek" control sera. Lyphochek comprises a range of assayed and unassayed lyophilised human control sera for both automated and manual procedures, in addition to a serum reference standard. All these materials are reported to be of outstanding consistency, reliability and stability.

Circle No. 51 on Reader Enquiry Card.

Acoustic emission system. EMI Electronics. The acoustic emission system from EMI is capable of predicting, detecting and locating fatigue cracks in large steel structures. The system uses a Data General mini-computer with a software package designed to provide a range of characterisation processes capable of distinguishing between background noise and genuine crack extension noise. All processing and recording equipment is mounted in a standard 6 ft×19 inch rack. An associated graphics display shows not only the relative positions of emissions but also analyses their characteristics and provides a display of emission density. The system is available for hire or for sale.

Circle No. 52 on Reader Enquiry Card.



The EMI acoustic emission system

Hoffman modulation contrast system now available for Nikon SC microscope. The Nikon SC microscope is now available in a model equipped with the Hoffman modulation contrast system. The system is especially useful in tissue culture studies. The images produced appear three-dimensional, and the system is not affected by the birefringence exhibited by many biological specimens. It uses a modified standard 40× objective, with special built-in modulator element, and a matching slit aperture which fits into a pre-aligned mounting on the condenser.

Circle No. 53 on Reader Enquiry Card.

Anti-human β_2 -microglobulin antibody. Sera-Lab announce the introduction of a monoclonal antibody to β_2 -microglobulin. The material complements two other monoclonal antibodies, directed against a marker on chromosome six, and these three antibodies will form a useful screening panel for the close mapping of HLA markers. The material is suitable for techniques using precipitation and also for labelling with radioisotopes.

Circle No. 54 on Reader Enquiry Card.

Digital image analysis system. Zeiss. A unique and simple quantitative image analysis system, called MOP, has been introduced Carl Zeiss. The special feature of this system is the interaction of the operator's discriminating judgment with the high-speed calculation ability and memory of its microcomputer. The images to be analysed can be in the form of photographs including X-rays, projections and drawings, or they can be directly evaluated through a microscope. The system consists of a compact hybrid microcomputer and a multi-purpose measuring tablet. It has the ability to accurately measure up to 8 parameters simultaneously and the capacity to summarise and store data in up to 20 separate channels. In addition, as a stand-alone system, it can determine percentages, averages, standard deviations, and area differentials. It is also capable of elaborate distribution analysis. The MOP is portable, requires no installation and practically no operator training.

Circle No. 55 on Reader Enquiry Card.

Ultra-fast film for tritium detection. LKB introduces a high-speed film for gross autoradiography of low-energy emitting radio-isotopes such as tritium and ^{125}I . The film, named LKB Ultra-film ^3H , is specially designed for direct high-speed autographic applications in autoradiography of tissue sections, electrophoresis and electrofocusing in thin layer polyacrylamide and agarose gels, and paper electrophoresis and thin layer chromatography. It is 12–64 times faster than conventional X-ray films for tritium detection. With its transparent, uncoloured backing it is also convenient for photocopying, making enlargements and direct display in overhead projectors.

Circle No. 56 on Reader Enquiry Card.

Rhodamine-conjugated immunoglobulin fractions of antisera. Accurate Chemical and Scientific Corporation announces the availability of five new tetramethylrhodamine-conjugated immunoglobulin fractions of antisera. Tetramethylrhodamine isothiocyanate isomer R has been used for the labelling. R 151, rabbit anti-human IgG specific for γ -chains; R 152, rabbit anti-human IgM, specific for μ -chains; R 154, rabbit anti-human κ light chains; R 155, rabbit anti-human λ light chains; R 156, swine anti-rabbit immunoglobulin. Free rhodamine has been removed from these products by gel filtration, and optimum labelled antibody molecules have been isolated by ion exchange chromatography.

Circle No. 57 on Reader Enquiry Card.

Metal film detectors for lasers. Rofin has introduced a new type of detector which consists of a metal film on an aluminium substrate and which has a very high power handling capability. This, in addition to its broad spectral response (visible to $>30\ \mu\text{m}$) makes it ideal as a general purpose laser detector system. A unique feature of the new device is that it can be used for position sensing. Position sensitivities in the μm range are routinely achieved. The detector has a flat response from D.C. to $>10\ \text{kHz}$, and will respond, with lower sensitivity, to MHz frequencies with rise times of less than $1\ \mu\text{s}$. Active areas up to $10''$ diameter are available.

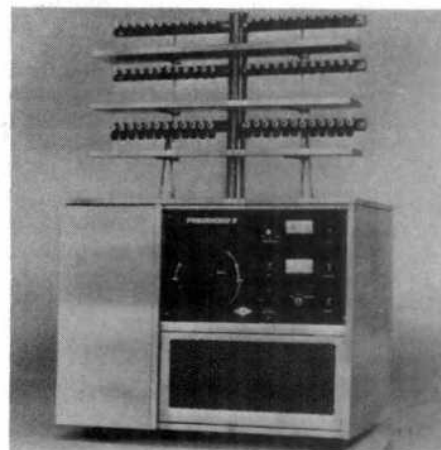
Circle No. 58 on Reader Enquiry Card.

Trace metal-free pipette tips. Brinkmann. Brinkmann Instruments have introduced trace metal-free pipette tips for use with Eppendorf pipettes, sizes 1 to $100\ \mu\text{l}$. The pipette tips are manufactured from non-wetting virgin polypropylene and contain no trace metals or other contaminants. These colourless tips are supplied in 20 sealed bags of 50 tips each inside a shipping/storage carton containing 1,000 tips.

Circle No. 59 on Reader Enquiry Card.

Freeze-drier. Techmation. A heavy-duty freeze-drier for research or pilot production has been introduced by Techmation. The VirTis Freezemobile II, with a total condensate capacity of 18 l, can achieve temperatures of $-55\ ^\circ\text{C}$ in less than 20 min as standard and $-85\ ^\circ\text{C}$ with optional cascade freezing. The condenser has a clear acrylic cover through which the user can observe the ice build-up during drying. An automatic hot gas quick defrost is available which allows the complete removal of the 18-l ice plug in 3 h. Five base models are available including one with a built-in shell freezing bath and twin condenser units.

Circle No. 60 on Reader Enquiry Card.



The VirTis Freezemobile II

nature CLASSIFIED ADVERTISEMENTS

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APPOINTMENTS VACANT

UNIVERSITY OF STRATHCLYDE

Applications are invited from graduates in Operational Research for a

LECTURESHIP

In the
DEPARTMENT OF
OPERATIONAL RESEARCH

Candidates should have teaching and research experience in Operational Research and/or substantial experience of Operational Research in the field.

Salary scale £3,883 to £7,754 (under review) with placing according to qualifications and experience. Superannuation benefit.

Application forms and further particulars (quoting 10/79) and enclosing a self-addressed envelope (9in by 4in), may be obtained from the Academic Appointments Officer, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW with whom applications should be lodged by March 31, 1979.

9822(A)

UNIVERSITY OF ZÜRICH INSTITUTE OF INORGANIC CHEMISTRY POSTDOCTORAL RESEARCH CRYSTALLOGRAPHER

Applications are invited for a post-doctoral position in X-ray-crystallographic work mainly on coordination compounds. Good training in crystallography and experience in single crystal structure determination is a necessity. Well equipped laboratory (including low temperature and high temperature X-ray-crystallography).

The initial appointment would be for one year, with extension possible for two or more years. Salary range Swiss francs 40,000 to 45,000 per year (current exchange: £12,000 to £13,400 per annum sterling).

Applications with curriculum vitae and names of two referees should be sent as soon as possible to Dr Erich Dübler, Institute of Inorganic Chemistry, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland.

9904(A)

The British Council invites applications for the following post:

UNIVERSITY OF SRI LANKA Colombo Campus SENIOR LECTURER IN FISHERIES BIOLOGY (Sri Lanka)

Qualifications. Candidates, men only, should hold Ph.D. and have at least 4 years postdoctoral experience in marine fisheries research and/or development. Knowledge of marine or freshwater aquaculture an advantage. Duties include teaching at postgraduate level and supervision of research.

Benefits: Personal allowance free furnished accommodation and other benefits. 2 year contract.

Salary: £5,681 to £7,707

Return fares are paid. Local contracts are guaranteed by the British Council. Please write briefly stating qualifications and length of appropriate experience, quoting reference number and title of post, for further details and application form to The British Council (Appointments), 65 Davies Street, London, W1Y 2AA.

9879(A)

UNIVERSITY OF CAMBRIDGE ANIMAL PRODUCTIVITY/ NUTRITION

Applications invited for a University Demonstratorship in Animal Productivity/Nutrition in the Department of Applied Biology. Qualifications—good Honours degree, research experience in some aspect of animal productivity related to nutrition and competence in biometry. Salary in scale £4,133 to £5,129. Pensionable under U.S.S. Limited contribution to removal expenses. Further details from and applications including full personal particulars, list of publications and names and addresses of not more than three referees to the Secretary, Faculty of Biology 'A' Appointments Committee, Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX later than March 31, 1979.

9891(A)

SEALE-HAYNE COLLEGE NEWTON ABBOT, DEVON LECTURER IN ANIMAL PRODUCTION

Applications are invited from suitably qualified graduates with teaching, research or appropriate industrial experience. Duties include teaching degree and diploma students.

Salary based on Scales of Salaries for Teachers in Establishments for Further Education, England and Wales, Lecturer Grade II (£4,101 to £6,558 per annum).

Applications to and further particulars from, The Principal, Seale-Hayne College, Newton Abbot, Devon, TQ12 6NQ.

9849(A)

THE UNIVERSITY OF BIRMINGHAM Faculty of Medicine and Dentistry DEPARTMENTS OF IMMUNOLOGY AND PHYS. RESEARCH ASSOCIATE

A good honours life sciences degree is required to assist in the department of *in vitro* and *in vivo* cytotoxic assays of antitumour antibody bound to cyclotron-produced emitting isotopes. Postgraduate experience with small animal models, immunology or radio handling techniques would be a plus.

The project, financed by the MRC, is for three years. Salary will be the Research Associate Range 1B scale—£3,384 to £4,882 superannuation. The maximum salary will be £3,634.

Further information may be obtained from the Assistant Registrar, Medical School, Birmingham B15 2TT with whom applications (three including a curriculum vitae) should be lodged not later than March 1979. Please quote reference IMM/179.

9888

TECHNICIAN (Grade 5) required in Department of Microbiology, University of Reading. Main duties are supervision of laboratory service section dealing with teaching and research in Microbial Physiology Biochemistry, equipment for tea and research, chemical stores according to qualifications and experience in range £3,186 to £3,726 (under review). Further details available on request. Apply, stating qualifications and experience and the names of two referees, to Ref T.N. 74A, to Assistant 1 (Personnel), University of Reading, Whiteknights, Reading, RG6 2AA.

9831

UNIVERSITY OF EDINBURGH DEPARTMENT OF MICROBIOLOGY

Applications are invited for post of

UNIVERSITY DEMONSTRATOR

in the Department of Microbiology. Candidates should normally be in their third year of graduate research in any field of Microbiology.

Salary scale £3,384 to £4,882 per annum.

Further details are available from the Secretary to the University, Old College, South Bridge, Edinburgh EH8 9YL, to whom applications (five copies), giving the names of three referees, should be sent not later than March 1979. Please quote Reference I.

9872C

MICROBIOLOGIST/ IMMUNOLOGIST

Bayer AG, West Germany, who have been active in pharmaceutical research for about 100 years, wish to appoint a person to establish a new unit within the Chemotherapy Department at their headquarters in Elberfeld, West Germany.

Applicants should have experimental experience both in microbiology and immunology and should preferably be medically qualified holding a higher degree. They should have sufficient experience to build up a new unit to follow a new line of therapeutic research. Applicants should be aged 30-40.

A knowledge of German would be an advantage but is not essential.

An attractive salary will be offered, together with additional benefits associated with a major Company.

Applications should initially be sent in confidence to:

Dr. H. B. Allen, Medical Director
Bayer UK Limited, Pharmaceutical Division
Haywards Heath West Sussex RH16 1TP

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CHEMICAL/RUBBER/POLYURETHANES
ENGINEERING PLASTICS/DYESTUFFS
TEXTILE FIBRES/CROP PROTECTION PRODUCTS
PHARMACEUTICALS/VETERINARY PRODUCTS

9903(A)

**JAMES COOK UNIVERSITY
OF NORTH QUEENSLAND
LECTURER**

IN THE DEPARTMENT OF
TROPICAL VETERINARY SCIENCE
(VETERINARY MICROBIOLOGY)

A vacancy exists for a Lecturer in Veterinary Microbiology in the Department of Tropical Veterinary Science. Applicants should have wide Post-graduate experience in Veterinary Microbiology relating to diseases of livestock. Expertise in Bacteriology is preferred and a background of research in Contemporary Immunological methods is essential. The appointee will be responsible for the presentation of graduate courses and the development of research on disease problems in food animals and will also be required to supervise research by graduate students undertaking M.Sc. and Ph.D. programmes.

The salary range for the position of Lecturer is \$A15,786 to \$A20,737 per annum and commencing salary will be determined according to qualifications and experience. Conditions of appointment include F.S.S.U. type superannuation, study leave, invalid pension scheme, housing assistance and allowance for travelling and removal expenses on appointment. Additional information and application forms are obtainable from the Registrar, Post Office, James Cook University, Qld 4811, Australia, or from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. Applications close March 31, 1979. 9821(A)

**NG'S COLLEGE HOSPITAL
MEDICAL SCHOOL
(University of London)**

Denmark Hill,

London SE5 8RX

**DEPARTMENT OF
CHEMICAL PATHOLOGY
RESEARCH FELLOW**

Applications are invited for the above post, which will be supported by a grant from the Cancer Research Campaign.

The successful applicant, who should have a Ph.D. degree or equivalent experience, would join a small group studying the mechanism of steroid hormone action, under the direction of Dr M. Norman.

The project will involve the use of human lymphoid cell line for the *in vitro* investigation of interactions between steroids and other drugs used in the treatment of leukaemia. Experience in the fields of tissue culture or mechanism of drug action would be desirable but not essential.

The appointment will be for three years, with a starting salary of £4,882 annum plus £450 London Weight Allowance. Further particulars may be obtained from the Secretary to the Medical School to whom applications, including full curriculum vitae and naming two referees, should be sent by March 23, 1979. 9868(A)

**UNIVERSITY OF ESSEX
LECTURESHIPS IN THE
DEPARTMENT OF BIOLOGY**

Applications are invited for two lectureships in biology tenable from October 1, 1979 (salary scale £3,883-£7,754 under review). One appointment will be for an animal biologist, preferably with interests in physiology and immunology, the other for someone interested in the molecular basis of development or cell biology.

Applications (nine copies), including curriculum vitae and the names and addresses of two referees, should reach the Registrar (AG/66/N), University of Essex, Wivenhoe Park, Colchester CO4 3SQ, from whom further particulars of the research and teaching are available, by March 16, 1979. 9864(A)

Drug Metabolism Section Heads Biosciences Research Centre Great Burgh, Epsom

As a result of the Company's expanding research activities, a new Drug Metabolism Unit is being established at Great Burgh. The Unit, which should be ready for occupation in July 1979, will contain three sections: Analytical Biochemistry, Chemistry and Metabolic Biochemistry. As part of the Clinical Pharmacology Department, the Unit's responsibilities will include the investigation of drug metabolism in experimental animals and man, with particular interest in the areas of biosciences and chemotherapeutic agents.

Two Section Heads are required (the Head of the Chemistry Section has already been appointed), who will assist the Head of the Unit in the equipping of the laboratories and the appointment of staff within their respective sections, before the Unit becomes fully functional.

Analytical Biochemistry

The Head of Analytical Biochemistry Section will be responsible for a team concerned with the development and application of methods for the quantitative analysis of drug substances and their metabolites in biological systems and for the design and initiation of pharmacokinetic studies. Candidates for this position should have broad experience in the use of H.P.L.C., G.L.C., GC-MS, automated analysis and other relevant analytical techniques used in modern biochemical analysis.

Metabolic Biochemistry

The Head of Metabolic Biochemistry Section will be responsible for a team investigating the metabolic fate of pharmacological agents in laboratory animals and man. The successful candidate will have had at least three years post-doctoral experience in the field, be well informed on the theoretical concepts involved and possess a substantial knowledge of relevant practical techniques.

The nature of the work in the Drug Metabolism Unit will necessitate considerable liaison between the three Section Heads, the Head of the Unit, and other scientific staff.

The positions which are open to male and female candidates will command a competitive starting salary and a range of other benefits including non-contributory pension and free life assurance. For an application form please telephone Burgh Heath 53344, Extension 208, or write to: **The Site Personnel Manager, Beecham Pharmaceuticals, Research Division, Biosciences Research Centre, Great Burgh, 1 Yew Tree Bottom Road, Epsom, Surrey.**

Beecham Pharmaceuticals

9910(A)

Pre and postdoctoral positions are available for studies on the protein synthesising system with special emphasis on structure and function of mRNP particles, ribosomes—topography, biogenesis and function—under various conditions of growth and development. Teaching opportunities available. Reply with resumé to: Professor Bruce H. Sells, Laboratories of Molecular Biology, Faculty of Medicine, Memorial University of Newfoundland. 9751(A)

APPLICATIONS are invited to fill three new permanent faculty positions in geophysics, ocean-oriented engineering and physical/meteorological oceanography to expand present twelve-person faculty. Six-credit graduate (M.Sc., Ph.D.) and undergraduate level teaching loads offer a relatively unencumbered opportunity to develop research interests in national and international areas while living in an ideal climate. Ph.D. degree required. Rank and salary open. Contact N. W. O'Hara, Head, Dept. of Oceanography and Ocean Engineering, Florida Institute of Technology, Melbourne, FL 32901. (305) 723-3701. Equal opportunity/affirmative action employer. 9847(A)

**UNIVERSITY OF LONDON
CHAIR OF
HUMAN ENVIRONMENT
tenable at the
Institute of Archaeology**

The Senate invites applications for the above Chair. Further details should be obtained from the Academic Registrar (N), University of London, Malet Street, London WC1E 7HU, before submitting applications (10 copies). Closing date: March 31, 1979. 9900(A)

Director

National Center For Toxicological Research Food and Drug Administration

The U.S. Food and Drug Administration is seeking candidates for the position of Director of the National Center for Toxicological Research (NCTR). The Center, an interagency facility, conducts research programs on the biological effects of potentially toxic chemical substances found in the human environment. The Director of NCTR provides leadership and direction to all Center operations and provides scientific liaison between NCTR and other Government agencies, private organisations and the worldwide academic, scientific and medical communities. The Center is located in Jefferson, Arkansas and has a staff of approximately 600 employees. The Center is well-equipped with sophisticated and modern laboratories to support advanced research in virtually all areas of toxicology.

FDA is looking for candidates of recognised scientific distinction and administrative ability. Candidates should possess an advanced degree in a relevant field of science (such as a Ph.D. in biochemistry, pharmacology, toxicology, or statistics, an M.D., or a D.V.M.). The position also requires appropriate research experience and an association with multi-disciplinary issues. This is a Civil Service position that pays up to \$47,500 per year in basic salary. The Director will also be eligible to receive performance bonuses and special stipends that may result in a higher total pay. This position has been designated for inclusion in the Federal Government's Senior Executive Service (SES), and the selectee will be required to enter the SES. Information on the SES will be available for interested applicants.

Send curriculum vitae and/or Standard Form 171 (Personal Qualifications Statement) and bibliography to:

HEW/Food and Drug Administration,
NCTR Search Committee,
c/o Ms. Patricia Sykes,
Rm. 9-89, 5600 Fishers Lane,
Rockville, Maryland 20857, U.S.A.
FDA/An Equal Opportunity Employer.

9784(A)

Department of Agriculture for Northern Ireland and The Queen's University of Belfast

Applications are invited in respect of the combined post of—

Head of Agricultural and Food Bacteriology Research Division of the Department of Agriculture; and

Professor of Agricultural and Food Bacteriology in the Faculty of Agriculture and Food Science, The Queen's University of Belfast.

which will become vacant in April 1979.

SALARY SCALE

The appointment will be made within the scale of £10,043-£11,300 which is subject to review with effect from 1 April, 1979.

There is a non-contributory pension scheme apart from 1½% deduction from salary to cover widows and dependants benefits in the case of male officers.

APPLICATIONS

Please write or telephone for an application and further details quoting job reference SB 129/79/NA to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585 ext 304).

Completed forms must be returned to arrive not later than 12 April, 1979.

9886(A)



**NORTHERN IRELAND
CIVIL SERVICE**

DALHOUSIE UNIVERSITY DEPARTMENT OF BIOLOGY

Faculty position: Forest Resource Ecologist, in Department of Biology with participation in the programme of the Institute for Resource and Environmental Studies.

Rank: Probably Assistant or Associate Professor. The individual appointed should be capable of teaching applied Plant Ecology and Forest and Land Resource Evaluation and Management to undergraduates and graduate students and of supervising graduate students in both the Master of Environmental Studies and the Master of Science programmes.

Research interests in Renewable Energy and Forest Management and Conservation, and in Forest Ecosystem Nutrient cycling. Ability to relate research to the needs of government, industry and the community.

Submit C. V. and 3 letters of reference by March 30, 1979 to Chairman, Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1. 9874(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL PEPTIDE SECTION (DEPARTMENT OF CHEMICAL PATHOLOGY) TECHNICIAN/SCIENTIFIC OFFICER

required in the Peptide Laboratory to take part in the synthesis of novel peptide hormone analogues. Applicants should possess H.N.C. or a degree in chemistry or biochemistry and have experience and/or interest in organic synthesis. The post is tenable for 18 months in the first instance, with a possibility of further extension.

Salary within the range £3,646 to £4,199 placing dependent on qualifications and experience.

Application forms may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS quoting reference number 4/203/N.

9810(A)

UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF BIOCHEMISTRY POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited from biochemists, molecular biologists or microbial geneticists to join a group investigating DNA repair and genetic recombination in *Escherichia coli*. The project is supported by the Medical Research Council until March 31, 1981. The work will involve a combination of biochemical and genetic techniques, and previous experience in nucleic acid biochemistry, molecular genetics, enzyme purification or DNA cloning would be advantageous.

Commencing salary will be within the scale £3,883 to £6,555 (under review) with full superannuation provision.

Applicants should send a curriculum vitae and the names of two referees to Dr P. T. Emmerson, Department of Biochemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU. 9848(A)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF VETERINARY PATHOLOGY TECHNICIAN

in the histopathology teaching and diagnosis laboratory. Candidates should possess HNC or equivalent and have wide experience in histopathological techniques. Salary in a range up to £4,365 p.a. (under review).

Application forms may be obtained from The Registrar, The University, Quote Ref: RV/510/N. 9881(A)

UNIVERSITY OF THE WEST INDIES JAMAICA

Applications are invited for the of LECTURER/ASSISTANT LECTURER IN THE DEPARTMENT OF BOTANY. Preference will be given applicants who possess a first degree with majors in Botany and hold graduate qualifications or have interest and teaching experience in Biology of the Lower Plant Group. The appointee will be required to take up duties as soon as possible. Scale: Lecturer—J\$8,913 to J\$13,136 p.a., Assistant Lecturer: J\$7,236 to J\$9,226 p.a. (£1 Sterling = J\$3. FSSU. Study and Travel Grant, furnished accommodation will be by the University at a rental of 20% of salary or a housing allowance of 20% of salary will be paid. For passages. Detailed applications (copies) with curriculum vitae naming three referees should be as soon as possible to the Registrar, University of the West Indies, M. Kingston 7, Jamaica. Applicants resident in the UK should also send copy to the Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 9883(A)

UNIVERSITY OF ADELAIDE

Applications invited from persons familiar with and experienced in NMR techniques and desiring to work on biologically relevant problems appointment for three years as RESEARCH FELLOW (X1905) (logical NMR Spectroscopy) to work on a project entitled "Aqu interactions between hormones phospholipid membrane constituent. The work is funded by an A.R.C. grant and will be supervised by Professor L. G. Paley, Department of Plant Physiology, Waite Agricultural Research Institute, to whom enquiries about the project may be addressed.

SALARY SCALE: \$A15,179 by (1) by 680 (2) to \$A17,220. economy class airfare and a contribution towards removal expenses will be provided. It is expected that there be provision for superannuation.

APPLICATIONS, in duplicate, including personal, education and professional details, and confidential letter of recommendation pertaining to position from three referees, should reach the Registrar of the University G.P.O. Box 498, Adelaide, S Australia, 5001 not later than March 31, 1979. 9869(A)

MONASH UNIVERSITY Melbourne, Australia DEPARTMENT OF PATHOLOGY AND IMMUNOLOGY JUNIOR RESEARCH FELLOW or RESEARCH FELLOW

Junior Research Fellow or Research Fellow with biophysics qualifications or relevant experience to help on a Becton-Dickinson FACS-11 fluorescence cell sorting in immunological and cancer research directed by Professor R. C. Nairn, Mc Medical School, Alfred Hospital. be interested in flow cytometry computer data analysis. Position available immediately. Salary: Junior Research Fellow \$A13,606 to \$A1 per annum; Research Fellow \$A1 to \$A19,940 per annum. Temporary housing. Application procedure available from the Academic Registrar, Monash University, Wellington 1 Clayton, Victoria, 3168, Australia. the Association of Commonwealth Universities (Apspts), 36 G Square, London WC1H 0PF. Enquiries to Professor R. C. Nairn, in University. 9870

**CITY OF LONDON
POLYTECHNIC
DEPARTMENT OF
BIOLOGICAL SCIENCES
Laboratory Superintendent—
Technician Grade 8(c)**

Applications are invited for the above post. The department runs both full-time and part-time courses at undergraduate and undergraduate level as well as having an active research programme.

Minimum qualifications required—N.C./D. or equivalent and at least 3 years' relevant experience.

Salary: £5,214 to £5,625 inclusive of London Weighting.

Application forms, which should be returned within two weeks of the appearance of this advertisement, together with the job description, can be obtained from the Secretary, Department of Biological Sciences, City of London Polytechnic, Old Castle Street, London E1 7NT. 9809(A)

**UNIVERSITY OF ABERDEEN
LECTURESHIP
IN DEPARTMENT
OF ANATOMY**

Applications are invited for the above post which will become vacant March 31, 1979. Candidates may have either medical or scientific qualifications and will be expected to, or to become, competent in teaching gross and microscopic anatomy to medical and science students. While area of research is excluded, candidates with interests in growth studies (human and/or experimental), endocrinology, neurobiology or cell biology will be especially welcome.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by March 23, 1979. 9842(A)

**UNIVERSITY OF
MANCHESTER
DEPARTMENTS OF
SURGERY AND PATHOLOGY
POSTDOCTORAL
IMMUNOLOGIST**

Required with experience in the techniques of Cellular Immunology as applied to patients. The post is for one year initially, but renewable. The objective is to study the nature of the immune deficit in injured and malnourished patients. A full-time technician in Immunology and adequate laboratory facilities are available for the project. Salary scale for Research Officer with starting point up to £5,604 per annum. Enquiries to Professor L. Ford, Department of Pathology, Oxford Building, Oxford Road, or Professor M. Irving, Department of Surgery, Hope Hospital, Eccles Old Road, Salford M5 8HD. Applications to Professor Ford by March 15. 9814(A)

**UNIVERSITY OF
NOTTINGHAM
DEPARTMENT OF
PHARMACY
LECTURERS IN
PHARMACEUTICS**

Applications are invited from Pharmacy Graduates for a Lectureship in Pharmaceutics. Experience in Physical Pharmaceutics or Pharmaceutical Technology would be considered an advantage.

The salary will be within the range £883 to £7,754 per annum. Further particulars and forms of application, returnable not later than March 19, 1979, from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Ref. 660. 9815(A)

The Daresbury Laboratory has a vacancy for a

COMPUTATIONAL SCIENTIST

to develop the theory of the electronic structure of surfaces and their scattering of electrons and photons, in collaboration with a group of scientists from various institutions: the universities of Coleraine, Leicester, Liverpool, Warwick and York, and the Daresbury Laboratory, all being involved in the collaboration.

The post will be based in the computational science group at Daresbury but will involve occasional visits to the other centres. A strong interest in the interaction between computational theory and the experimental results being obtained in LEED, photoemission from solids and EXAFS will be expected. Applicants should possess a Ph.D degree, preferably in the theory of the solid state, or expect to obtain one during 1979.

An IBM 370/165 computer is installed at the Laboratory providing comprehensive facilities. The laboratory also provides two central facilities for the universities: a purpose-built X-ray and ultra-violet source and a 30MV tandem Van de Graaff to be used for nuclear structure experiments.

Appointments will be made at salaries between £3,695 and £6,216 depending on age, ability and experience. Posts are normally available for a fixed term of 3 years and are superannuable.

Closing date: 1st April 1979.

For further information please write to or telephone the head of the theory group, Dr. J. B. Pendry (Tel. No. (0925) 65000, Extension 223). Applications, from either sex, should be sent together with curriculum vitae and the addresses of two referees, quoting reference number DL/654 to

The Personnel Officer

DARESBUURY LABORATORY Science Research Council Daresbury, Warrington WA4 4AD

9823(A)

**UNIVERSITÄT
KAISERSLAUTERN**

Im Fachbereich Biologie ist folgende Stelle zu besetzen:

**PROFESSOR (C 4) FÜR
BIOTECHNOLOGIE**

Bewerber sollen in der Lage sein, biologische und biochemische Erkenntnisse in praktisch verwertbare Techniken umzusetzen; daher kommen hauptsächlich Arbeitsgebiete aus der angewandten Mikrobiologie in Frage. Habilitation oder entsprechende fachliche Qualifikation ist Voraussetzung. Die Bereitschaft zur Zusammenarbeit mit den vorhandenen biologischen Arbeitsgruppen und den Fachbereichen Chemie, Maschinenwesen und Elektrotechnik wird vorausgesetzt. Neben einer Beteiligung an der Grundausbildung der Diplombiologen und Lebensmittelchemiker soll der Bewerber bei der Ausarbeitung einer Studienrichtung für Biotechnologie massgeblich mitwirken.

Bewerbungen sind mit den üblichen Unterlagen (Lebenslauf, Wissenschaftlicher Werdegang, Forschungsziele, Schriftenverzeichnis, bisher durchgeführte Lehrveranstaltungen) bis zum 15. Juni 1979 zu richten an das Dekanat des Fachbereichs Biologie, Universität Kaiserslautern, Postfach 3049, 6750 Kaiserslautern.

9908(A)

**SHEFFIELD AREA HEALTH AUTHORITY (TEACHING)
SOUTHERN DISTRICT (TEACHING)**

**GROUP DEPARTMENT OF HAEMATOLOGY
NON-MEDICAL SCIENTIFIC OFFICER
(Basic Grade)**

Applications are invited for the above post from candidates possessing an appropriate 1st or 2nd class honours degree in a biological science. The successful applicant will be required to work in the hospitals' Blood Transfusion Laboratories, the main one of which is in the new Hallamshire Hospital. Previous transfusion experience is not required but an interest in hospital work is necessary.

Whitley Council Terms and Conditions of Service apply. Salary Scale (Probationer—new entrants to the National Health Service) £3,486 to £4,275 per annum.

(Post-Probationer—applicants with at least 2 years National Health Service experience) £3,793 to £4,899 per annum.

Candidates wishing to view the Laboratories should telephone Mrs M. S. Lepp, Principal Non-Medical Scientific Officer, Royal Infirmary, Sheffield S6 3DA (Tel: 0742 20977, ext. 400).

Applications stating age, qualifications, experience and the names and addresses of two referees to:

District Personnel Officer,
B Floor, Hallamshire Hospital,
Glossop Road,
Sheffield S10 2JF

by March 12, 1979.

9877(A)

BIOZENTRUM

University of Basle

Department of Cell Biology

Applications are invited for a

**Professorship in Cell Biology
from fall 1979**

Duties include teaching in general Cell Biology (eight hours per week) and research in Cell Biology with emphasis on membrane phenomena.

Applications stating publications, teaching experience, curriculum vitae etc. should be submitted before May 31, 1979, to the Dean of the Faculty of Science.

Address:

Dekanat der
Philosophisch-Naturwissenschaftlichen
Fakultät der Universität Basel
Klingelbergstrasse 70
CH-4056 Basel (Switzerland)

9832(A)

**The International
Atomic Energy Agency**

seeks

**HEAD FOR THE INTERNATIONAL LABORATORY
OF MARINE RADIOACTIVITY IN MONACO**

He should be a Senior Scientist (10-15 years' experience) with knowledge of the oceans, particularly radioactivity. Duties include administration of the Laboratory and responsibility for scientific programme.

Emoluments US\$ 47,000 per annum, tax-free. Additional allowances if married. Travel and removal expenses paid. Six weeks' annual leave. Initial contract two years. Send curriculum vitae under VN 16/79 to IAEA, Division of Personnel, PO Box 590, Vienna, Austria. 9862(A)

DUNDEE COLLEGE OF TECHNOLOGY

Department of Molecular & Life Sciences

**SENIOR LECTURESHIP IN MAMMALIAN
PHYSIOLOGY**

Applicants should have an honours degree and a higher degree in mammalian physiology, together with suitable research or industrial experience. The person appointed will be required to make a major contribution to the teaching of animal physiology in degree, HND and HNC courses, he/she will also be expected to make a substantial contribution to course development, research, staff development and departmental administration.

Salary on the scale £7,155 to £7,962 (bar) to £9,042 with initial placing dependent upon approved prior experience. Financial assistance towards the cost of removal expenses may be payable.

Further particulars and application form obtainable from the Administrative Assistant (Establishment), Dundee College of Technology, Bell Street, Dundee DD1 1HG, to whom completed application forms should be returned by March 16, 1979. 9827(A)

**THE CITY UNIVERSITY
DEPARTMENT OF
CHEMISTRY
LECTURER**

Applications are invited for a Lectureship tenable in the above Department from April 1, 1979 or as soon as possible thereafter. Candidates should be INORGANIC CHEMISTS with an enthusiasm for teaching and with active research interests.

Salary will be on the scale £3,883 to £7,754 per annum (under review) plus £450 per annum London Allowance.

Application forms and further details are available from the Deputy Academic Registrar, The City University, Northampton Square, London EC1V 0HB, telephone 01-253 4399, ext. 338. Please quote reference C/232/N. Closing date March 31, 1979.

9824(A)

**TECHNICIAN/RESEARCH
OFFICER**

Required to assist in research into the control of animal cell division. Main duties include cell culture work and performance of experiments concerned with elucidating the mechanisms by which cell division is controlled. Techniques used include ³H-thymidine autoradiography, time-lapse microcinematography, cell-cell fusion.

HNC/Degree required. Salary range £3,261 to £4,902 plus L.W. For application form telephone Mrs Nelson, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2 on 01-242 0200, ext. 305. 9840(A)

**UNIVERSITY OF
CALIFORNIA AT DAVIS
ZOOLOGY DEPARTMENT
POSTDOCTORAL
RESEARCH ASSOCIATE**

A postdoctoral research position is available for a cellular physiologist to investigate both transcellular ion currents and intracellular ion concentration changes during the activation and early development of vertebrate eggs. Both the extracellular vibrating probe (J. Cell Biol. 63: 614) and recessed-tip intracellular ion-specific microelectrode techniques are being used in this research. Some experience with electrophysiology is desired. This appointment will be for two to three years. Salary: \$11,000/yr. Closing Date: April 1, 1979.

Send résumé and names of three references to: Dr. R. Nuccitelli, Zoology Department, University of California, Davis, CA, 95616, U.S.A. Equal Opportunity/Affirmative Action Employer. 9875(A)

BIRZEIT UNIVERSITY

An independent Arab University in the Israeli-occupied West Bank of Jordan has the following faculty openings for the academic year 1979/1980: BIOLOGY—BIOCHEMISTRY, CHEMISTRY (PHYSICAL, INORGANIC, ORGANIC), MATHEMATICS (ALGEBRA, ANALYSIS, MATHEMATICAL STATISTICS), PHYSICS (Experimental and Theoretical). English is the basic language of instruction and candidates should have a Ph.D. preferably or a master's degree with experience. Starting annual salary for Ph.D. is 2,700 Jordanian Dinars (current exchange rate 1 JD equals about £17). Optional summer teaching extra fringe benefits include Relocation allowance (15 per cent of salary). Transportation also provided. All applications should be addressed to Dean of the Faculty of Science, Birzeit University, Birzeit, West Bank Via-Israel. 9909(A)

**THE QUEEN'S
UNIVERSITY OF BELFAST
LECTURESHIP
IN BIOCHEMISTRY**

A lectureship in the Department of Biochemistry is available from October 1, 1979, or such other date as may be arranged. The lecturer will be expected to teach at various levels and to engage in research, preferably collaborative, in fields already active in the Department, details of which are set out in a departmental prospectus which is available. Initial placing, which will depend on age, experience and qualifications, will be made at one of the first three points on the scale for lecturers (effective October 1, 1978 but subject to further review). £3,385, £4,134, £4,383 rising to £7,755 with contributory pension rights under the FSSU/USS. The appointment will be subject to a period of probation of up to three years. Further particulars are available from the Personnel Officer, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland. Closing date: April 16, 1979. (Please quote Ref. 79/N.) 9887(A)

**NATO
Advanced Study
Institute**

Metal Ions in Biological Systems
Portugal 17-30th September 1979

Information and application forms may be obtained from

Prof A. V. Xavier

Centro de Química Estrutural

Universidade de Lisboa

Complexo Interdisciplinar

Instituto Superior Técnico

Lisboa 1

Portugal

9883(A)

The Research Organisation of Internationally recognised Europe Pharmaceutical Group located in Pa requires a

**TECHNICAL
RESEARCH OFFICER**

The high success rate and rapid growth of this pharmaceutical group is related to the quality of people they employ and their continued commitment to research and development.

To strengthen the research effort the research organisation requires professional and highly motivated scientists who will be responsible for:

- Advancing on the implementation of the GLP standards within research and within a proposed new research centre.
- Assisting with the planning and organisation of research projects.
- Organising the collation and storage of research data.

Applicants can be either male or female and should be a graduate in biological sciences. It is essential that the candidate should have experience of the pharmaceutical industry.

A realistic salary can be expected and relocation assistance will be available together with other financial benefits.

All applications will be treated with the strictest confidence and should be sent to 'Nature' who will forward. 9895(A)

MEDICAL RESEARCH COUNCIL

MRC PNEUMOCONIOSIS UNIT
Llandough Hospital, Penarth,
South Glamorgan, CF6 1XW

Applications are invited for post in statistical section of this Unit. The work involves statistical counselling and the design and analysis of experiments in the different fields of activity of the Unit—clinical medicine, physiology, radiology, pathology, immunology, physics, biochemistry, demography, and the extraction of information from data, already existing, in industry and elsewhere, bearing on occupational lung diseases.

Applicants could be recent graduates in mathematical statistics, graduates with some years experience in medical statistics, or graduates wishing to engage to medical statistics from some other field of biometry.

Further details may be had on application to the Director, at above from. 9838(A)

COMMONWEALTH AGRICULTURAL BUREAUX Vacancy for SCIENTIFIC INFORMATION OFFICER

at the
Commonwealth Bureau of Plant
Breeding and Genetics
Department of Applied Biology
Cambridge CB2 3DX

ties include scanning the world literature dealing with the breeding, genetics and cytology of economic plants, preparing abstracts for publication in *Plant Breeding Abstracts*, and answering enquiries within this field. A degree either in biology, agriculture or genetics or in human is required. Applicants coming with scientific and linguistic aptitudes will receive prior consideration. Capacity to read foreign languages other than German will count as additional qualification. Ability to write good English is essential.

Salary. The salary is in the scale £39 to £5,448 p.a. plus a compulsory allowance (taxable but not pensionable) of 3.3% of basic salary to offset personal contribution to pension scheme. Starting salary according to qualifications and experience. Promotion to higher scales on merit.

Application forms and full particulars from: Executive Director, Commonwealth Agricultural Bureaux, Rothamsted House, Farnham Royal, Bucks SL2 3BN.

Closing date for applications: March 1979 9839(A)

NEUROPATHOLOGIST/ PHYSIOLOGIST/BIOLOGIST

required by the Biology section of the Medical Research Council Cyclotron Unit. The successful applicant will be a group investigating responses of small tissues in animals which are relevant in the treatment of cancer. Studies will include effects of both ultraviolet and X-rays and certain adjunct treatments used in radiotherapy. The post will be concerned primarily with damage to nervous tissue but may extend to other tissues. The applicant must have a First or Upper Second Class degree in an appropriate subject and at least 3 years' postgraduate research experience or a degree in medical or veterinary sciences relevant experience is desirable but not essential. The post is a three year term appointment. Salary, according to age and experience, in the range £6,081 to £6,530 (inclusive of pay supplements and London Weighting), plus 5 weeks annual leave. Please write, giving qualifications and curriculum vitae, to:

Miss A. Pires, MRC Cyclotron Unit, Hammersmith Hospital, Duane Road, London W12 0HS Tel: 01-743 4394, ext. 103. 9892(A)

PRECEPTOR Harvard University

Applications are invited for the position of preceptor to organise and supervise laboratories for two introductory courses (introductory organismic and evolutionary biology, comparative animal physiology and anatomy). The Ph.D. or equivalent research/teaching experience is preferred. This position is renewable on an annual basis for 5-8 years. Starting date: July 1, 1979. Applications should be addressed to the Chairman's Office, Department of Biology, Harvard University, Cambridge, Mass. 02138. Harvard is an Equal Opportunity/Affirmative Action Employer. 9846(A)

THE POLYTECHNIC OF CENTRAL LONDON DIVISION OF SCIENCE LECTURER II IN PLANT ECOLOGY

Salary: £4,575 to £7,032 inclusive of London Allowance. Applicants should be Honours graduates in Botany, Biology or Environmental Science with at least a Ph.D. or equivalent research experience in Quantitative Plant Ecology. The successful candidate will be expected to teach aspects of Ecology and Plant Diversity at Honours Degree and postgraduate level and to participate in research.

Application form and further details from the Establishment Officer, PCL, 309 Regent Street, London W1R 8AL (01-580 2020 ext 212).

Closing date: Three weeks after the appearance of the advertisement 9850(A)

UNIVERSITY OF CALIFORNIA DEPARTMENT OF VITICULTURE AND ENOLOGY

The Maynard A. Amerine Professorship

An Endowed Chair From
The Ernest Gallo Foundation
Applications are now invited for the Maynard A. Amerine Professorship. Applicants should have made research contributions of significance at the professional level in fields that include or are pertinent to enology, viticulture, or the fermentation sciences. The appointee will be expected to contribute to both undergraduate and graduate instruction and to provide intellectual leadership in the Department, the College, and the Davis Campus.

The formal appointment will be a permanent position as a tenured member of the Department of Viticulture and Enology at one of the higher steps of the rank of full professor.

Applications, in the form of a letter describing research and teaching interests and background, together with a curriculum vitae and three letters of recommendation, should be submitted by May 15, 1979, to:

The Chair
Search Committee for the Amerine
Professorship c/o College of Agricultural and Environmental
Sciences
228 Mink Hall
University of California
Davis, CA 95616

The University of California is an affirmative-action/equal-opportunity employer. 9853(A)

POSTDOCTORAL POSITION

for 2 years available immediately. Studies on gene expression of enveloped animal viruses, vesicular stomatitis and Rous sarcoma, viral membrane biogenesis and morphogenesis (P.N.A.S. 74, 1516, 75, 715, 76, 570). Starting salary \$11,600. Ph.D. in biochemistry, molecular biology or microbiology preferred. Send résumé and three references to Dr H.P. Ghosh, Professor, Department of Biochemistry, McMaster University, Hamilton, Ontario L8S 4J9 Canada. 9854(A)



The ICI Corporate Laboratory, situated at Runcorn, Cheshire, has the following vacancies for research scientists:—

BIOCHEMIST/ PLANT PHYSIOLOGIST

Two scientists are required to join a small team within the Bioscience Group, investigating aspects of the regulation of cereal development, with a view to the development of chemicals with novel plant growth regulatory activity. Applicants should possess a degree or equivalent qualification in a subject which includes knowledge of biochemistry or plant physiology. Practical laboratory experience will be an advantage. (Ref. BS1).

THEORETICAL CHEMIST/ PHYSICIST

A research scientist is required to join a small theoretical group within the Corporate Laboratory, to investigate aspects of solid state physics. A PhD in some branch of theoretical physics or chemistry, together with computational experience is essential. (Ref. PT1).

If you are interested in either of these vacancies, please write, not later than March 9th, quoting reference number, and including a full curriculum vitae, to:

Miss S E Bird
Personnel Officer
ICI Corporate Laboratory
PO Box 11
The Heath
RUNCORN Cheshire

9830(A)

WELLCOME MUSEUM HISTORY OF MEDICINE

Assistant Keeper

to take part in the transfer of objects (illustrating the history of medicine) from the Wellcome Trustees to the Science Museum and to be responsible for maintaining and developing a number of collections. This involves seeking new material, historical research, answering enquiries, and general administration. An important part of the duties includes working on particular aspects of setting up new permanent galleries and documentation of the objects.

Candidates must have a degree with first or second class honours, or an equivalent qualification, in a relevant subject and the ability to explain medical and scientific matters clearly to the lay public. Experience in museum work at a high level, medical or related qualifications/experience, and/or knowledge of the history of medicine, advantageous.

SALARY: as AK I £6855—£8820 or AK II £3575—£5305. Level of appointment and starting salary according to age, qualifications and experience. Non-contributory pension scheme.

For full details and an application form (to be returned by 30 March 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants., RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote G(12)382. 9833(A)

SCIENCE MUSEUM



LEICESTER UNIVERSITY

FACULTY OF SCIENCE

Chair of Physics

Applications are invited for an additional Chair of Physics tenable from October 1, 1979 or an agreed later date. Candidates should be experienced in some branch of experimental physics.

Further particulars from the Registrar to whom applications should be sent by April 21, 1979. Candidates in the U.K. should submit 15 copies of their application (overseas candidates may submit one copy). 9867(A)

RESEARCH OFFICER/ TECHNICIAN

required by the Medical Research Council Cyclotron Unit at Hammer-smith Hospital. The work will consist mainly of the study of subcellular changes in cell and tissue systems after exposure to radiation and other toxic hazards. The aim is to gain a fuller understanding of post-treatment changes in normal and malignant tissues. Previous experience of biochemical and/or cell culture techniques would be advantageous. Candidates should possess a degree or equivalent qualification in a relevant discipline. Salary, according to age and experience, in the range £3,615 to £5,256 (inclusive of London Weighting).

Please write, giving qualifications and curriculum vitae, to: Miss A. Pires, MRC Cyclotron Unit, Hammer-smith Hospital, Duane Road, London W12 0HS. 9893(A)

THE INSTITUTE OF CHEMISTRY

invites applications for TWO FACULTY POSITIONS

in organic geochemistry. Applicants should have the Ph.D., and research interest in petroleum geochemistry. The salary will be commensurate with experience and qualifications (minimum: \$22,000).

Applications including a curriculum vitae and the names of three references should be addressed to:

Dr Carlos López Eyzaguirre
Instituto de Química
Facultad de Ciencias
Universidad Central de Venezuela
Apartado 10098
Caracas-Venezuela

9905(A)

WELSH NATIONAL SCHOOL OF MEDICINE (University of Wales) DEPARTMENT OF MEDICAL BIOCHEMISTRY

RESEARCH OFFICER

required for a DHSS-funded appointment (renewable annually for up to 3 years) in the above Department at Health Park, Cardiff.

The project involves the development of chemiluminescent compounds as alternative labels to radioactive iodine in immunoassay. The aim is to establish clinically useful methods for measuring a variety of peptides and small molecules using luminescence. Candidates should have a degree in Biochemistry or Chemistry and the position would be suitable for someone wishing to study for a higher degree. Starting salary up to £3,384 per annum.

Further particulars (quoting ref. No. C38/4/26) from the Registrar and Secretary, Welsh National School of Medicine, Health Park, Cardiff, CF4 4XN to whom applications should be submitted by March 31, 1979. 9859(A)

QUEEN MARY COLLEGE University of London PHYSICS DEPARTMENT

Applications are invited for appointment as

LECTURER

from October 1. Preference may be given to candidates with research interests in Experimental High Energy Physics or the application of physics to the Design and Development of Techniques of measurement or detection. Salary scale £3,975 to £8,250 per annum (under review) plus £450 London Allowance. Further details and application forms available from The Registrar (N), Queen Mary College, Mile End Road, London E1 4NS, to be returned by April 11. 9808(A)

CHAIRPERSON, PROFESSOR OF CHEMIS DEPARTMENT OF CHEMIS

State University of New York Albany—Applications are invited for this position to start on September 1979 or as soon thereafter as possible. The Department of Chemistry commitment to continuing development of outstanding programs search and teaching and seeks a person to provide leadership in areas and to guide the future of the department. The applicant should be a chemist of senior with an established reputation search and interest in teaching administration. Each applicant send a resume and the names of three references to: J. A. Z. Chairman, Search Committee, Department of Chemistry, State University of New York at Albany, Albany, New York 12222. Applications should be completed before March 30, 1979. EO/AA employer. 974

THE UNIVERSITY OF MANCHESTER EXPERIMENTAL OFFICE DEPARTMENT OF PHARMACY

The person appointed will be responsible for the development co-ordination of analytical techniques in research dealing with pharmacokinetics, drug metabolism and pharmacology. The techniques primarily involve the use of chromatography, spectroscopy and radioactivity. Successful candidate will also help and run kinetic experiments on animals and man, and help in assessing the data using computer techniques. Applicants must be able in either chemistry, biochemistry or a related science, with a minimum of two years' relevant experience. Initial salary in the range £3,384 to £5,604, depending on U.S.S. Particular application forms (returnable by 20) from the Registrar, The University, Manchester M13 9PL. Quote 38/79/N. 981

UNIVERSITY OF NEWCASTLE UPON TYNE THE MEDICAL SCHOOL APPOINTMENT OF LECTURER IN PHYSIOLOGY

Applications are invited for a Lectureship in Physiology (Medical Non-Medical) in the Department of Physiology. The Department responsible for the teaching of PHYSIOLOGY & PHYSIOLOGICAL BIOCHEMISTRY to Medical, and Pure Science students. Appointment will be from the date on or after October 1, which may be agreed. Ample opportunity exists for a medically qualified person to engage in interdisciplinary clinical research and teaching.

The appointment will be made on the Lecturers' Pre-Clinical Grade (£5,129 to £7,754) or Lecturers' Pre-Clinical Higher (£7,268 to £8,257) in the case of a medically qualified person. In the case of a person not medically qualified, appointment will be to the Lecturers' Non-Medical Grade (£3,883 to £5,129). The appointment will be made on a suitable point on these scales according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

Further particulars may be obtained from the Senior Assistant Registrar, The University, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU to whom applications (three copies) together with the names and addresses of three referees, should be sent not later than April 2, 1979. quote reference N. 981

LIVERPOOL UNIVERSITY MEDICAL SCHOOL SENIOR RESEARCH ASSISTANT

join a group working on the development of new probes for identifying illular antigens in the electronmicroscope. The post is funded by a 3-year R.C. grant and is suitable for someone with a chemical/biochemical background wishing to gain experience in a cell biological area. Applicants could have a Ph.D. in biochemistry or a related subject.

The initial salary will be in a range of £5,129 per annum. Applications, together with the names of two referees should be received not later than March 23, 1979, by The Registrar, The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Note Ref: RV/505/N. 9866(A)

POSTDOCTORAL RESEARCH ASSOCIATE POSITION

To study role of poly(adenosine phosphoribose) synthesis in eukaryotic DNA repair processes. Experience with DNA repair processes. Experience with DNA repair, nucleic acid and rromatin chemistry desirable. Send curriculum vitae and references to Dr A. Berger, Hematology-Oncology Division, Jewish Hospital, Washington University School of Medicine, 216 S. ngshighway, St. Louis, Missouri, 110.

Equal Opportunity Employer. 9860(A)

CANCER CHEMOTHERAPY
Postdoctoral Research Training offered jointly by the Departments of Medicine, Mendelsohn, M.D. and S. B. Holl, M.D.) and Chemistry (The Athletic Mouse Research Center, N. O. aplan, Ph.D.), University of California, San Diego. The successful candidate (M.D. or Ph.D.) will be able to study various chemotherapies on human tumor lines in athymic mice and correlate them with clinical cases at the University Hospital. For the next year the annual stipend ranging from \$10,000 to \$13,200 commensurate with experience is available from training grant. The Fellow will be expected to apply for own support for subsequent two years. Closing date for application is April 30, 1979; appointment date is August 1, 1979. Apply to: Dr. N. O. Kaplan, Q-058, University of California, San Diego, La Jolla, CA 92093. The University of California is an Affirmative Action employer. 9861(A)

UNIVERSITY OF ABERDEEN TECHNICIAN (Grade 5)

required to look after the Electron microscope Unit in the Department of Botany to maintain, operate and repair specimens for a Philips EM301 transmission electron microscope and Cambridge Instruments S600 scanning electron microscope. The unit, which is used for teaching and research, is well equipped with ancillary apparatus including a freeze-drying machine, a sputter-coater and critical point drier and has its own photographic darkroom. Applicants should hold an HNC (or equivalent qualification) and should have had considerable experience in electron microscopy or in photography and electronics.

For suitably-qualified candidate, salary on scale £3,186 to £3,720 (under review).

Applications from recent graduates interested in training in electron microscopy will be considered for appointment against this post; salary for a candidate during initial training period would be on scale £2,346 to £499 (under review).

Applications giving date of birth, educational qualifications and previous experience should reach the Secretary, University Office, Regent Walk, Aberdeen, AB9 1FX, by March 22, 1979. Please quote Ref. No. 50/79. 9873(A)

UNIVERSITY OF ABERDEEN DEPARTMENT OF MEDICINE POSTDOCTORAL RESEARCH FELLOW

Applications for the above post are invited from biological scientists with a knowledge of transplantation immunology. The successful applicant will join a team investigating the role of gene products of the major histocompatibility complex, especially HLA-D and HLA-DR matching, in renal allograft survival.

Commencing salary will be £4,382 per annum on the Range 1A Scale for Research and Analogous Staff, plus U.S.S. The post is grant supported and is available for three years from September 1, 1979, or from a suitable date to be negotiated.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by March 23, 1979. 9841(A)

UNIVERSITY OF LONDON CHAIR OF BOTANY tenable at Imperial College of Science and Technology

The Senate invites applications for the above Chair, tenable in the Department of Botany and Plant Technology.

The present strengths of the Department lie mainly in plant physiology, plant pathology and timber technology. Fields that could now with advantage be strengthened include ecology or physiological ecology, all areas of genetics, and microbiology. Candidates with interests in other fields may also be considered.

The Professor of Botany will be eligible for appointment as Head of the Department of Botany and Plant Technology when Professor A. J. Rutter retires on August 31, 1979.

Applications (10 copies) must be received not later than March 19, 1979, by the Academic Registrar (N), University of London, Malet Street, London WC1E 7HU, from whom further particulars should first be obtained. 9899(A)

MEDICAL LABORATORY SCIENTIFIC OFFICER (OR JUNIOR)

required for varied endocrine analytical work in Department of Gynaecology (Biochemistry Laboratory). HNC standard needed or "A" level chemistry for junior post. Apply giving age and details of qualifications and experience to Dr. M. G. Brush, St. Thomas's Hospital Medical School, London, SE1 7EH. 9843(A)

AGRICULTURAL RESEARCH COUNCIL INSTITUTE OF ANIMAL PHYSIOLOGY

Babraham, Cambridge, CB2 4AT

Applications are invited for a post, financed by the M.R.C. for 3 years commencing October 1, 1979. This project involves studies of the transport, metabolism and secretion of vasoactive agents using endothelial and smooth muscle cells in culture as well as isolated vascular preparations. It forms part of an integrated programme of vascular physiology, under the supervision of Dr J. L. Gordon, in the Department of Cell Biology. Candidates must have a good honours degree in physiology, biochemistry or a related subject, and experience with any of the above techniques would be advantageous. Preference will be given to candidates possessing a Ph.D. or equivalent. Salary in scale £4,101 to £5,448 or £5,154 to £6,898, depending on experience. Applications, including C. V. and names of two referees, should be sent as soon as possible to the Secretary of the Institute, quoting Ref. J. L. G. Closing date April 5, 1979. 9844(A)

Royal Botanic Gardens, Kew

Botanists/ Biologists

The Gardens, while primarily a scientific institution, also provide an important public amenity. The scientific work is concerned with the classification, growth, structure and uses of plants, utilising the rich collections of preserved material in the Herbarium and Museums and the unique collection of living plants in the Gardens.

Anatomical Investigations

This post is in the Jodrell Laboratory which provides facilities for anatomical, cytological and physiological research. The botanist/biologist appointed will join a team working on systematic and experimental plant anatomy. This will involve identifying plant fragments and organs, developing new techniques and providing a technical advice service to visiting research workers. The work will involve the use of optical and scanning electron microscopes.

Candidates, normally aged under 27, should have an Honours Degree or equivalent in a discipline which has included a specialist study of botany. An interest in natural history and geography would be an advantage.

Reference: SB/18/AH.

International Conservation Work

The Conservation Unit works in close co-operation with the Threatened Plants Committee of the International Union for Conservation of Nature and Natural Resources. The botanist/biologist appointed will collate national and international information on threatened plants and floras with a view to formulating conservation schemes. In addition, the successful candidate will assist with Kew's work of advising the public on plant conservation.

Candidates, normally aged under 27, should have a good Honours Degree or equivalent in an appropriate discipline which has included a specialised study of both botany and geography. An interest in nature conservation and the diversity of the plant kingdom is highly desirable. Reference: SB/19/AH.

Appointment as Scientific Officer (£3110-£4690) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 30 March 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours).

Please quote appropriate reference.

**Science
Group**
CIVIL SERVICE

9871(A)

UNIVERSITY OF CRETE

The Departments of Mathematics and Physics are having openings for visiting appointments at all ranks. Greek speaking mathematicians and physicists are invited to apply. Applications for regular appointments will be formally solicited soon.

All correspondence including letters of recommendation should be sent by the end of April 1979: to Ms Sophia Fotiti, University of Crete, Solonos 71, Athens 143, Greece. 9851(A)

Research Microbiologist

Guinness is seeking a graduate microbiologist to join the present research team in its Dublin Brewery.

The successful candidate will have proven ability in either academic or industrial research. Detailed knowledge of fermentation technology is highly desirable. He or she will be expected to carry out independent work in problem solving and in the application of

innovative approaches to fermentation microbiology.

An attractive starting salary will be negotiable, based on experience and qualifications. Conditions of service are excellent and include four-and-a-half weeks' annual vacation, non-contributory pension, profit-sharing scheme and assistance with relocation expenses.

Please write giving full details of age, qualifications and experience to:

Dr. R.B. Gilliland,
(Ref. MI 79)
Manager, Research & Development,
Arthur Guinness Son & Co. (Dublin) Ltd.
St. James's Gate, Dublin 8.

GUINNESS



9825(A)

UNIVERSITY OF DAR ES SALAAM—TANZANIA

Applications are invited for the following posts in the
DEPARTMENT OF ZOOLOGY

LECTURER/SENIOR LECTURER IN ZOOLOGY
(ENTOMOLOGY/PARASITOLOGY)

Applicants should hold a Ph.D. with research and several years of teaching experience in Entomology and Parasitology. Appointee will be required to organise undergraduate course in applied entomology and animal parasitology and to teach and supervise research in his/her subject area at postgraduate level; to organise and carry out research in Entomology and Parasitology. Further, he/she will be expected to work in collaboration with other members of the Department in an integrated research programme.

LECTURER/SENIOR LECTURER IN WILDLIFE
ECOLOGY AND MANAGEMENT
(POPULATION ECOLOGY)

Applicants should hold a Ph.D. with research and teaching experience in Population Ecology. Appointee will be required to organise undergraduate courses in Animal population ecology and to teach and to supervise research in his/her subject area at postgraduate level; to plan and carry out research in Animal ecology relevant to Tanzania. Further, he/she will be expected to work in collaboration with other members of the Department in an integrated research programme.

SALARY SCALES (salaries exclusive of 50 per cent expatriation allowance). Senior Lecturer ET1,980 to ET2,480 per annum. Lecturer ET1,620 to ET1,980 per annum (ET1 equals £1 22 sterling). The British Government may supplement salaries in range £4,356 to £4,782 per annum (sterling) for married appointees and £2,754 to £3,018 (sterling) for single appointees (reviewed annually and normally free of tax) and provide children's education allowances and holiday visit passages. Family passages; F.S.S.U. biennial overseas leave. Detailed applications (two copies) with curriculum vitae and naming three referees to be sent direct to Chief Academic Officer, University of Dar es Salaam, P.O. Box 35091, Dar es Salaam, Tanzania, by April 8, 1979. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 9857(A)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF HISTOLOGY AND CELL BIOLOGY (MEDICAL) TECHNICIAN

To join group using immunological methods to study a variety of cellular activities. Experience in methods for culturing lymphocytes or radioimmunoassay an advantage but not essential.

Salary in a range up to £2,880 p.a. (under review). Application forms may be obtained from The Registrar, The University, P.O. Box 147, Liverpool, L69 3BX. Quote Ref: RV/509/N. 9880(A)

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF BIOCHEMISTRY Applications are invited for a post of LECTURER IN BIOCHEMISTRY

tenable from October 1979

The successful candidate will be expected to be interested in mammalian and medical biochemistry and to participate in teaching and research in these areas. A medical degree would be an advantage although is not essential. The Department provides courses for science, medical and dental undergraduates. Excellent facilities are available for most types of biochemical research.

Salary within the range £3,883 to £7,754 p.a. (plus USS), depending on age, qualifications and experience.

Applications (2 copies) naming 3 referees should be sent by March 30, 1979 to Miss L. R. Heyes, Senate Registry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, from whom further particulars may be obtained. 9779(A)

THE ANIMAL VIRUS RESEARCH INSTITUTE PIRBRIGHT

Applications are invited for a person to work with a group engaged on the structure and replication of animal viruses. The successful candidate will be expected to work on the proteins of the viruses, with the aim of relating structure to antigenic activity. Applicants should have a first/second class honours degree in Biochemistry or related subject, with at least 2 years post graduate research experience. The post is in the HSO grade (£4,101 to £5,448). House available for suitable married applicant. Non-contributor superannuation scheme.

Application forms and further particulars from the Secretary, The Animal Virus Research Institute, Pirbright Woking, Surrey, GU24 0NF. 9890(A)

UNIVERSITY OF NEWCASTLE UPON TYNE BIOCHEMISTRY RESEARCH DEMONSTRATORSHIP

Applications are invited for a post of DEMONSTRATOR in the Department of BIOCHEMISTRY for candidates who possess, or have submitted for, a Ph.D. in Biochemistry for a period of up to three years tenable as soon as possible. Research projects currently in progress include membrane transport of nucleic acid precursors, acquisition and modification of enzyme conformation, DNA repair and genetic recombination, triglyceride and lipoprotein metabolism, enzymology of resistance to antibiotics and biotransformation. Duties will also include teaching (approx. 2 hours per term) by lectures, tutorials and practical classes.

Salary will be at an appropriate point on the Grade IB (bar) scale £3,384 to £4,882 per annum, according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

Further particulars may be obtained from the Senior Assistant Registrar, The University, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU to whom applications (two copies), together with the names and addresses of three referees, should be lodged not later than March 31, 1979. Please quote reference N. 9813(A)

UNIVERSITY COLLEGE, BOTSWANA

Applications are invited for the post of LECTURER in ENVIRONMENTAL SCIENCE in the DEPARTMENT OF GEOGRAPHY. The appointee will be expected to organise and teach courses in Hydrology and Soil Science at BSc Part II level. The appointee will assist with the introductory courses in aspects of the physical environment to a wide range of students. He/she will also be encouraged to pursue research in his/her field. Salary scale, P5,652 to P9,492 p.a. (£1 sterling = P1.65). The British Government may supplement salary range £2,478 to £2,724 p.a. (sterling) for married appointee or £924 to £940 p.a. (sterling) for single appointee (reviewed annually and normally free of tax) and provide children's educational allowances and holiday visit passages. Family passages; inducement allowance 30%, gratuity 25% at end of each 2 year tour, biennial overseas leave various allowances. Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent Registrar, University College Botswana, PB 0022, Gaborone, Botswana Southern Africa by April 17, 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road London W1P 0DT. Further details may be obtained from either address. 9884(A)

**ROYAL FREE HOSPITAL
SCHOOL OF MEDICINE
(University of London)
DEPARTMENT OF
BIOCHEMISTRY AND
CHEMISTRY
POSTDOCTORAL
RESEARCH ASSISTANT**

Applications are invited for work with Professor J. A. Lucy on membrane fusion, supported by a research grant for three years from the Medical Research Council. Experience of work with proteins and proteinases would be an advantage.

Commencing salary within range £3,909 to £4,408 plus £430 London allowance.

Further particulars from the School Secretary, R.F.H.S.M., 8 Hunter Street, London WC1N 1BP, tel. 01-57 5385, ext. 54, to whom applications (two copies) with the names of two referees should be submitted as soon as possible, quoting reference MRC. 9812(A).

**THE UNIVERSITY OF
THE WEST INDIES
TRINIDAD**

Applications are invited for the post of LECTURER/ASSISTANT LECTURER in ANALYTICAL CHEMISTRY in the DEPARTMENT OF CHEMISTRY. Salary scales (under review): 1977/78: Lecturer: TT319,071 £29,799 p.a. Assistant Lecturer: TT15,480 to £16,974 p.a. (£1 sterling TT\$4.80). FSSU Unfurnished accommodation if available at 10% or rented at 12½% or housing allowance of 20% of pensionable salary, family passages. Study and Travel Grant. Detailed applications with curriculum vitae and naming three referees should be sent direct to Secretary, U.W.I., St. Augustine, Trinidad as soon as possible. Applicants resident in the UK should also send copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0JT. Further details may be obtained on either address. 9885(A).

**CHELSEA COLLEGE
University of London
BIOPHYSICS**

TECHNICIANS GRADE 4 and GRADE 2B required to assist in research and teaching activities within the Biophysics section of the Basic Medical Sciences Group.

Experience of biophysical, bioengineering and/or biochemical techniques would be an advantage.

Salary scales (under review):
Grade 4 £3,420 to £3,867 per annum inclusive
Grade 2B £2,994 to £3,345 per annum inclusive

Enquiries and application forms from a Secretary, Biophysics Laboratory, Chelsea College, 17a Onslow Gardens, London SW7 3AL. Tel. 01-589 4438. 9882(A).

**THE FEDERAL UNIVERSITY
OF PARÁIBA**

João Pessoa, BRAZIL

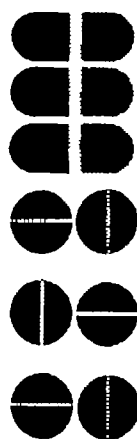
FACULTY POSITIONS

in the Chemistry of Natural Products and in Pharmacology

Applications are invited for a number of positions from individuals holding doctoral degree in the above areas. The successful candidates will participate in research and teaching of the course in natural products chemistry and pharmacology.

Salary: US\$20,000 to US\$22,000 as fringe benefits.

Send curriculum vitae, list of publications and names of three referees to Director, Laboratório de Química Farmacológica, Universidade Federal da Paraíba, 58000-João Pessoa-Paraíba-Brazil. 9876(A).



Pharmacologist Gastrology Ware, Herts

An opportunity has arisen in our Pharmacology Department for a graduate pharmacologist/physiologist with experience of the alimentary system.

The vacancy is within the Gastrology Project team which is studying new drugs for treating gastric ulceration. The successful candidate will supervise routine testing of new compounds and conduct basic research into new areas of alimentary function.

Applicants must have spent several years working on the gastro-intestinal tract and should possess a Ph.D. degree. Graduates with comparable experience will also be considered.

This is an immediate vacancy and graduates who will be completing their studies later this year should not apply.

The post carries a competitive starting salary which will reflect the age, experience, and qualifications of the successful candidate. Other benefits include bonus, pension and productivity schemes, extensive sports and social facilities, and where appropriate, assistance with relocation expenses.

Please apply to: Ian Collins, Ph.D., Personnel Manager, Glaxo-Allenburys Research (Ware) Ltd., Ware, Herts. SG12 0DJ. Tel. Ware 3232. 9898(A).

Glaxo-Allenburys Research (Ware) Limited

**MEDICAL RESEARCH
COUNCIL**

Radiobiology Unit, Harwell

**MAMMALIAN GENETICIST
OR CYTOGENETICIST**

A scientist is needed to join a group studying the genetic effects of radiation and other mutagenic agents in mammals. The emphasis is on methods of exploring the sensitivity to mutagens of germ cells of different sexes, developmental stages and species in the intact animal. Formal genetics and cytogenetics relevant to the mutagenesis work are also included. Candidates with experience of genetics of higher organisms, or of handling rodent chromosomes, at either meiotic or mitotic stages, would be at an advantage.

Possible subjects of research include

- comparison of the sensitivity to mutagens of various mammalian species, with particular emphasis on methods of studying chromosome changes in oocytes and early embryos,
- studies of the causes of chromosomal non-disjunction in mammals,
- factors affecting the sensitivity of oocytes to mutagenesis.

There are excellent research facilities. The Unit also includes groups studying cell mutation, cytogenetics, pathology, and effects of radionuclides.

Applicants should hold a relevant good honours degree, with three years' research experience, and preferably a Ph.D. Salary and conditions of service according to M.R.C. non-clinical scientific scales. Hostel accommodation for single, or housing for married, people may be available.

Applications, with curriculum vitae and names of two referees, should be sent to Administrator, M.R.C. Radiobiology Unit, Harwell, Didcot, Oxon. OX11 0RD, from whom further particulars may be obtained. 9733(A).

**UNIVERSITY OF SUSSEX
SCHOOL OF BIOLOGICAL
SCIENCES**

**CYTOGENETICIST/
CELL BIOLOGIST**

Preferably with some postdoctoral experience of current techniques in genetic toxicology using mammalian cells, required to join MRC funded project on assessment of risks associated with pre-natal exposure to mutagens and carcinogens.

The appointment will be made at a starting salary in the range £3,883 to £4,382 p.a. on the Research Fellow 1A scale (under review) with superannuation benefits, and the post is tenable for 3 years. Starting salary dependent on age and experience.

Applications, (including curriculum vitae and details of 2 referees) should be sent to Professor Robin Cole, Developmental Genetics Laboratory, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, as soon as possible. 9880(A).

**UNIVERSITY OF OXFORD
DEPARTMENT OF
PHYSICS**

Clarendon Laboratory

SURFACE PHYSICS

ELECTRON MICROSCOPY

A Physicist with experience of electron microscopy is required to work as a postdoctoral research officer in a group concerned with studies of the physics of surfaces, particularly their mechanical and chemical properties, using both scanning and transmission electron microscopy. The group receives substantial support from industry, with which it has close links. The post is for a period of three years and involves a limited amount of demonstrating. Salary scale £3,384 to £4,466, under review, starting point according to age, e.g. £3,883 at age 24.

Application, with curriculum vitae and names of two referees, should be sent to Dr J. Wilks, Clarendon Laboratory, Oxford OX1 3PU, from whom further details may be obtained. 9836(A).

**MEDICAL RESEARCH COUNCIL
Clinical and Population Cytogenetics Unit
Edinburgh
MOLECULAR OR CELL BIOLOGIST**

A vacancy exists for a molecular or cell biologist to work with a small team studying the molecular organisation and function of human chromosomes. One area of interest involves the use of restriction enzymes in the study of DNA composition and variation in man and experience with relevant techniques, or in the handling of cultured mammalian cells and chromosomes, would be an advantage, but not essential. Candidates should have a Ph.D. or equivalent and the appointment will be made at an appropriate level on Medical Research Council scientific scales. Applications, including names of two referees, should be sent to the Administrative Officer, M.R.C. Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, by March 31, 1979. 9834(A).

SCIENCE, ENGINEERING AND TECHNOLOGY

The British Council

OVERSEAS CAREER SERVICE

The British Council, which has a responsibility for Britain's cultural and educational relations overseas, will have some vacancies in 1979. The Council is represented in over 80 countries. Overseas Career Service staff must expect to serve wherever they are posted, and to spend two-thirds or more of their working lives abroad.

Staff joining the Overseas Career Service bring with them a wide variety of skills and experience and most appointments are made with a view to eventual promotion on merit to senior managerial posts. The Council has a continuing need for a proportion of new staff with qualifications and experience in science and engineering, as well as in the other specialist subjects of interest to the Council: English language teaching and librarianship for example.

We should like to hear from you if you have a background in science or engineering and some postgraduate experience in research, industry, education or administration. An essential requirement is the personal and managerial qualities which overseas representation demands.

Recently, most successful candidates for the Overseas Career Service have been between 25 and 32 years of age, but we should be glad to hear from younger or older candidates who have the necessary background. Only in exceptional cases is appointment offered to candidates over the age of 40.

The initial salary is in the region of £5,300, possibly higher for particularly well-qualified candidates, and terms and conditions of service are in line with those of comparable organisations in the public sector: free accommodation and overseas allowances, including children's education allowances, are provided while overseas.

For further details and an application form, please write or telephone quoting C1 to Staff Recruitment Department, The British Council, 65, Davies Street, London W1Y 2AA, telephone 01-499 8011, extension 3041.

9878(A)

THE UNIVERSITY OF HULL CURATOR OF THE GEOLOGICAL COLLECTIONS

Applications are invited for the post of Curator in the Department of Geology, tenable from September 1, 1979. The person appointed will be responsible for curation of the Department's teaching and research collection of rocks, minerals, fossils, maps and pamphlet collection, and the work will include the design and execution of exhibits. An honours degree in geology is required.

Salary will be on the Other Related Staff Grade 1B scale: £3,384 to £4,882 (bar) (under review), initial placing depending on age and qualifications.

Applications giving details of age, qualifications and experience, together with the names of two referees, should be sent by April 30, 1979 to The Registrar, The University of Hull, Hull, HU6 7RX from whom further particulars may be obtained.

9858(A)

NATURE CLASSIFIED
will accept your
advertisement
up to 3.30 p.m.
Friday preceeding
Thursday's issue

Analytical Chemist (Clinical Pharmacology)

A chemist with wide theoretical and practical experience in the assay of pharmaceutical products in biological fluids is required to develop and utilize methods of analysis in human pharmacological studies. Applicants should have at least 3-5 years post doctoral experience in a pharmaceutical or medical field. The International Pharmaceutical Research Institute is located near Munich and has new analytical laboratories. Spoken German is not essential but applicants should hold a passport from an EEC country and would be paid according to age and experience. Applications together with supporting material to Professor C. Kozma, Iphar, Lena-Christ-Strasse 2, D-8022 Muenchen-Gruenwald, West Germany.

9852(A)

ASSISTANTSHIPS

THE POLYTECHNIC OF CENTRAL LONDON

School of Engineering and Science
Bio-Organic Research Group

RESEARCH ASSISTANTS IN GENETICS, MICROBIOLOGY, BIO-CHEMISTRY AND CHEMISTRY (4 POSTS)

Applications are invited from graduates with an appropriate good Honours Degree to undertake research on the following projects:

Post 1 mutagenesis in antibiotic-producing *Streptomyces* (supported by Beecham Pharmaceuticals Ltd.)

Post 2 genetic studies of the yeast *Saccharomyces lipolytica* (supported by SRC)

Post 3 chemical identification of antibiotics produced by micro-organisms

Post 4 combined antibiotic and heavy metal resistance of bacteria found in freshwater and sewage

All assistantships are tenable for a maximum period of three years.

Successful candidates will be registered for a Ph.D. with the CNA.

Applications stating the project(s) of interest and including a curriculum vitae and names of two referees to Dr. G. Holt, Division of Science, PCL, 115 New Cavendish Street, London W1M 8JS. (Tel.: 01-486 5811, ext. 6367.)

9894(P)

UNIVERSITY OF CALGARY GRADUATE ASSISTANTSHIPS

(Teaching and Research)

Available for students proceeding to the M.Sc. and Ph.D. degrees in all branches of CHEMISTRY and BIO-CHEMISTRY. The assistantships are valued at \$7,000 to \$8,000 p.a. and include remission of program fees. Travel allowance also available.

For further information write to:

Department of Chemistry
Chairman of Graduate Studies
UNIVERSITY OF CALGARY
Calgary, Alberta, Canada
T2N 1N4

Please indicate field of interest.
9855(P)

UNIVERSITY COLLEGE LONDON

CHEMISTRY DEPARTMENT

Applications are invited for an S.R.C. Postdoctoral Assistantship to study ligand chemistry in transition metal clusters, in particular additions to aromatic bridging ligands and dnmr behaviour. The appointment will begin on October 1, 1979 for one year, with possible renewal for a second. Initial salary in range £3,909 to £4,158 plus £450 London Allowance. Applications with curriculum vitae and names of two referees, should be sent to Dr A. J. Deeming, Department of Chemistry, University College London, 20 Gordon Street, WC1H 0AJ.

9856(P)

UNIVERSITY OF OXFORD DYSON PERRINS LABORATORY POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a postdoctoral research assistantship supported by the S.R.C. to further develop and exploit a new method for measuring kinetic isotope effects (including those for 'heavy' atoms) with high accuracy. Preference will be given to candidates with experience in kinetics or in synthesis with stable isotopes.

The appointment is for two years, commencing October 1, 1979, or at an agreed date. The salary will be on the Research Support Grade Scale 1A, initially not higher than £4,130 p.a. (under review), plus U.S.S. benefits.

Applications (one copy), with curriculum vitae and the names and addresses of at least two referees (overseas applicants should ask referees to write directly to the address below) should be sent not later than March 31, 1979, to Dr M. J. T. Robinson, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY, from whom further information may be obtained.

9801(P)

GRADUATE ASSISTANTSHIP leading to M.Sc. and Ph.D. in Chemical Engineering. Stipend up to \$7,200 p.a. tuition for qualified candidates. Active and stimulating research program. Write Dr. John A. Roth, Department of Chemical Engineering, Vanderbilt University, Nashville, Tennessee 37235. Equal Opportunity Employer.

9835(P)

UNIVERSITY OF CALGARY GRADUATE ASSISTANTSHIPS

(Teaching and Research)

Available for students proceeding to the M.Sc. and Ph.D. degrees in branches of CHEMISTRY and BIO-CHEMISTRY. The assistantships are valued at \$7,000 to \$8,000 p.a. and include remission of program fees. Travel allowance also available.

For further information write to:

Department of Chemistry
Chairman of Graduate Studies
UNIVERSITY OF CALGARY
Calgary, Alberta, Canada
T2N 1N4

Please indicate field of interest.
9377(P)

UNIVERSITY OF DURHAM DEPARTMENT OF APPLIED PHYSICS AND ELECTRONICS Applications are invited for an S.R.C. POSTDOCTORAL RESEARCH ASSISTANTSHIP

tenable for 3 years from May 1, 1979 or as soon as possible thereafter. Applicants should have qualifications in physics, materials science, electron or a related subject.

The successful applicant will be a team working on Electroluminescence in Zinc Sulphide in the group directed by Dr J. Woods, and will be concerned with the fabrication and evolution of light emitting diodes and larger area devices.

Initial salary on National Research Range 1A in the range £3,883 to £4,382 (under review), plus superannuation.

Applications (three copies) naming three referees, should be sent March 21, 1979, to the Registrar & Secretary, Science Laboratories, South Road, Durham DH1 1LE, from whom further particulars may be obtained.

9897(P)

FELLOWSHIPS

THE ROYAL SOCIETY
BRITISH GAS SENIOR
RESEARCH FELLOWSHIP

Applications are invited by the Council of the Royal Society for The Royal Society — British Gas Senior Research Fellowship for original scientific or technological research in a field of mathematics, chemistry, physics or engineering (including metallurgy and materials science, fluid mechanics, thermodynamics, combustion, heat transfer, biochemistry, and computer science) related to the production, transmission and use of natural gas and its substitutes.

Candidates, who must be citizens of the United Kingdom or the Commonwealth, should supply the usual personal details and two referees. Testimonials will not be considered. Candidates should state the subject of the proposed research and where it will be undertaken. It is expected that the successful candidate will spend a significant proportion of his or her time in direct collaboration with industry, evidence of existing contacts would be helpful. Applicants should obtain and give evidence of consent from the head of department or other authority in the university and/or industrial establishments in which the research will be done.

The appointment which will be subject to the Society's general regulations governing research appointments, will be tenable for five years from October 1, 1979 (or another date to be arranged), and may exceptionally be renewable for a further two years, such renewal being considered at the end of the fourth year of tenure. Applicants should preferably be under 45 on October 1, 1979. The present stipend scale is £8,031 by £252 to £8,787 by £249 to £9,036 per annum, and the point of entry will be determined by Council. Superannuation benefits will be provided. Some provision for research expenses will be available.

Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG, and should be received not later than March 31, 1979.

9819(E)

THE ROYAL SOCIETY
SENIOR RESEARCH
FELLOWSHIPS

Applications are invited by the Council of the Royal Society for up to three Royal Society Senior Research Fellowships for research in any scientific subject, established by funds from the Parliamentary Grant-in-aid. The appointments will be tenable in any appropriate university department or research institution in the United Kingdom approved by the Council of the Royal Society. Candidates, who must be citizens of the United Kingdom or the Commonwealth, must supply the usual personal details and give two referees. Testimonials will not be considered. The subject of the proposed research, and where it would be done, together with the name of the head of the department, whose consent should first be obtained, must be stated.

The appointments, which will be subject to the Society's general regulations governing research appointments, will be tenable for five years from October 1, 1979 (or another date to be arranged) and may be renewable for a further two years. Applicants should preferably be under 45 on October 1, 1979. The present stipend scale is £8,031 by £252 to £8,787 by £249 to £9,036 per annum, and the point of entry will be determined by Council. Superannuation benefits will be provided. Some provision for research expenses will be available.

Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG, and returned by March 1, 1979.

9820(E)

UNIVERSITY OF ESSEX
DEPARTMENT OF CHEMISTRY
POSTDOCTORAL
RESEARCH FELLOWSHIP

Applications are invited for a position as post-doctoral research assistant from October 1, 1979 to work with Dr. J. G. Tillet and Dr. R. M. G. Roberts in the field of organosulphur chemistry. This project is funded by the Science Research Council and will involve a kinetic study of the metal ion catalysed cleavage reactions of cyclic and open-chain disulphides. Appointment will be for two years and the salary will be on the Research Fellow 1A scale (plus superannuation).

Applications should reach the Chairman, Department of Chemistry, University of Essex, Colchester CO4 3SQ, from whom further particulars may be obtained by March 31, 1979.

9865(E)

UNIVERSITY OF
BRITISH COLUMBIA
Vancouver, B.C., Canada
POSTDOCTORAL
FELLOWSHIP

(two years) available immediately for research on the mechanisms of bacterial attachment to hard and soft tissue with emphasis on isolation and characterisation of cell surface components involved in bacterial attachment reactions.

Salary \$11,700 for first year and \$12,500 for second.

Send curriculum vitae and names of three referees to:

Dr B. C. McBride
University of British Columbia
Microbiology Department
2075 Westbrook Mall
Vancouver, B.C. V6T 1W5

9837(E)

THE ROYAL SOCIETY
THE BRITISH PETROLEUM
COMPANY LIMITED
SENIOR RESEARCH
FELLOWSHIP

Applications are invited by the Council of the Royal Society for The Royal Society — British Petroleum Company Limited Senior Research Fellowship, established by means of a five-year grant from the British Petroleum Company Limited for research in a subject field associated with the exploration, production and transport of fossil fuels and/or with the physical, chemical and mechanical properties of materials used in these operations. The Fellow will be expected to have an interest in the application of his research to practical problems. The appointment will be tenable in any appropriate university department or research institution in the United Kingdom approved by the Council of the Royal Society. Candidates, who must be citizens of the United Kingdom or the Commonwealth, must supply the usual personal details and give two referees. Testimonials will not be considered. The subject of the proposed research, and where it would be done, together with the name of the head of the department, whose consent should first be obtained, must be stated.

The appointment, which will be subject to the Society's general regulations governing research appointments, will be tenable for five years from October 1, 1979 (or another date to be arranged). Applicants should preferably be under 45 on October 1, 1979. The stipend scale will be £8,031 by £252 to £8,787 by £249 to £9,036 per annum, and the point of entry will be determined by Council. Superannuation benefits will be provided. Some provision for research expenses will be available.

Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG, and returned by March 31, 1979.

9818(E)

NEWCASTLE UPON TYNE
POLYTECHNIC
DEPARTMENT OF
ELECTRICAL ENGINEERING
AND PHYSICAL ELECTRONICS
FELLOWSHIP IN
PHOTOVOLTAIC SOLAR
ENERGY

The Fellow will collaborate with existing research groups in both a techno-economic study of the viability of photovoltaic power generation in a variety of economic and climatic conditions and in the development programme of sputtered thin film solar cells. Post tenable for five years, salary depending upon experience.

Burnham F.E.: Principal Lecturer
Scale: £7,047 to £8,844 per annum.

For further particulars and application form, returnable by March 15, 1979, please send a stamped addressed foolscap envelope to the Staffing Officer, Newcastle upon Tyne Polytechnic, Ellison Building, Ellison Place, Newcastle upon Tyne NE1 8ST.

9902(E)

UNIVERSITY OF
DUNDEE
POSTDOCTORAL
RESEARCH FELLOWSHIP
DEPARTMENT OF
CHEMISTRY

Applications from appropriately qualified candidates (either sex) are invited for a Postdoctoral Research Fellowship available for one year in the first instance but with the possibility of subsequent renewal for one further year.

The person appointed will work on an investigation of the photochemistry of some benzotriazine-3-N-oxides with Dr W. M. Horspool and Dr A. W. Murray (either of whom will be pleased to answer informal enquiries).

Initial salary £3,883 per annum (Research and Analogous Staff Range 1A, Point 1). Superannuation under U.S.S.

Applications, quoting Reference EST/29/79J and including a curriculum vitae and the names of two referees should be lodged with The Secretary, The University of Dundee, Dundee DD1 4HN by March 22, 1979.

9628(E)

STUDENTSIPS

THE ANIMAL VIRUS
RESEARCH INSTITUTE,
PIRBRIGHT
A.R.C. RESEARCH
STUDENTSHIP 1979

Applications are invited for an Agricultural Research Council Studentship to join a group working on the structure of foot-and-mouth disease virus RNA and the biochemistry of virus mutants as they relate to the function of the RNA. The Studentship will be tenable for up to 3 years from October 1979 and the successful candidate will be expected to read for a higher degree. Applicants should possess, or expect to obtain, a good honours degree in a Molecular Biological subject.

Application forms and further particulars may be obtained from the Secretary, The Animal Virus Research Institute, Pirbright, Woking, Surrey, GU24 0NF, applications should be received by March 31, 1979.

9845(F)

THE MEDICAL COLLEGE
OF ST. BARTHOLOMEW'S
HOSPITAL
(University of London)

Applications are invited from good honours graduates and those expecting to graduate in 1979 for

POSTGRADUATE
STUDENTSIPS

in Biochemistry. Successful candidates will be registered for the degree of Ph.D. Topics include: Molecular Enzymology, Endocrinology, Metabolic Biochemistry, Biochemical Carcinogenesis and Medicinal Chemistry. Enquiries to: Professor E. M. Crook, Department of Biochemistry, The Medical College of St Bartholomew's Hospital, Charterhouse Square, London, EC1M 6BQ.

9907(F)

LECTURES



Mental Health Foundation

The Sir Geoffrey Vickers Lecture
entitled

INNOVATIONS IN SOCIAL PSYCHIATRY
will be given by

PROFESSOR JOHN WING

Director, MRC Social Psychiatry Unit
The Institute of Psychiatry, London
at the

Edward Lewis Lecture Theatre
Middlesex Hospital Medical School
Cleveland Street, W.1

on

Tuesday, 1st May 1979 at 6.00 p.m.

The Chair will be taken by

The Hon. Robert Loder

Chairman of the

Research Committee of the Foundation

NO TICKET IS NECESSARY

(There are no parking facilities)

9826(K)

COURSES AND CONFERENCES

IId Announcement

IVth Colloquium :

HORMONES AND CELL REGULATION

(03/10/1979—3 p.m. to 06/10/1979—1 p.m.)

The 'Institut National de la Santé et de la Recherche Médicale' is organising its annual meeting on cellular regulation at the Bischoffsberg (near Strasbourg). The aim of the Conference is to present reviews of recent advances in general lectures and communications of hot unpublished results by posters, both being analysed in general discussions.

Organisers:

J. NUNEZ, H. DENTON, J. E. DUMONT, D. HEPP, B. JEANRENAUD, I. MCINTYRE, S. MOREL, G. SCHULTZ, H. J. VAN DER MOLEN.

Preliminary programme:

General lecture: J. P. EBEL (Strasbourg)

Cyclic Nucleotides: J. G. HARDMAN (Nashville), Z. SELINGER (Jerusalem), S. VAN HEININGEN (Edimburg), G. ROSSELIN (Paris)

Mechanisms not related to cyclic nucleotides: H. DEWULF (Leuven), R. H. MITCHELL (Birmingham), K. JACOBS (Heidelberg)

Insulin: P. DE MEYTS (Leuven), H. BLUNDELL (London)

Steroids: C. B. BOYD (Edimburg), GEHRING (Heidelberg), K. AHREN (Göteborg)

Opiates: B. HAMPRECHT (München), C. KORDON (Paris)

Differentiation: N. LE DOUARIN (Paris), R. KRAM (Brussels), L. HOUEBINE (Jouy en Josas).

Participants can present posters related to the topics of the Conference.

Afternoons are free with a wide-range of leisure activities (tennis, table-tennis, etc.).

The general attendance is limited to 100 persons.

Registration fee: 200 F.F. Room and board can be obtained at the site of the Conference. Provisional prices, 725 F.F. (double room), 900 F.F. (single room). A limited number of fellowships will be available to help young investigators who have a special interest in the program and who cannot otherwise attend the Symposium.

Investigators interested in attending the meeting and/or presenting a poster should submit an application form to the secretariat:

Dr J. NUNEZ—INSERM—Unité de Recherche sur la Glande Thyroïde
79, av. de Général Leclerc, 94 BICETRE—FRANCE

9740(C)

EMBO COURSE ON MEMBRANES & THE CYTOSKELETON

October 1-10, 1979

European Molecular Biology Laboratory Heidelberg, F.R.G.

The course will be organised by the Membrane Group at EMBL and will comprise a series of lectures (1-3 October) and practical work (4-10 October).

The teaching staff will include: M. Bretscher (U.K.), J. Goldstein (U.S.A.), W. Franke (F.R.G.), R. Henderson (U.K.), R. Kemler (France), J. Rothman (U.S.A.), G. Palade (U.S.A.), P. Peterson (Sweden), T. Pollard (U.S.A.), P. Robbins (U.S.A.), G. Schatz (Switzerland), W. Sebald (F.R.G.) and K. Weber (F.R.G.);

and the following staff members of EMBL: B. Dobberstein, H. Garoff, G. Griffiths, A. Helenius, B. M. Jockusch, D. Louvard, K. Simons, G. Warren and H. Weiss.

Practical work will include: Membrane reconstitution; biosynthesis of membrane proteins *in vitro*; immunofluorescent microscopy and electron microscopy of the cytoskeleton.

A maximum of 36 students will be able to attend. Applications should include a short curriculum vitae and a description of present research interests. For further details write to:

Graham Warren,
EMBL,
Postfach 10.2209,
D-6900 HEIDELBERG, F.R.G.

The closing date for applications is May 1, 1979. 9906(D)

THE NEW YORK ACADEMY OF SCIENCES

Conference on

REGULATORY FUNCTIONS OF INTERFERONS

New York City, October 23-26, 1979

The New York Academy of Sciences is planning to sponsor a four-day Conference on interdisciplinary aspects of interferon research. The Conference is co-chaired by J. Vilcek (New York University), I. Gresser (Institut de Recherches Scientifiques sur le Cancer, Villejuif) and T. C. Merigan (Stanford University). The Conference will cover: immunoregulatory actions of interferons; regulatory effects of interferons in lymphocytes, macrophages and other cells; interferons as lymphokines; cell growth inhibitory and antitumor actions of interferons; genetics of the interferon system; purification and properties of interferons; molecular mechanisms of interferon action.

To supplement invited lectures, the organising committee will accept suitable poster session presentations. The deadline for the submission of abstracts to be considered for poster presentation is June 1, 1979. The abstracts should include the name of the author(s), institution and 100-200 word summary of work to be presented in the poster. Send abstracts for poster session and inquiries about details of the program and registration to:

Conference Director,
New York Academy of Sciences,
2 East 63rd Street,
New York, NY 10021.

9757(C)

UNIVERSITY OF LONDON
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M.Sc.

in

HUMAN AND
APPLIED PHYSIOLOGY

A one-year course to train graduates in measuring and studying the physiological functioning of man in normal and abnormal environments. The course would equip students to specialise in the applications of physiology to man in industrial, service and clinical situations and the teaching of human physiology. Topics studied include:—basic measurements; life support systems; exercise; locomotor; postural; climatic; high altitude and hyperbaric physiology.

October 1, 1979 to September 30, 1980.

Applicants should have a good degree in Physiology or be medically qualified.

Further details and application form from: Dr S. Rowlands, King's College, Strand, LONDON WC2R 2LS, quoting reference N 1/3.

Closing date for applications: March 31, 1979. 9811(D)

UNIVERSITY OF BRISTOL
M.Sc. (MEAT SCIENCE)

This course occupies one calendar year and leads to the degree of M.Sc. (Meat Science) by examination and dissertation. Candidates should have either an honours degree in the biological sciences or a degree in veterinary science. Applicants with other qualifications, if combined with previous industrial research or experience, may also be considered.

The course provides a systematic programme of study at an advanced level covering the whole field of meat science. Instruction will be provided by the staffs of the University School of Veterinary Science and of the Agricultural Research Council Meat Research Institute, which are both situated on the University's estate at Langford, about 14 miles south of Bristol. Accommodation is available in halls of residence.

Starting with the production of meat animals, the course extends through the scientific bases of all the operations involved in the production of meat and meat products, and includes consideration of the economics of the industry and of its controlling legislation. The objectives of the course are to equip graduates to apply the principles of meat science within the industry, to operate effectively in posts with responsibility for its legislative control or to provide them with the foundation for a career in meat research.

For further particulars please apply to:

The Registrar and Secretary,
(Ref. CYF),
University of Bristol,
Senate House,
Bristol BS8 1TH.

9901(D)

WORKSHOPS

WORKSHOP:

Wetlands and Estuarine
Processes and Water Quality
Modelling

Sponsor: U.S. Army Corps of
Engineers, Waterways Experi-
ment Station.

Date: June 18-20, 1979, New
Orleans, La., U.S.A.

Abstracts to be submitted
before March 16, 1979, to:
Dr Peter Hamilton, Science
Applications, Inc., 4900
Water's Edge Drive, Suite
255, Raleigh NC 27606,
U.S.A.

9896(V)

ASSOCIATESHIPS

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THE UNIVERSITY COLLEGE
OF WALES, ABERYSTWYTH
Edward Davies Chemical
Laboratories

POSTDOCTORAL
RESEARCH
ASSOCIATESHIPS

Applications are invited from
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in collaboration with Professor
Graham Williams.

One post will be concerned with
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The second post will be concerned
with research into the molecular
interactions (including complex-
formation) of polymer systems, as
studied using modern electro-
optical and dielectric techniques.

The posts are for up to two years
from August 1, 1979. The starting
salary will be up to point 3 of the
Range 1A Scale (£3,883 to £4,382,
interim award).

Applications, enclosing a curri-
culum vitae and the names of two
referees should be submitted by
April 6, 1979 to Professor Graham
Williams, Edward Davies Chemical
Laboratories, University College of
Wales, Aberystwyth SY23 1NE.
Dyfed. 9829(O)

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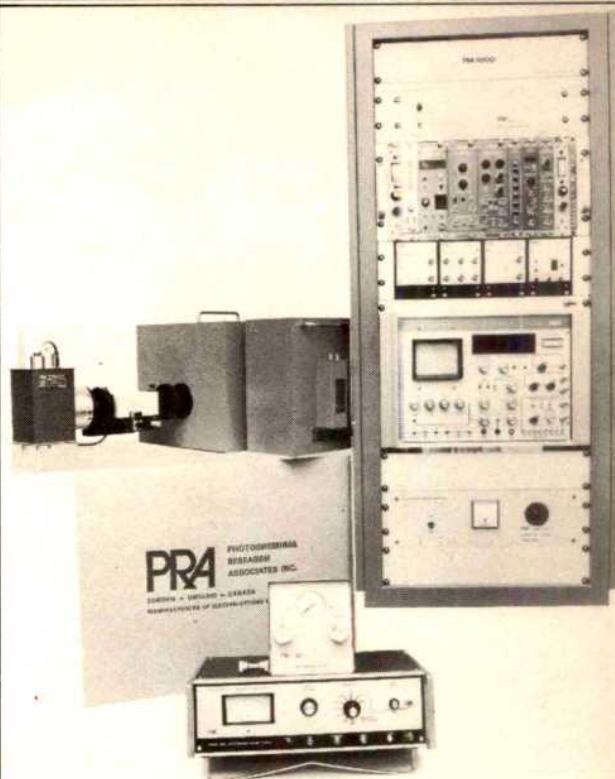
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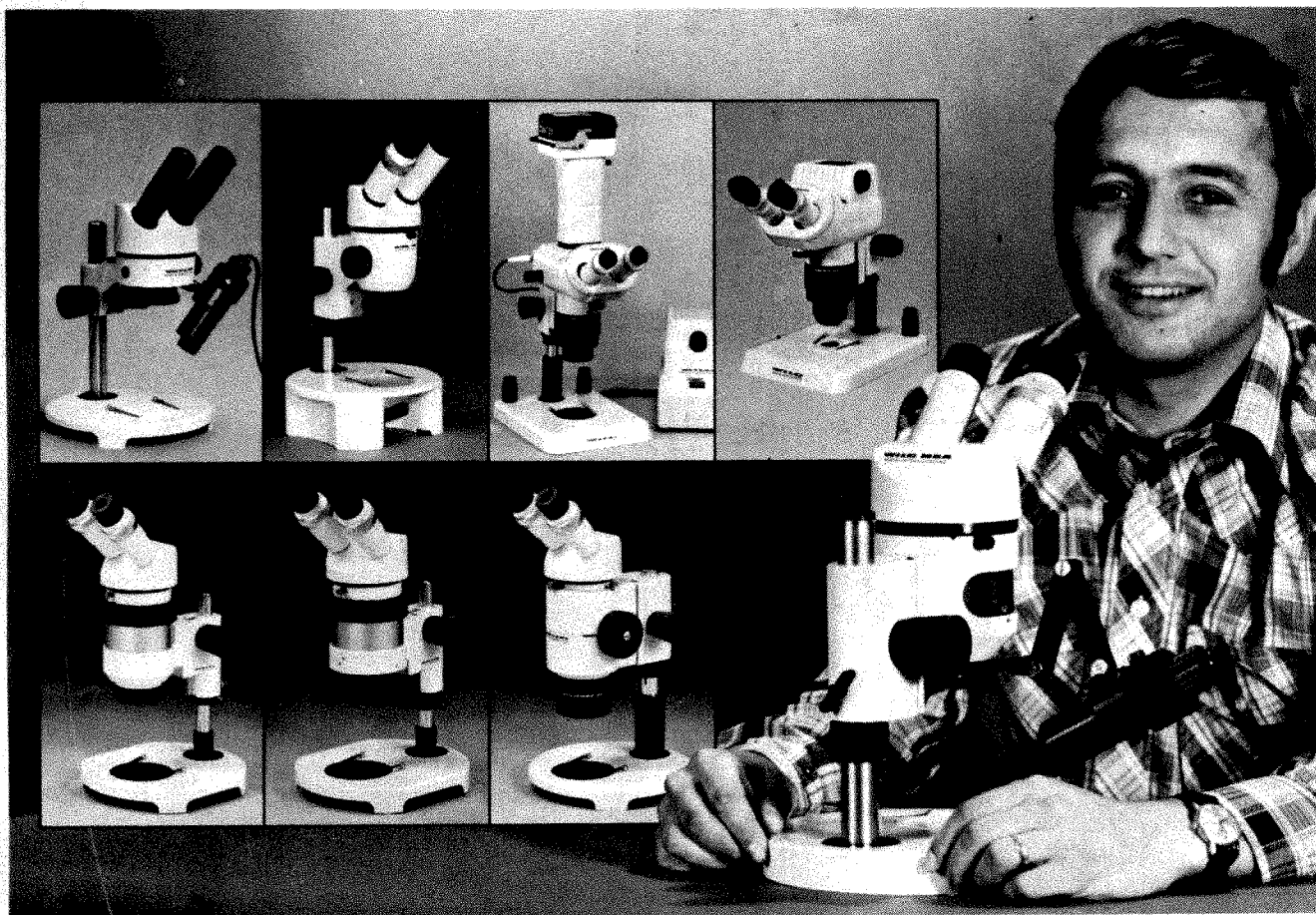
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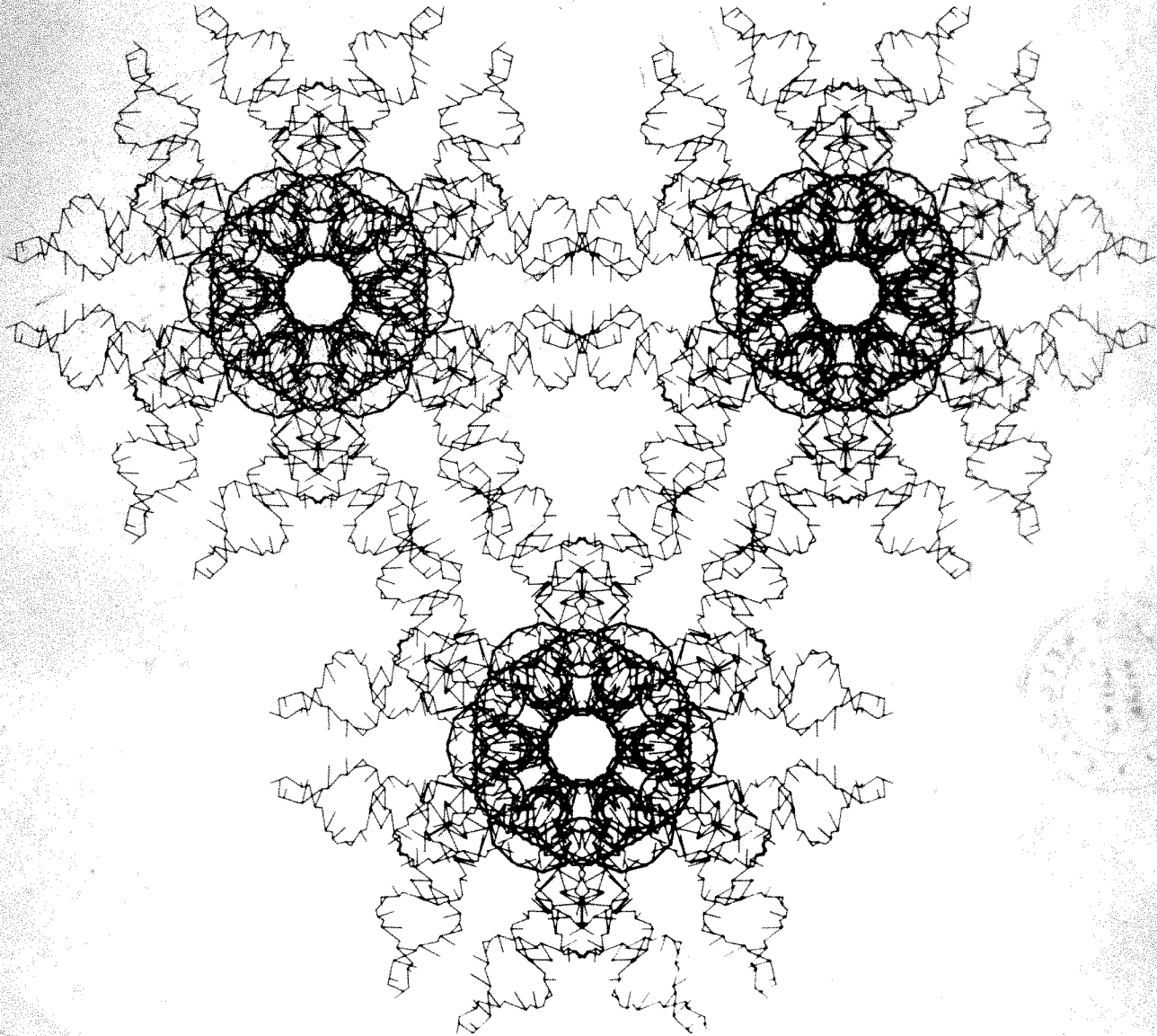
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Cover picture

The crystal structure of a eukaryotic
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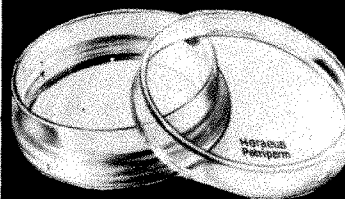
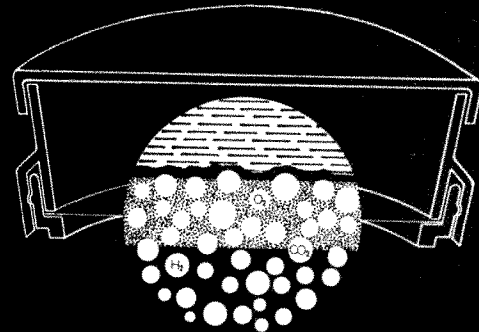


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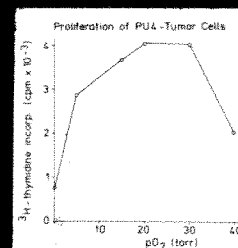
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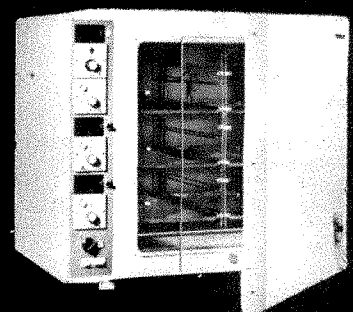
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Guide to authors

● Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.

● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

● Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.

● 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

Articles should be accompanied by an abstract of not more than fifty words. Letters should begin with a paragraph giving the background and main conclusions in terms intelligible to as wide a readership as possible.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; $\exp(a)$ is preferred to e^a if 'a' is more than one character.

References are indicated by superscripts in the text. See any contemporary *Nature* for style, but note: (i) only one reference number need be used if the reference is to several papers by identical authors.

(ii) first and last pages of references should be cited.

Abbreviations should follow the *World List of Scientific Periodicals*, fourth edn (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished' should be incorporated in text.

Artwork should be sent with the manuscript and clearly marked with author's name and the figure number. Line drawings should be either photographic prints or in Indian ink on heavy cartridge paper, tracing paper or similar materials. Most figures are reduced to one column width so originals should be about as wide as a page of *Nature*. To enable figures, particularly maps, to be edited in the same style as the text, they should contain only essential material. Ideally, an unaltered original and three lettered copies should be provided; labelling on half-tones should, if possible, be avoided entirely.

Magnifications quoted should be for the figures as submitted. We are always glad to see artwork for possible use on the cover, but cannot guarantee its return.

In order to save on postal expenses we return only the top copy and artwork of manuscripts that we cannot publish.

Nature's publishing policy is outlined in 258,1 (1975) and 264, v, 11 Nov. (1976).

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applied excellence

Advanced techniques in routine use at The Radiochemical Centre

Described below are just two examples of the many up-to-date techniques, which have been pioneered or applied for routine use at The Radiochemical Centre. These developments are part of our constant endeavour to maintain our position at the forefront of the specialised field of tracer methodology, so that we can continue our supply of radiochemicals of the highest quality and technical specifications.

Distribution of labelling in tritium compounds

Modern techniques for the production of tritiated compounds are more sophisticated than those used in the early days of tritium labelling, and produce compounds labelled in specific positions rather than generally labelled. Nevertheless, it is necessary for many tracer applications of tritium compounds to know the *precise* position and configuration of the tritium labels. Traditional chemical methods of doing this are tedious and time consuming and subject to considerable error, and so the routine supply of such information has until recently not been possible.

The Radiochemical Centre, in collaboration with the University of Surrey, has developed over the past eight years the technique of tritium nuclear magnetic resonance (tnmr) spectroscopy for this purpose. This method is much quicker and more accurate than the traditional chemical or biochemical methods for determining distribution of tritium labelling.

It is now used routinely to establish the distribution of tritium labelling produced by the usual methods of tritiation employed at The Radiochemical Centre. We supply accurate details as to the position and configuration of the tritium labels for an increasing number of our labelled compounds.

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[G-³H]VINBLASTINE Code TRK.507 17 44% 11' 8% 12,13' 46% 14' 2%	

The latest of our publications is as follows:
A. RAWI, J. M. A., BLOXIDE, J. P., ELVIDGE, J. A., JONES, J. R.,
CHAMBERS, V. E. M., CHAMBERS, V. M. A. and EVANS, E. A.,
Steroids vol. 28 (3), p. 359-375, 1976.

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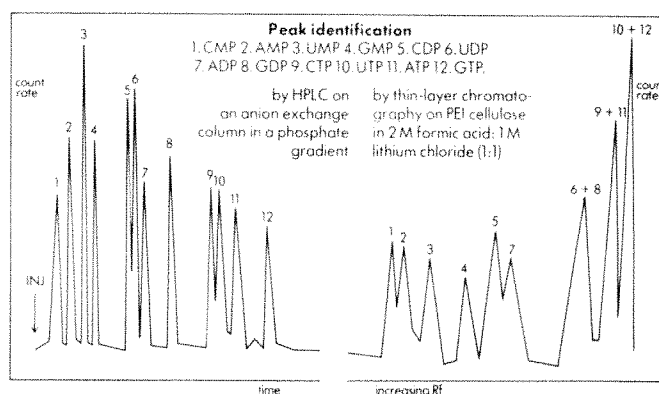
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Work aimed at developing the applications of this method to radiolabelled compound separations is still in progress, but The Radiochemical Centre is already using the technique in many of its production processes, and in analytical applications. The result is purer compounds for the customer and greater efficiency of working.

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1465-11-77

nature

8 March 1979

Better information about development

WHEN the United Nations Conference on Science and Technology for Development (UNCSTD) comes to an end in Vienna in late August, what will it leave behind for the future? Many hope it will not spawn another UN agency or office, as there are enough of them already. But in individual countries there could be a valuable legacy: as a result of preparations for UNCSTD many nations have brought together a wide range of people—administrators, politicians, industrialists, trade unionists, academics—to discuss national problems, be they those of rich or poor countries. If these networks survive and continue to talk to each other then UNCSTD will not have been in vain.

Britain has kept such a low profile both domestically and internationally over the UNCSTD meeting that to talk about networks having emerged from any national debate on the subject would be entirely fatuous. And yet there is at present the possibility of raising public awareness about development questions.

The Overseas Development Ministry recently commissioned a survey on public attitudes towards overseas development and found a substantial decline in support for British aid since the last such survey (in 1969).

Whilst only a few per cent are against richer countries giving help to poorer countries, 39% of those interviewed were against Britain herself giving aid. And few were able to give even an order of magnitude estimate of how much the country spends on overseas aid, generally overestimating the figure. As interviews progressed and those interviewed were told Britain's actual expenditure, a significant shift in

attitudes occurred and support for more expenditure grew substantially.

Part of the reason for ignorance and distrust of overseas aid could simply be low public awareness of what is involved in an aid programme. Such a conclusion would be reinforced by comparative statistics on how much Britain spends on education about development: Britain's spending on education about overseas development has until recently amounted to about £215,000 per annum—about 0.3p per capita. This contrasts very unfavourably with countries such as Germany and Canada (spending 2p and 7p per capita respectively), and doesn't bear comparison at all with expenditure of 10p per capita in the Netherlands or 15p in Sweden. Now spending is going up enormously; a recent ODM working party on the subject recommended a budget of £2.7 millions for development education by 1982 (*Overseas Development Paper 14*, HMSO) and the government has accepted these proposals.

Universities should take advantage of this new scheme. By no means all ignorance of the problems of the developing world is confined to those who never benefited from higher education; indeed most students are unlikely to be made to think at all about these matters during their formal coursework except, perhaps, in a handful of lectures in an optional course on science and society. Better informed graduates in science and technology might make for more soundly-based judgements later in life. The financial means for providing better information are now available. Who will take them up? □

JET—an insoluble salary problem

READERS of the classified advertisement section of *Nature*—most of our readers tell us they turn to it first—will not be unaware of wide differentials in salary. If you work in a British university, or for the British civil service, the salary scale will be tightly defined to the fourth significant figure of a very modest sum. Find a post in a continental university and the salary doubles, at the least. The cost of living goes up too, of course, although few would deny that, taken all in all, the scientist in England is ill-paid in comparison with his continental colleagues.

It is the posts in international organisations which really raise the eyebrows, however. Many of these positions are either tax-free or subject to very modest taxes indeed. Recently, for instance, readers will have noticed such choice offerings as the directorship of an internationally sponsored laboratory in Monaco, no less, at £24,000 per annum, tax free. This is hardly an unusual sum; researchers with several years' experience can command the same at CERN.

What happens, however, when internationally sponsored laboratories, with their traditionally high tax-free salaries come to Britain where the natives are ill-paid? The answer is—real recruitment problems, as those working on the Joint European Torus (JET) programme are finding out in their early days at Culham.

At the professional level, roughly one-third of the em-

ployees at JET are British, the rest are from other participating nations; at the senior technician level Britain supplies about 50% of the staff. The United Kingdom Atomic Energy Authority pays the salaries of Britons at its own standard rates subject, of course, to British taxes. Euratom supports the rest, who pay only a very modest 'community tax'.

Were Euratom to pay at the same rate as it does in Brussels, say, the differential between British and non-British employees' pay would be grotesque, so a figure of 62% of the Brussels rate has been established. Italians, for instance, still find this sort of salary attractive, but the same cannot be said for Germans—so there are differential recruitment problems. And even so, the 62% figure means that the net salaries of non-British employees are between 1.5 and 2 times greater than those of British counterparts. It is generally accepted that a factor of 1.3 to 1.5 is adequate compensation for living in a foreign country; there is little doubt that higher figures are ultimately going to be bad for morale.

At present, even if there are recruitment problems, the high level of motivation of the staff makes the salary question less important. But JET is a long-haul project, and the salary issue will not go away unless something quite remarkable happens to Britain and the pound sterling. □

'Emergency' ban on 2,4,5-T herbicide in US

Clive Cookson reports from Washington on swift reaction to a study linking 2,4,5-T with an increase in miscarriages

THE US Environmental Protection Agency has taken emergency action to ban the use of the controversial herbicide 2,4,5-T, after receiving new evidence linking it to a high incidence of miscarriages in Oregon.

"Studies completed only days ago show a high miscarriage rate immediately following the spraying of 2,4,5-T in the forests around Alsea, Oregon," explained EPA deputy administrator Barbara Blum. "This alarming correlation comes at a time when seven million pounds of 2,4,5-T are about to be used to control weeds on power line rights-of-way and in pastures, and to manage forests across the nation".

These uses will be stopped immediately by "emergency suspension"—the most drastic action EPA can take against a herbicide or pesticide, and the first time it has ever been invoked. The closely related herbicide, Silvex, which is used primarily to kill weeds on suburban lawns, is also covered by the order. But the remaining legal uses of 2,4,5-T, on open ranges and rice fields, are not included and may continue, the EPA said, "because they appear at this time not to involve human exposure comparable to the suspended uses".

However, the Environmental Defense Fund has rejected this argument and petitioned EPA for a total ban on all uses.

2,4,5-T [2,4,5-trichlorophenoxyacetic acid] and Silvex [2-(2,4,5-trichlorophenoxy)propionic acid] are both contaminated with small amounts of 'dioxin' (TCDD), which produces birth defects, miscarriages and tumours in animals, even at extremely low concentrations. The dioxin is thought to be responsible for their ill effects on animals.

For eight years, US environmentalists have been fighting legal and administrative battles against the continued use of 2,4,5-T (which was applied as a military defoliant in Vietnam and was widely blamed for the high incidence of birth defects there). But the EPA claims that its latest study is the first to link the herbicide clearly to ill effect on humans.

The agency commissioned the study last year when nine women from Alsea wrote to say they had had miscarriages after the national forest there was sprayed with 2,4,5-T. Scientists from Colorado State University and the University of Miami medical school compared miscarriages in the Alsea basin (western Oregon) with a control

population in rural eastern Oregon. They reported that:

- The miscarriage rate in the Alsea area was significantly higher than in the control area, where 2,4,5-T was not sprayed.
- The rate peaked dramatically in June in each of the six years studied, two or three months after the annual spring spraying. The spontaneous abortion index in June over the period 1972-77 was 130 per 1,000 births in Alsea and 46 per 1,000 in the control area.

Dr Blum said: "It's a remarkable correlation. While it is not proof of a cause and effect relationship, it is highly suggestive, particularly in light of animal data, and gives great cause for concern". She estimated that four million people across the United States would be protected by the emergency action.

The EPA claims that the short-term economic impact of the ban will be slight, because alternative herbicides are available for use on pasture and rights-of-way, and only 0.2% of the country's commercial forest area is sprayed annually. The long-term cost to forestry of a permanent ban could be \$7m-12m a year, because other herbicides are less effective and more expensive.

Dow Chemical is the leading manufacturer of 2,4,5-T and Silvex. A few

Dioxin: the 10-year battle that began with Agent Orange

Alastair Hay traces the steps leading up to the EPA ban

EVER since the late 1960s when the herbicide 2,4,5-T was found to contain the extremely toxic contaminant 2,3,7,8-tetrachlorodibenzodioxin (dioxin), there has been much concern over its continued use. It has already been banned in some European countries.

According to Mr Frank Parsons of the EPA's Pesticide Programme, 2,4,5-T is the biggest single issue the agency has had to deal with so far. In April last year, the agency issued its "Rebuttable Presumption against Registration and continued Registration of Pesticide Products containing 2,4,5-T", and interested parties were given 45 days in which to submit evidence supporting or opposing the use. An additional 60 days grace was granted following a request from one

of the major manufacturers for more time to present its case.

Parsons says that the EPA has received over 2,700 submissions on 2,4,5-T ranging from a 2,300-page memorandum from Dow Chemical—the major manufacturer of 2,4,5-T—supporting its use, to individuals claiming they were sprayed with the herbicide and their "hair dropped out". Asked how seriously the agency considered such comments, Parsons said that if enough of these claims are made "we can't ignore them." But the main protagonists in the debate are the chemical manufacturers and environmental pressure groups.

During the Vietnam war, Dow supplied most of the 2,4,5-T used by US forces in their defoliation programmes in Vietnam. It was used at that time in a 1:1 combination with 2,4 dichlorophenoxyacetic acid, called Agent Orange.

In 1969, Agent Orange was reported to be teratogenic (causing foetal abnormalities) in a study published by the US Bionetics Research Laboratory. Although it was subsequently shown that it was the dioxin con-

taminant—sometimes present in quantities in excess of 30 ppm—which was teratogenic and not 2,4,5-T itself, the Bionetics study led the US Government to withdraw Agent Orange from Vietnam and to restrict 2,4,5-T use in the US to forestry and clearing rights of way.

It was the high dioxin contamination which marked 2,4,5-T as a teratogen. The EPA reasoned, therefore, that reducing this contamination to less than 0.1 ppm might render it safe. However, as more evidence about dioxin's toxicity accrued, the agency was forced to review the situation. In 1972, Dow obtained a temporary court injunction preventing EPA from taking further action against 2,4,5-T. The following year, this was overturned by the US Court of Appeal. When the first hearings were held by the EPA in 1974 they proved inconclusive: there was insufficient data supporting allegations that dioxin in 2,4,5-T presented a health risk, and an order proposing the cancellation of 2,4,5-T was withdrawn.

The latest evaluation of the herbicide has thus been a continuation of

other firms also make them. Although the emergency suspension took effect from the moment it was announced on 1 March, the companies can appeal to have it lifted. When the suspension issue is settled, "cancellation hearings" will begin, to determine whether the herbicides should be banned permanently. That will take years to decide. □

EPA disputes Dow claim

The Environment Protection Agency has disputed claims by Dow Chemical that some dioxin compounds found in the environment are the result of normal combustion processes. The claim was made by the chemical company last November as part of its defence against charges that pollution associated with its herbicide production plant at Midland in Michigan was resulting in trace elements in fish in the Tittabawassee River.

Dow claimed that, since it had found dioxins associated with a number of combustion sources including car exhaust and charcoal grills, dioxins were formed by normal combustion processes. But EPA says this claim was based on purely circumstantial evidence. It points out that evidence submitted by Dow to the EPA shows dioxin levels two to four times higher in Midland than in other locations checked. It also criticises Dow for using analytical techniques and procedures which have not yet been corroborated by other scientists. □

Dow admits 'poor' lab results

THE US Environmental Protection Agency (EPA) has not only been reviewing the safety of the herbicide 2,4,5-T; it is also engaged in a major collaborative programme with US scientists to compare laboratory measurements of 2,3,7,8-tetrachlorodibenzodioxin (dioxin) — a toxic contaminant present in the herbicide.

Central to the EPA's concern about dioxin is the poor reproducibility of dioxin measurements. In an attempt to find out which laboratories were reliable the EPA induced five labs to take part in a collaborative programme. The results of this collaboration were reviewed at a recent meeting in Washington organised by the agency.

The potential threat posed by 2,4,5-T is that its dioxin contaminant will enter the human food chain. US scientists claimed recently that dioxin had been detected in beef fat and human milk. The same two vehicles were therefore chosen by the EPA for its programme. Fat and milk samples were 'spiked' with dioxin in quantities ranging from 0–8 ppt (parts in 10^{12}) and extracts prepared suitable for analysis. The extracted samples, together with known standards—all prepared by the EPA Pesticide Monitoring Laboratory at Bay St Louis, Mississippi—were randomised and sent to the five participants: the Dow Chemical Company; Harvard University; the University of

Nebraska; Wright State University, Ohio; and the EPA Health Effects Research Laboratory (HERL) in North Carolina.

If proof were needed that there is inter-laboratory variation, then this study provided it. Only two laboratories performed well in the study—those of Dr Michael Gross of Nebraska and Dr Matthew Meselson of Harvard. The EPA's own laboratory results were regarded as reasonable by scientists present at the meeting but those of the other two were felt to be poor—Dow's being the less accurate.

The fact that Dow Chemical performed badly in this collaborative programme has not passed unnoticed by some scientists in the field. They argue that it should have been Dow, a company with a long-standing interest in dioxin measurements, which came out at the top of this league table. Dr Warren Crummett, of Dow, confessed that his results were "poor". "They were worse than normal", he told *Nature*. According to Crummett, some of the blame lies with the method of dioxin extraction chosen by the EPA. Had Dow done its own extracting procedure, then, Crummett was convinced, his laboratory's results would have been much better.

Of the five laboratories, only Gross's performed well in identifying 'false positives' and 'false negatives'. Discon-

the earlier reviews, but with the difference that all sides have been claiming sufficient evidence to support their case. Mr Gary Jones, a spokesman for Dow Chemical told *Nature* in an interview before the EPA ban was announced that the company's view was that "if, with all the confidence we have on the safety of 2,4,5-T, we can't say it is safe, then we can't prove the safety of aspirin".

The company felt that the attack on 2,4,5-T is part of a campaign by environmental groups whose campaign will not stop with the banning of the herbicide. Dow sees itself as defending an attack on the chemical industry by environmentalists who, Jones said, "want to go back to windmills". Jones added that Dow was defending 2,4,5-T not because the herbicide is a large revenue earner—sales worth \$12m represent only about 0.2% of the company's total annual income—but because it is "science which should be determining 2,4,5-T safety, not emotion".

The basis of Dow's argument is that dioxin at 0.1 ppm in 2,4,5-T presents no human health hazard. The company has been defending this

claim not only before the EPA but also in a New York federal court. Dow and five other US manufacturers of 2,4,5-T are being sued for \$10m by the estate of a 28-year-old Vietnam veteran, Paul Reutershan, who died in December last year of terminal cancer of the colon.

Reutershan, a helicopter crew chief in the Vietnam war, flew on several herbicide spraying missions during which Agent Orange was used. Ten years later when he was diagnosed as having cancer, Reutershan was convinced that dioxin was the cause. Following his death, a new suit was brought on his behalf as well as on that of "all American servicemen whose health has been damaged, or is likely to be damaged, because of contact with Agent Orange". The new suit has been filed by a veteran attorney of chemical contamination cases, Victor Yannacome Jr, and is likely to prove a test case.

There is little comfort for the litigants, however, from the Veterans Administration (VA). The VA has reviewed some 500 claims from ex-servicemen—mainly from the Chicago area—that exposure to 2,4,5-T has

damaged their health. But, a spokesman said, the administration has not been able to prove that "any of these claims are herbicide-related".

Environmental groups such as the Environmental Defense Fund insist that the very presence of dioxin renders the use of 2,4,5-T dangerous. For these groups the fact that dioxin has been shown in three separate studies to be a carcinogen, and that it is always present as a contaminant in 2,4,5-T—albeit at a low concentration—is sufficient to proscribe its use in any circumstances.

Balancing these conflicting arguments was apparently a difficult exercise for the EPA. Before the ban was announced, the Agency admitted that it was practically impossible to do a "precise reckoning" of the risks and benefits of the herbicide. One official of the Agency confessed that the "political climate is terrific on this issue" and that it did complicate matters. However, as he saw it, the EPA's role was to review the evidence and if the Agency made both the environmentalists and industry unhappy then "we are probably doing our job".

certainly the other four all failed to single out enough of these 'false' values. There was some agreement among those present at the Washington meeting that dioxin values close to the detection limit—regarded as being 9 ppt—did complicate matters and that perhaps some false positives and negatives were unavoidable.

In future, the researchers will probably abide by the gentleman's agreement practised so far that only values which are at least 2½ times greater than the noise levels will be regarded as true measurements. This decision could well eliminate some of the false predictions. Some scientists—notably those at Dow—feel that dioxin estimations lower than 9 ppt must, therefore, still be treated with scepticism. Others still disagree, and argue that it depends on the methods of estimation.

Contamination of the dioxin extracts by other chlorinated hydrocarbons—PCBs, for example—makes interpre-

tation more difficult. Dow argue that this could be one reason for their poor results and that their extraction would produce a "cleaner" sample. However, contamination is not the only problem. There is still no measure of agreement between the practitioners on the methods to be used to estimate the recovery of the final dioxin sample.

Dioxin chlorinated with ³⁷Cl is used in most cases for this purpose: the tracer is normally incubated with samples before extraction, but there are still doubts in the minds of some of the researchers that this tracer 'equilibrates' with the natural dioxin to a sufficient extent so that estimates of the final recovery are indeed accurate.

There are doubts too about mass spectrometry readings and views differ as to whether two dioxin ions with a selected mass from natural dioxin (320 m/e, 322 m/e) should be measured and the results averaged or whether one mass reading is sufficient. The

results in the EPA review are more or less independent of the method of measurement—good and poor results were recorded by both mass spectrometry methods.

Although some of these views have still to be resolved there was still general agreement at the meeting that the EPA should publish the results of this collaborative programme as soon as possible and that it should be subject to peer review. However, whether the reviewers agree with the consensus of opinion expressed at the meeting that dioxin analysis has become far better in the past few years remains to be seen.

What they will undoubtedly say is that there ought to be even more collaboration between laboratories to ensure greater reproducibility of results. As for the EPA, it will probably be given credit for initiating the first major stage in this collaboration.

Alastair Hay

Chemicals in food: new US framework proposed

A NEW framework for regulating toxic substances in food, which would give greater discriminatory power to the Food and Drug Administration, and allow the benefits as well as the risks of a particular substance to be taken into account, has been suggested by a study group of the National Academy of Sciences.

The proposal is made in a report published last Friday by the Academy's Institute of Medicine and National Research Council, prepared at the request of Congress following the controversy two years ago over whether or not saccharin is carcinogenic, and if so what should be done about it.

According to the panel of scientists, lawyers and public policy experts which prepared the report, the proposed framework—under which a substance could be placed in a category of high, moderate or low risk, with discrimination given to the FDA over handling substances in each category—would effectively separate the scientific assessment of risk from its social consequence.

In a letter submitting the report to Health Secretary Mr Joseph Califano, for example, Dr Philip Handler, president of the academy, says: "estimation of risk is a scientific matter, albeit not always readily feasible. Decision concerning the acceptability and management of a given risk is an intrinsically political question to be returned to the polity for determination."

Not all of the panel, however, agreed that the distinction is a tenable one. A minority report, disputing the suggestion that toxic food substances can

be divided into three categories according to varying degrees of risk, says that there is "no scientifically defensible way" of doing this.

The statement, signed by five of the 37 members of the committees responsible for the report, says that "the ability of science to quantify human risk has not advanced sufficiently since the formulation of the Delaney Amendment [which banned all food additives shown to cause cancer in laboratory animals] to permit the construction of a scientific rationale for such a scheme."

The report forms half of a two-part study carried out by the NAS under the terms of an act passed by Congress in 1977, which placed an 18-month moratorium on a ban on saccharin following the disclosure that it had been found carcinogenic in laboratory animals. The first part of the report was published last November, and concluded that saccharin was indeed a potential human carcinogen, although its potency was probably low compared to other known cancer-causing agents, such as cigarettes.

The second report is concerned with the public policy implications of this and similar findings for the regulation of food safety. It recommends a single policy applicable to all foodstuffs, additives and contaminants, and says that regulatory agencies should be able to do more than simply ban or not ban a particular substance.

"This report suggests that a realistic policy would be to weigh the estimated level of risk of a substance in our food supply against the perceived benefits of its use, and to employ informed

judgment as a basis for regulatory decisions," according to Dr David A. Hamburg, president of the Institute of Medicine.

A major problem presented by current law, the report says, is that most cases of both health and non-health benefits are excluded from regulatory consideration. "It is better to have benefits defined, evaluated and openly considered when that is possible", it says.

Speaking in Washington last week Professor Don K. Price, one of the panel members responsible for the report, denied that by shifting the focus of decision-making from Congress to a regulatory agency, the process would become more vulnerable to outside pressures. "The committee felt that the present scheme, which in theory asks the FDA to make a purely scientific decision, has not worked very well, and in many cases political and other considerations have been smuggled in—or, as in the saccharin case, have given rise to other problems", he said.

"This new system would require regulatory agencies to get a statement on the scientific aspects from a research institution, and the subsequent decision-making process can then be made clear, public and open, so that you can deal with it better than if you ignored outside pressure".

The committee made no particular recommendation for the marketing of saccharin, and the minority statement disagreed with the view of the majority that "a total immediate ban of saccharin would not be a sound regulatory step at the present time."

David Dickson

2,000 US scientists boycott USSR

IN what one participant called a "grass-roots" protest at the treatment of dissidents by the Soviet authorities, over 1,800 US scientists have signed a pledge to "withhold all personal co-operation with the Soviet Union" until Yuri Orlov and Anatoly Shcharansky, the two USSR scientists gaoled last summer, are released.

A further 600 US scientists have signed a separate pledge that they will not attend international conferences in the Soviet Union, will collaborate with and attend lectures by Soviet visitors only if they are invited, will oppose any enlargement of scientific and technical exchange programmes between the USSR and the US, and will "campaign against the transfer of sophisticated Western technology to the Soviet Union".

Signatures on the two petitions have been gathered by the Berkeley-based organisation Scientists for Orlov and Shcharansky, set up last summer when the trials of the two dissidents was taking place. SOS, as the organisation is known, says that its policy is to take concrete actions that deprive the USSR of some of the benefits of American science and technology, particularly in areas such as computing where the US has a considerable lead.

"The actions taken by SOS are a completely spontaneous response to Soviet policy, and are not connected to any position or policy of the US Government," Professor Kurt Gottfried, professor of physics at Cornell University, said last week.

"Our opposition to Soviet oppression is a limited response to Soviet actions, not a search for confrontation. Despite our commitment to human rights we view any linkage between human rights and the effort to achieve arms control as irresponsible," Professor Gottfried said, adding that SOS fully supported President Carter's efforts to negotiate a second Strategic Arms Limitation Treaty.

Gottfried was echoing Sakharov, who recently declared that human rights should not be made a *sine qua non* of the signing of the SALT agreement. While supporting Sakharov's opinion, John Macdonald, Q.C., Yuri Orlov's London-based "defence lawyer" nevertheless feels that the signing of SALT could lead to the release of political prisoners such as Orlov and Shcharanskii. It could well provide an opportunity for President Carter to press home the human rights issue—and for Mr Brezhnev to make a gesture which the Soviet Union could afford.

David Dickson and Vera Rich

Yugoslavia hits nuclear snag

PROPOSALS to build Yugoslavia's second nuclear power station on the island of Vir (see map) are meeting with opposition from the island's inhabitants, as well as the municipal authorities and 'socio-political organisations' of the nearby resort town of Zadar on the central Adriatic coast.

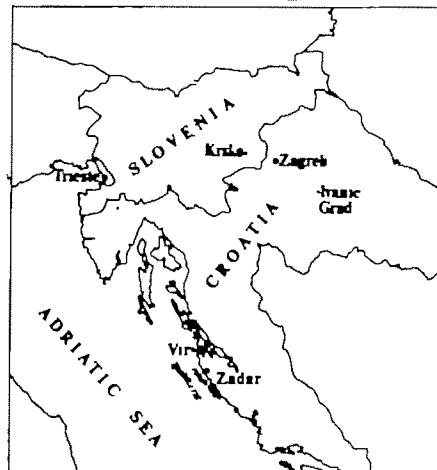
The local inhabitants and authorities seem more interested in preserving their amenities as a tourist area than in the alleged advantages which the plant would bring to the area—which is notably short in energy.

The vigorous opposition to the nuclear power station comes only a few weeks after a series of major power cuts so severe that even long-standing atheists were observed buying votive candles from church-porch stalls. Yugoslavia's major power source is hydroelectricity, and the dry autumn and early winter led in December to cuts of up to eight hours a day.

It was just such a drought, six years ago, that led to a big drive for alternative sources of energy. Five foreign enterprises competed for the contract for the first Yugoslav power station. Westinghouse won, and work began at Krsko, near the Slovenia-Croatia border.

The target date for completion was January 1979, but construction was consistently behind schedule.

The delays, however, did not relegate



nuclear power in the minds of the Yugoslav planners. By April 1978, the power industry was talking in terms of 12 nuclear power stations with a total annual capacity of 66,000,000 MWh by the end of the century.

So far, however, the Krsko station is still not operational and the siting of the second is in doubt. The final decision on the Vir site has not yet been reached—but local opposition suggests that a better choice might be Ivanicgrad, which not only has the necessary water resources, but is also a major industrial centre, where the local population may be less likely to raise environmental issues.

Vera Rich

UK must support talent, says minister

MRS SHIRLEY WILLIAMS, UK secretary of State for Education and Science, is concerned that not enough young scientists are finding jobs—because most available posts are filled by people in their thirties and forties who are unlikely to move on. "If science in Britain isn't to sink gently into the second class", she told the Association of British Science Writers earlier this week, "then we will have to try to make room for each generation of outstanding talent."

Making more money available to the research councils should help solve the problem, she said, and that was one of the reasons behind the 4% increase in the science budget announced at the end of last year. Another reason for increasing the science vote was to take the pressure off the SRC, whose budget had been "blocked up by existing commitments" and to encourage the funding of interesting or important work by the other research councils.

A white paper is to be published this week, said Mrs. Williams, about the 'customer-contractor' principle whereby a portion of research council funds is administered by ministries, which

commission research relevant to their needs. For the system to work, says Mrs Williams, "commissioned research has to be handled on a basis of stability and on a relatively long lead time". Problems occur if the level of research commissioned by a ministry is suddenly changed.

There is also a limit to that level and, according to Mrs Williams, the Agricultural Research Council's budget has already reached it. The increase in the science vote, however, should relieve some of the research council's dependence on commissioned research.

Mrs Williams is also concerned that the UK does not make the most of its innovative ideas. "It is sad", she says, "that one so often sees the brilliant idea worked on in a university laboratory being patented in another country and then sold back to us." She would like to see small companies encouraged to take up new ideas and the establishment of a "small enterprise board" which would assume for enterprise a similar role to that taken by the National Research Development Corporation for technology.

Judy Redfearn

news in brief

Reductions urged in medical radiation exposures: The elimination of all unnecessary medical and dental exposure to radiation was "critically important" to protect the health of the public, US Secretary for Health, Education and Welfare, Mr Joseph A. Califano, said last week.

Exposures resulting from X-rays, radiation therapy and nuclear medicine accounted for almost 90% of all the man-made radiation to which the general population was exposed, Mr Califano said, adding that he had ordered efforts by the DHEW through the Food and Drug Administration to reduce such exposures to be speeded up.

Commenting on Mr Califano's order, Dr John C. Villforth, director of the US Government's Bureau of Radiological Health, said that medical radiation should be cut to half of present levels.

"Approximately 30% of all medical exposures are unnecessary," said Dr Villforth, adding that many were done for non-medical reasons, such as protecting doctors from malpractice suits. The radiation dosage used in "necessary" medical procedures could be cut by another 30%, using the most recent X-ray films and machines.

However Mr Califano said that the US government was not yet prepared to recommend a reduction in permissible occupational exposure levels which has recently been suggested by a number of US scientists following growing evidence of the potential dangers of low levels of radiation previously treated as safe.

"We do not believe that the science is adequate to justify further reductions" he said, although stressing that more scientific research was needed to settle the issue, and that the problem was a "major public health issue."

Mr Califano released draft working papers which have been prepared by an Interagency Task Force on the Health Effects of Ionising Radiation, set up by President Carter last year. Reviewing past and current research, the task force states that "existing knowledge is insufficient to provide an unequivocal answer to the low-dose question", and adds that a definitive answer may never be found.



"I see Peterson's having his influence!"

Head of OTA resigns to head wildlife society: Mr Russell Peterson, former governor of Delaware, has resigned as director of the US Congress' Office of Technology Assessment to become president of the National Audubon Society. Mr Peterson's departure after only 13 months of a six-year appointment is a major blow to an agency which has been plagued with political problems. Under his leadership it had appeared to be entering a period of relative stability and productive activity.

Nuclear safety issue heats up: For ten years Dr Stephen Hanauer, assistant director and senior technical safety official of the US Nuclear Regulatory Commission has kept a 'nugget' file on reactor safety incidents. The Union of Concerned Scientists obtained a copy of the file under the Freedom of Information Act and has published a 95-page summary. Among its contents are tales of safety parts installed upside down, thickly painted and varnished rods or pumps sticking, frozen pipes, clogged drains, faulty

soldering, radioactive cleaning dust, accidentally bumped switches causing overflows and a \$45m fire that firefighters were afraid to put out because of the danger of spreading plutonium throughout the area. The US Atomic Energy Commission commented that "the absence of more serious effects is largely the result of luck".

In the UK, Friends of the Earth is pressing the Secretary of State for Energy to fulfill his promise to release the complete report of the Health and Safety Executive on the safety of pressurised water reactors. Only a summary was released on 29 July 1977. FOE cites the scandal surrounding the Rasmussen Report in the US which was issued in summary form and has been subsequently found so unreliable that the Nuclear Regulatory Commission dissociated itself from it. The Rasmussen summary included predictions that an accident at the Enrico Fermi reactor on 6 October, 1966, when part of a core fused, should have happened only once in a million years, and the same techniques predicted that the accident on 5 June 1970 when the 600 megawatt Dresden 2 reactor near Chicago went out of control and discharged radioactive iodine into the environment should have occurred only once in 10^{14} years. FOE stresses that "there is a clear cut need for public access to the Health and Safety Executive's detailed reactor studies".

INFCE halfway through: The International Nuclear Fuel Cycle Evaluation Programme established at the Western nations summit meeting in London in 1977 to study the hazard of nuclear proliferation is now halfway through its life with a report to be issued "sometime in 1980". INFCE began with some acrimony between the US and the UK over the danger of proliferation from nuclear reprocessing. The UK government is committed to reprocessing while American opinion is opposed to it. This difference in outlook colours the discussions of the twelve working parties. The US tries to win support for an extensive system of intermediate spent fuel storage deposits while the UK argues that such a system will be unnecessary if there is extensive reprocessing. Other preliminary studies indicate that there is no preferred "proliferation resistant" design: all proposed nuclear fuel cycles are equally hazardous. Factors included in the analyses are: extent of reprocessing, number of plutonium storage sites, and physical organisation of the fuel cycle (where fuel is to be stored in relation to the reactor), how buildings are to be laid out and the number of people to be involved. These "technical" issues are proving to be relatively unimportant compared to the political problem of proliferation.

Wave power cost estimates cut: Wave power designers at Wavepower Ltd in Southampton have found improvements in the design and manufacture of the Cockerell raft (30 November 1978, page 433) that may reduce projected costs from 26p per kwh to 6p per kwh. The original Cockerell raft was built in three sections with two hinges. The centre section contained a costly and complex pumping system and gearing train needed to drive the turbine. A new single hinge design eliminates the centre section and replaces the pumping system with a simple large-vaned pump that will be part of the raft structure. A more sophisticated but standard turbine will be used to effect power take-off. "We have thrown away a lot of steel and mechanical engineering" says Jim Platts, Senior Project Engineer at Wavepower. The new design will cut costs from 26p to 12p per kwh and a further reduction to 6p can be achieved by mass production methods based on techniques used in oil pipeline manufacture. Additional savings must be found, however,

Let us stop regulating DNA research

By J. D. Watson

The Royal Society critique (*Nature*, 15 February 1979, page 510) of the proposed new approach by Britain's Genetic Manipulation Advisory Group (*Nature*, 9 November 1978, page 102) to recombinant DNA regulation is correct on at least one point. We do not have the facts on hand to make the quantitative risk assessments to decide whether a proposed experiment should be done under maximal, minimal, or no precautions.

Assigning numbers in the manner suggested by Dr Sydney Brenner implies that we have some real measure of prospective risk. However, to my knowledge there is no evidence that any prospective recombinant DNA experiment poses any realistic threat to any scientist who uses the tools of his trade, much less to society itself. I thus do not see any logical way to arrive at such numerical risks, and the whole GMAG exercise strikes me as having no more validity than quantitative religion.

To be sure some experiments like putting *E. coli* genes back into *E. coli* are thought safe by virtually all informed individuals. In contrast, the insertion of the gene for the botulinus toxin into *E. coli* might strike some competent scientists as risky. But how much additional peril will be generated beyond that involved with working with botulinus organism itself is not obvious.

The *a priori* guesses that different knowledgeable minds will give may at best tell us something about their respective neuroses or lack of them. Each of us has different thresholds of fear, be it rational or irrational. For my part, I already see so many unambiguous hazards, like dioxin, that I remain incapable of adding still one more unless I see some real numbers. Recombinant-DNA-induced diseases to me fall in the category of UFOs or witches. Others may take

them seriously, but they should not expect me to join in. I would not spend a penny trying to see if they exist and would regard as pure folly the decision to spend sizable sums of money on such projects.

I am thus appalled by the recent proposals of the Honourable Joseph Califano and Shirley Williams that their respective governmental departments (HEW and the Department of Education and Science) initiate extensive experiments to probe the presumptive safety of recombinant DNA experiments. The variety of prospective experiments is so legion that we have no possibility of checking even a tiny fraction for their potential danger.

'Recombinant-DNA-induced diseases, to me, fall in the category of UFOs or witches'

Moreover, the answers we could obtain would have no real carry-over to other even closely related situations. So we would be spending vast sums of money (Shirley Williams has proposed tens of million pounds) merely to give the facade of responsibility. In fact, we would be irresponsible by diverting money away from projects which unambiguously might advance science, if not society itself.

We must thus be careful not to undertake risk assessment experiments merely because they can be done. Here I part company with the Royal Society's position that we should see, for example, whether non-pathogenic strains of *E. coli* can be converted into pathogens through random insertion of foreign DNA. Such data are very unlikely to affect the way we work with recombinant DNA. Unless we directly take DNA

from pathogenic bacteria, the chances of a positive answer are low. But even if we were to generate a new pathogen, it is not obvious that it would pose any special risk.

Almost totally lacking in the past six years of seemingly never-ending conjectures about the dangers from recombinant DNA experimentation is recognition of the fact that the world is already filled with large varieties of pathogenic microorganisms of many types. Moreover, they are constantly mutating to give us new forms that we have not seen before. Some are constantly making us ill, and sometimes even doing us in. For the most part, however, we can fight them at least to a draw, and, of necessity, most of us do not spend much time worrying whether the next time they will get us. Otherwise we would be completely paralysed from any constructive behaviour.

Instead of continuing to waste masses of paper and the time of countless individuals who have real jobs to carry out, I believe we should quickly and resolutely abandon any form of recombinant DNA regulation. Concurrently our national leaders should announce that they will help push DNA research as fast as our national and corporate treasuries can permit.

At the same time we should, of course, remain alert to the rare possibility that one or more recombinant DNA research workers will come down with a disease we have not seen before. If that happens we naturally would have a story that deserves serious journalism, and for the first time there would be real facts for the risk assessor. But until that situation arises, and I'm doubtful that it ever will, we must make clear to the public that there is no more reason to fear recombinant DNA than there is to panic about the Loch Ness Monster. □

if wave power is to be competitive with the estimated 1p-2p per kwh cost of windpower and with the standard fossil fuel costs of 1½p per kwh.

American Institute of Physics wins tax exemption: The American Institute of Physics, the umbrella organisation for nine separate physics societies, has won its appeal against a tax ruling that would have deprived it of tax exempt status.

Many of the institute's activities, including the publication of a number of journals, had been placed in jeopardy by a decision taken last year by the Manhattan District Office of the Internal Revenue Service that it was not entitled to

tax exemption as a scientific, educational and charitable organisation.

The institute filed a protest in December against this ruling, which is thought to have been made on the grounds that the institution provides a specialist service to its member societies which is not available to the general public.

In a letter received by the institute last week, the national office of the IRS said that it had considered the institute's appeal, and had decided in its favour. "The entire physics community will be pleased with the Internal Revenue Service ruling", said Professor Philip Morse, chairman of the AIP's governing board.

'Fortunately for us, plants are very adaptable and exist in great diversity . . . they could thus continue indefinitely to supply us with renewable quantities of food, fibre, fuel and chemicals'

Professor D. O. Hall of King's College, London University, on biological solar energy conversion for fuels

BIOLOGICAL solar energy conversion via the process of photosynthesis produces every year stored energy in the form of biomass which is about ten times the world's annual use of energy. The amount of proven fossil fuel reserves below the earth is only equal to the present standing biomass (mostly trees) on the earth's surface while the total fossil fuel resources are probably only ten times this amount (Table 1). This massive-scale capture of solar energy and conversion into a stored product occurs with only a low overall efficiency of about 0.1% on a world-wide basis but because of the adaptability of plants it takes place and can be used over most of the earth.

It is not widely appreciated that one-sixth of the world's annual fuel supplies are wood fuel and that about half of all the trees cut down are used for cooking and heating. In the non-OPEC developing countries, which contain over 40% of the world's population, non-commercial fuel often comprises up to 90% of their total energy use. This non-commercial fuel includes wood, dung and agricultural waste and because of its nature is seldom thoroughly considered. Total wood fuel consumption is probably three times that usually shown in statistics, and about half of the world's population relies mainly on wood for their cooking (four-fifths of total household energy use) and heating. Furthermore, supply statistics of non-commercial energy can be out by factors of 10 or even 100.

The so-called 'second energy crisis' has arisen as a result of the diminishing supplies of non-commercial or traditional fuels such as wood, dung and straw. This has led to increasing deforestation, damage to ecosystems and rural poverty. Fuels-from-biomass programmes are now being considered in many developed and developing countries of the world but the biggest difficulty with implementing them seems to be the simplicity of the idea—the solution is too simple for such a complex problem!

In the past, photosynthesis has given us coal, oil and gas, fuelwood, food,

fibre and chemicals. The relative use of these fixed carbon sources has varied over the years and will undoubtedly do so in the future. However, now with abundant oil, the products of present-day photosynthesis are mainly evident to the developed world as food. We should re-examine and if possible, re-employ the previous systems; but, with today's increased population and standard of living, we cannot revert to old technology and must instead develop new means of using present-day photosynthetic systems more efficiently.

Each year plant photosynthesis fixes about 2×10^{11} tonnes of carbon with an energy content of 3×10^{11} J; this is about 10 times the world's annual energy use and 200 times our food-energy consumption. The efficiency on land may be about 0.2–0.3% overall, whereas average agriculture may be about 0.5% efficient. These efficiencies represent stored energy and not just the initial conversion efficiencies so often quoted in other energy systems.

All the atmospheric CO_2 is cycled through plants every 300 years, all the O_2 every 2,000 years and all the H_2O every 2 million years. The magnitude and role of photosynthesis is largely unrecognised principally because we use such a small fraction of the fixed carbon and don't realise the important recycling phenomena—any interference in this latter role from pollution could have serious consequences. The increasing content of CO_2 in the atmosphere (a 25% increase over the past 125 years) has become a worrying problem. However, there is no doubt that renewable biological (and photochemical) systems for fuel production will not contribute to the atmospheric CO_2 concentration and biomass may just help alleviate some of the problems by acting as a temporary carbon sink.

Fortunately for us, plants are very adaptable and exist in great diversity—they could thus continue indefinitely to supply us with renewable quantities of food, fibre, fuel and chemicals. If the impending liquid fuel problem which is predicted within the next 10 to 15 years comes about, we may turn to



plant products sooner than we expect. Let us be prepared!

Solar/biological systems could be realised to varying degrees over the short and long term. Some, such as the use of wood, biological and agricultural wastes, and energy farming, could be put into practice immediately, whereas others may never become practicable. Photobiological systems can be tailored to suit an individual country taking into consideration total available energy, local food and fibre production, ecological aspects, climate and land use. In all cases the total energy input (other than sunlight) into any biological system should be compared with the energy output and also with the energy consumed in the construction and operation of any other competing energy producing system.

Biomass

Solar energy is a very attractive source of energy for the future but it does have disadvantages. It is diffuse and intermittent on a daily and seasonal basis, thus collection and storage costs can be high. However plants are designed to capture diffuse radiation and store it for future use. Thus there is much serious thought (and money) being given to ideas of using biomass as a source of energy—especially for liquid fuels, but also for power generation. I am aware of biomass programmes in the UK, Ireland, France, Germany, Denmark, Sweden, USA, Canada, Mexico, Brazil, Australia, New Zealand, India, Philippines, Thailand, Israel and China.

The advantages and easily identified

problems with biomass systems are summarised in Table 2; the long term advantages are however very considerable. The programmes vary in their emphasis, and show that most of the research and development should be done locally. Such R & D is an ideal opportunity to encourage local scientists, engineers and administrators in one field of energy supply. Even if biomass systems do not become significant suppliers of energy in a specific country in the future, the spin off in terms of benefits to agriculture, forestry, land use patterns and bioconversion technology are, I think, significant.

In Table 3 I have attempted to summarise biomass products and their costs to give an idea how close some technologies are to economic reality. One must be fully aware of the assumptions involved before extrapolating or drawing firm conclusions from these figures.

USA

The USA has a very large R & D programme on biomass which had a 1977/1978 budget of \$22m and a proposed budget of \$42m for 1979. Burwell in an article called "Solar biomass energy: an overview of US potential" gives a good general picture and presents some of his own ideas of the best systems for the future. The 1975 US use of energy was 71×10^{14} J; the total standing forest inventory however has an energy content three times this annual energy use. The total annual biomass growth of commercial forests is 9.3×10^{14} J of which 6.6×10^{14} J is potentially collectible. Cropland agriculture produces energy totalling about 12×10^{14} J annually of which about 40% is represented by residues left on the land. Grain crops alone produce about 7.1×10^{14} J annually of which 5.9×10^{14} J is the net collectible energy yield, 3.8×10^{14} J of this being in the form of residues. A detailed analysis of "potentially usable biomass residues" shows that of the residues currently collected, 2.1×10^{14} J could be obtained from urban solid wastes, 1.0×10^{14} J from animal feedlots, canneries, wood manufacture, and so on. Uncollected residues such as cereal straw, corn stalks and logging residues could contribute 5×10^{14} J per annum. Burwell considers that major opportunities for energy provision lie in the use of forest residues and improved management of productive forest land. He also makes the interesting point that 60% of US cropland is dedicated to the production of livestock—and this excludes the contribution that 282 million hectares (38% of mainland US area) of pasture and rangeland make to livestock support. There are thus large areas of land which could be used in the future

for the production of biomass.

The concept of intensive silviculture biomass farms or energy farms has been one subject of detailed analysis. Fast-growing deciduous trees which coppice (resprout from stumps) have been examined, as have numerous other trees including those which fix nitrogen to ammonia. The use of agricultural residues as a source of energy in the US has been the subject of another major evaluation. A national total of 430 million dry tons is available each year, made up of 280m tons of crop residues, 120m tons of residues from logging and 30m tons from animal manure. (Excluded from these figures were residues from food processing, grazing animals, and hay and forage crops.) Theoretically this residue could supply 31% of the electricity, 20% of the natural gas, or 8% of the oil requirements of the US. This is very unlikely ever to happen since at

'If the impending liquid fuel problem comes about, we may turn to plant products sooner than we expect . . . Let us be prepared'

present 20% of the residues are used and 58% are returned to the soil, leaving only 22% classified as excess. However, these percentages will change as other uses (energy, chemicals, and so on) and prices emerge. At present electricity production from residues is cost-competitive at five study areas, process steam at seven areas and synthetic natural gas (compared to propane costs) at two areas.

Another US study says that adaptive systems should be encouraged which will "modify themselves to meet evolving needs and constraints". Such adaptive systems contrast with energy farms and the use of agriculture residues—but of course, the three systems can blend into each other depending on circumstances. Thus biomass intermediates (besides food) are processed into fuels or materials depending on relative price levels. Two examples cited are corn and sugarcane production. It is "theoretically possible to reorganise the present US corn biomass system to permit the production of 10 to 18 $\times 10^9$ l of ethanol (or its equivalent in other fermentation products) while obtaining the same quantity of end-use food products in the form of beef, poultry and pork". The ethanol could be blended at 10% with a $\frac{1}{4}$ of the US gasoline. Sugar cane produces numerous byproducts such as molasses, alcohol and bagasse which can be used

for power generation, fermentable substrates and substitute wood. The study concludes that "fuels from biomass may possibly supply 10% or more of a curtailed US energy consumption" and that "knowing when and where to switch from emphasis on food and materials to emphasis on fuels is just as important as knowing how to produce the fuels."

Canada

Canadian studies on the large scale production of methanol from biomass show that by 2025 between 4 and 42% (depending on total energy use) of the transport fuels could be provided by such methanol. Methanol represents a rather unique fuel combining the portability of liquid petroleum products and the clean, even-burning characteristics of natural gas. The Federal Government recently announced a \$180m programme on biomass R & D and also provided incentives to industry to use wood and forest residues to provide energy.

Australia

In Australia, *Eucalyptus*, cassava (*Manihot*), *Hibiscus*, napier grass (*Pennisetum*) and sugarcane have been selected as potentially the most desirable high-yielding crops which can be harvested over the whole year. It was shown that alcohol produced from cassava (starch-rich) is an economically viable system, but that if processing to destroy cell walls is required the costs become too high. The cost of alcohol from cassava is calculated to be Australian \$250/tonne from a 100,000 tonne/year batch-process plant which compares favourably with the current market price of alcohol (Australian \$275/tonne) as an industrial solvent. Recent studies show that over half Australia's liquid fuel requirements could be produced from specifically-grown crops and agricultural residues.

New Zealand

With its very efficient agriculture, low population density and the fact that it uses a large proportion of its foreign exchange for petroleum purchases it is natural that New Zealand is considering biomass as a source of fuels. Current work shows that fodderbeet for ethanol and wood for methanol may be economic sources of liquid fuels.

Philippines

A feasibility study has shown that a 9,100 hectare fuel wood plantation "would supply the needs of a 75 MW steam power station if it were not more than 50 km distant". The investment requirements and cost of power produced looks favourable and competitive with oil-fired power stations of similar capacity. The best species of fast growing tree seems to be the "giant ipil-ipil" (*Leucaena leucocephala*) which fixes



Fodder beet in New Zealand: ideal for fermentation to alcohol.

nitrogen to ammonia.

Europe

In Europe a number of countries are conducting feasibility studies of the potential which biomass may have for supplying a source of energy and fuels in the future. Trial plantings of alder, willows, poplars, etc. are being undertaken in addition to assessing energy yields from agricultural residues, urban wastes, techniques of conversion, waste land and forest potentials and algal systems.

Little has been published as yet but a recent study (Project Alter) in France by "Le Groupe de Bellevue" proposes that in the long term France could produce liquid and solid fuels, comprising 11% and 14% of its total energy requirement respectively, from biomass sources. Land use constraints will be a problem but considering Europe as a whole, its past vegetation history, with its diverse climates and land use patterns, and its already burgeoning food (including liquid) surpluses there may be far greater potential for biomass production than is commonly imagined.

Brazil

By far the most ambitious biomass programme which has been planned is that in Brazil for the production of alcohol from sugar cane, sorghum, cassava and other crops. This National Alcohol Programme (PNA or Proalcool) was established in November 1975. The alcohol will be used to blend with petrol—a mixture of up to 20% (by volume) requires no adjustment to the engine (over the past 10 years the State of Sao Paulo with over 1.3 million cars has varied the alcohol content of its petrol from 0.4 to 13.5%—and 18% in 1978—depending on the availability of alcohol and price of molasses). Up to August 1977, 141 new alcohol distilleries were authorised by Proalcool which would require an investment of about \$900m and would supply 3.2×10^9 litres of alcohol by 1980, about a fifth of the projected gasoline requirement. By 1985 total production of alcohol could reach 5 to 10×10^9 litres with a total investment of about \$3.15 billion. An economic

analysis of the production of alcohol from sugar cane and cassava calculated selling prices as fuel, ex-distillery, of \$0.33/1, 81% of the present retail price of gasoline on a volume basis. Brazil is clearly embarking on an ambitious programme of fuel import substitution using the natural advantages of land and climate which it has—and it may be a very useful demonstration to other countries. The indirect benefits such as saving foreign exchange, creating new employment, encouraging domestic technology and industry, and reducing pollution, are great.

Sahel region

The problem of deforestation and desertification has highlighted the lack of fuelwood in most countries of this region. It is a scarcity which is reaching crisis proportions in large parts of South Asia, the Sahel, Andean countries, Central America and the Caribbean.

The per capita requirement for cooking alone is about 0.5 m³ of wood/year. Total fuelwood requirement is probably about 1 ton which is equivalent to about 400 kg coal per person per year—equivalent of a 3 inch lump of coal per person per day. Demand from urban areas and industry to use char-

coal instead of the more expensive kerosene and oil is diverting woodfuel from rural areas, resulting in increasing forest and scrub destruction (often long distances from villages) and use of dung as a fuel instead of as a fertiliser. A recent Dutch study of the Sahel region points out two possible solutions: decreasing fuelwood demand by using stoves which reduce consumption by 70%, and increasing the supply of fuelwood by establishing "forest plantations". Costs of reforestation and fuelwood production have been calculated for the tree species suggested and the conclusion is that "under the conditions assumed it is an economically feasible activity". Naturally there are institutional problems which impinge on agriculture and other practices of the society but if such countries are to achieve even a modicum of internal fuel production they should seriously consider such biomass systems and set up fuelwood plantations as soon as possible.

India

A study in Tamil Nadu, South India, on the possibility of growing Casuarina (a nitrogen fixing tree) as a source of fuel for a power plant generating 100 MW electric has shown favourable

Table 1 Fossil fuel reserves and resources, biomass production and CO₂ balances

Proven reserves						Tonnes coal equivalent	
Coal	..	..	..	..	..	5×10^{11}	
Oil	..	..	..	..	..	2×10^{11}	
Gas	..	..	..	..	..	1×10^{11}	
						$8 \times 10^{11} \text{t}$	$= 25 \times 10^{21} \text{J}$
Estimated resources						Tonnes coal equivalent	
Coal	..	..	..	..	..	85×10^{11}	
Oil	..	..	..	..	..	5×10^{11}	
Gas	..	..	..	..	..	3×10^{11}	
Unconventional gas and oil	..	..	..	..	..	20×10^{11}	
						$113 \times 10^{11} \text{t}$	$= 300 \times 10^{21} \text{J}$
Fossil fuels used so far (1976)	..	..	..	..	..	$2 \times 10^{11} \text{t carbon}$	$= 6 \times 10^{21} \text{J}$
World's annual energy use	..	..	..	..	..		$3 \times 10^{20} \text{J}$
							$(5 \times 10^9 \text{t carbon from fossil fuels})$
Annual photosynthesis							
(a) net primary production	..	..	..	..	..	$8 \times 10^{10} \text{t carbon}$ $(2 \times 10^{11} \text{t organic matter})$	$= 3 \times 10^{21} \text{J}$
(b) cultivated land only	..	..	..	..	..	$0.4 \times 10^{10} \text{t carbon}$	
Stored in biomass							
(a) total (90% in trees)	..	..	..	..	..	$8 \times 10^{11} \text{t carbon}$	$= 20 \times 10^{21} \text{J}$
(b) cultivated land only (standing mass)	..	..	..	..	..	$0.06 \times 10^{11} \text{t carbon}$	
Atmospheric CO ₂	..	..	..	..	..	$7 \times 10^{11} \text{t carbon}$	
CO ₂ in ocean surface layers	..	..	..	..	..	$6 \times 10^{11} \text{t carbon}$	
Soil organic matter	..	..	..	..	..	$10-30 \times 10^{11} \text{t carbon}$	
Ocean organic matter	..	..	..	..	..	$17 \times 10^{11} \text{t carbon}$	

These data, although imprecise, show that (a) the world's annual use of energy is only 1/10 the annual photosynthetic energy storage, (b) stored biomass on the Earth's surface at present is equivalent to the proven fossil fuel reserves, (c) the total energy stored as fossil carbon only represents about 100 years of net photosynthesis, and (d) the amount of carbon stored in biomass is approximately the same as the atmospheric carbon (CO₂) and the carbon as CO₂ in the ocean surface layers.

economic and social benefits. In direct competition with coal a payback period for such an energy plantation would be from 15 to 30 years. An equivalent of 110 hectares is required to generate 1 MW electricity; about 11,000 labourers would be required, and the overall energy output/input ratios are high.

China

During the 1970s biogas plants have been perfected and installed at a rapid rate. It is estimated that there are now (1977) 4.7 million in operation and the methane so produced is used for cooking, crop drying, power generation and various other purposes. In Szechwan province alone, 17 million people use biogas for cooking and lighting; in some areas 80% of all rural households are served by biogas.

Algal systems

The idea of using algae and bacteria in biological solar energy systems is not new but has received more serious attention over the past few years. One advantage of such microbial systems is that they can be technologically sophisticated or simple depending on local conditions.

Many liquid and semi-solid wastes from houses, industries and farms are ideal for the growth of photosynthetic algae and bacteria, and under good conditions rapid growth with about 3–5% solar conversion efficiency can be obtained. The harvested algae may be fed directly to animals, fermented to produce methane, or burnt to produce electricity. Simultaneously, waste can be disposed of and water purified; it is estimated that such algal systems are half to three-quarters as expensive as conventional waste disposal systems in California. The harvested biomass can be fermented to methane, while the residues would contain virtually all the nitrogen and phosphorus of the algal biomass, so providing a good agricultural fertiliser. One acre of algal



The Sahel: scarcity of firewood has reached crisis proportions.

ponds could supply the fertiliser required by 10 to 50 acres of agriculture.

Future photosynthesis

One of the 'problems' with photosynthesis is that it requires a whole plant (or alga) to function—and the problem with whole plant photosynthesis is that its efficiency is usually low (less than 1%) since many limiting factors of the environment and the plant itself interact to determine the final overall efficiency. Thus a task for photosynthesis of the far future is to try to select and/or manipulate plants which will give higher yields with acceptable energy output/input ratios. We need much more effort placed on studies of whole plant physiology and biochemistry and their interactions with external (environmental) factors. Already this type of research is being increasingly funded by both industrial and government organisations.

Examples of the type of research which is being done or needs to be done are: photosynthetic mechanisms of carbon fixation; factors in bioproductivity; genetic engineering using plant cell tissue cultures; plant selection and breeding to overcome stresses (drought, temperature or salinity); selection of plants and algae with useful products such as oil, glycerol, waxes, or pigments; nitrogen fixation and metabolism and its regulation by

photosynthesis.

Synthetic photosynthetic systems and photochemistry

Since whole plant photosynthesis operates under the burden of so many limiting factors (internal and external) would it be possible to construct artificial systems which mimic certain parts of the photosynthetic process and so produce useful products at higher efficiencies of solar energy conversion? (A 13% maximum efficiency of solar energy conversion is considered a practical limit to produce a storable product). I think that this is definitely feasible from a technical point of view but it will take some time to discover whether it could ever be economic. Plants in fact perform at least two unique reactions upon which all life depends and which have not yet been emulated in artificial (synthetic) systems: the splitting of water by visible light to produce oxygen and protons (hydrogen) and the fixation of CO₂ into organic compounds.

Photosynthesis is the key process in the living world and will continue to be so for the continuation of life as we know it. The development of photo-biological energy conversion systems has long-term implications. We might well have an alternative way of providing ourselves with food, fuel fibre and chemicals in the next century.

Suggested time-table

for recommendations

● *Next 10 years*—fuels from residues, trees and existing crops; more efficient use of existing biofuels; demonstrations and training.

● *10–20 years*—increased residue and complete crop utilization, local energy crops and plantations in use.

● *After 20 years*—energy farming; improved plant species; artificial photo-biology and photochemistry. □

Table 2 Biomass systems

Advantages	Problems
1. stores energy for use at will	1. land use competition
2. renewable	2. land areas required
3. dependent on technology already available, with minimum capital input	3. supply uncertainty in initial phases
4. can be developed with present manpower and material resources	4. fertilizer, soil and water requirements
5. reasonably priced	
6. ecologically inoffensive, and safe	
7. does not increase atmospheric CO ₂	

Table 3 Predicted biomass energy products costs compared to conventional—USA (1981 \$)

Product	Cost from biomass (\$/10 ⁶ BTU)	Conventional cost (\$/10 ⁶ BTU)	Biomass Conventional
Methanol	8.4–15.9	8.4	1.0–1.9
Ethanol	15.0–36.3	19.6	0.8–1.9
Medium BTU gas ..	4.7– 7.4	3.0–5.0	0.9–2.5
Substitute natural gas	4.8– 7.3	3.0–5.0	1.0–2.4
Ammonia	5.8–11.4	7.4	0.8–1.5
Fuel oil	3.6– 7.9	3.2	1.1–2.5
Electricity	0.03–0.14(\$/KWH)	0.03–0.06(\$/KWH)	0.5–4.5

This article formed part of a study done by the author for a UNESCO study on "Fundamental World Energy Problems" and a UNEP project on "Photosynthesis in Relation to Bioproductivity". References are not included but the author can supply details—also refer to article in "Solar Energy" (1979).

correspondence

Jobs gloom: a military solution?

SIR,—The problems of job opportunities and job security in science are serious (21/28 December, page 743). But solutions which may be highly desirable for individuals may be disastrous for science itself. While there are many exceptions, for most scientists the decline in research productivity between the years of 35 and 45 is unequivocal. Unfortunately this decline is only rarely matched by a compensating increase in teaching or administrative skills.

Although it may be anathema to most scientists to look to the military for an answer, I suggest that in this area it has much to teach us. Anyone becoming an army officer accepts that the career grade is Major and that, depending on the country concerned, those who are not promoted above this grade will retire between 40 and 45. Each level of promotion buys a few extra years until Field Marshals are appointed for life. A similar system in science would provide a long term solution to the present ills. It would work as follows.

Far more junior posts than are at present available would be created but these would automatically terminate at age 40. A gratuity equal to three years salary or a modest pension would then be provided. Retirement could be taken as early as 35 when a one year gratuity would be given.

Promotion above the basic career grade would depend on a realistic assessment of research, teaching and administrative contributions. Each promotion rung would buy an extra five years. Perhaps half the entry would be eliminated at the first hurdle and another half at the second, with relatively little attrition thereafter. The few highly productive scientists who remain active into old age would become 'Field Marshals' and would not be expected to retire.

The advantages of this system are many. As in the army, people would enter science knowing that unless they did very well their career would end at 40. This would eliminate the unenthusiastic at the beginning and provide a notable stimulus to research. Many junior posts would be available and the system would not be wrecked by a preponderance of individuals making little or no contribution. Institutions would not be reluctant to make early appointments for fear of having to maintain a person's employment until 65 or 70. In most cases scientists would know by the age of 35 whether or not they were going to be successful and could then begin to plan second careers in the comfortable knowledge that they would have three years in which to make adjustments. As with the military, organisations would develop which specialised in placing scientists in appropriate positions in industry or government. Industry, government, and hence science would all benefit from this influx of people with a genuine scientific background and not simply a first degree in science.

I do not underestimate the anguish

such a system would cause in the transition period. But some such radical approach is essential if we are not to replace the current problems of individuals by the much more serious one of an almost totally stagnant scientific community.

Yours faithfully,

DAVID F. HORROBIN

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No rabies virus at Glasgow

SIR,—I should be grateful for the opportunity of correcting an error which has appeared in your journal (11 January, page 81). This was a statement in which the Department of Virology at the University of Glasgow was cited as a laboratory holding a Category A Pathogen, namely rabies, as at 12 December 1978. This, I am happy to tell you, is not the case, as the small stock of rabies virus held by that department was destroyed in June 1977. It had, in fact, never been used for active research, and had remained in deep freeze for eight years before being destroyed.

I imagine that your source of information for this article was the draft of the Shooter report. I am informed by the Scottish Home and Health Department that they anticipate being able to update and correct this situation before the report is finally published.

Your faithfully,

ANDREW D. RANKINE

*Court Offices,
University of Glasgow, UK*

Reassess hazards of recombinant DNA

SIR,—The comments (15 February, page 509) by a committee of the Royal Society on the recent published statement of the Genetic Manipulation Advisory Group will be widely welcomed and supported. However, I should like to comment on one of the suggestions they make.

It is argued that there is an urgent need for more research to evaluate the risks associated with the ingestion of organisms containing recombinant DNA. Before devoting yet more resources to the general hypothesis that recombinant DNA synthesised *in vitro* poses novel hazards to man, is there not a prior need to reconsider in which areas of recombinant DNA research there remain sound arguments indicating that the hypothesis should be taken seriously? The committee has properly criticised the Genetic Manipulation Advisory Group for publishing what are often called hazard scenarios without adequate argument or evidence supported by citation of relevant literature. But could not a similar criticism be made with equal validity against the hazards hypothesis in its entirety? The report of the working party chaired by Lord Ashby which was set up to evaluate this hypothesis made a number of assertions in favour of it but none of them was documented in the way the Royal Society committee advocates. Admittedly this report was written in response to the possibilities suggested in

the Berg letter were more emotional than rational. All the more reason, now that four years have passed, for a cool reassessment of the questions before we embark on experimentation.

The most urgent need in 1979 surely is for a re-examination of the hazards hypothesis to determine which, if any, aspects of it remain credible enough to deserve further attention on scientific grounds. This reassessment can only begin if those who remain convinced of hazards are prepared to state their case in published form with the rigour urged by the Royal Society committee, and in a manner that permits rebuttal like any other scientific argument.

If such a reassessment suggests that areas of significant risk remain then let us devote resources to quantifying the risks in these areas. If, on the other hand, this reassessment suggests that the hypothesis is unfounded or if no one is prepared to argue the case to the contrary, then should not those biologists who have collectively exploited their authority to open the so-called debate on the hazards of genetic engineering be urged to use their authority to close it in like manner. The danger in what the Royal Society proposes is that it could lend further weight to a hypothesis that may not deserve it.

Yours faithfully,

R. H. PRITCHARD

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Energy forecasts mislead

SIR,—Your discussion of energy forecasts (18 January, page 162) misses the point. It is easy to forecast a low consumption of energy, and all too easy to make this forecast come true. The price of nuclear powered electricity has risen partially because of delays caused by those predicting low consumption of energy; as a result, demand has fallen. In a recent visit to England I noted that the head of a small college lived in a house I rejected as unsuitable as a research lecturer in 1952. This contraction in housing standards, and with it a contraction in demand for fuel, has reached other parts of society in the past few years.

The challenge is not to predict a low energy consumption and make it come true; but to work toward a just society, where the well being and convenience we have often correlated with energy consumption can be achieved with minimum financial, ecological and environmental costs. None of these costs have a one-to-one relationship with energy production or consumption, and to argue otherwise may be counter-productive. Those energy producers who concentrate solely on meeting "demand" have led us astray. Those who, like Mr Leach, are constantly trying to tell us that we don't need as much are equally guilty of keeping our concentration in the wrong place.

Yours faithfully,

RICHARD WILSON

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news and views

It is only five years since the first reports that lymphoid cells from unimmunised mice were able to kill certain lymphoid target cell lines (Herberman *et al. J. natn. Cancer Inst.* 51, 1509, 1973). Since then, despite a deluge of papers dealing with the phenomenon, both in rodents and in man, little solid evidence has emerged to convince the cynical immunologist that this spontaneous or natural killing has the three attributes of immunological respectability—specificity of effect, specificity of immunisation, and specific memory. Moreover, the popular term, 'natural' killing only serves now to remind us how little is really understood of the role in nature of this function so readily studied *in vitro*.

Early results suggested that natural killer (NK) effector cells in the mouse lacked surface markers of both thymus-derived (T), and bone-marrow-derived (B) lymphocytes, and were neither phagocytic nor adherent to plastic. This null cell appeared to lyse preferentially lymphoid tumour cells expressing antigens coded by RNA tumour viruses (retraviruses), leading to the suggestion that NK cells were an *in vivo* surveillance mechanism against retravirus-induced tumours. For tumour immunologists this was an attractive hypothesis as it provided a substitute for the discredited T cell as a surveillance mechanism.

As is so often the case, unfortunately, later data have complicated the issue. No firm answers have yet emerged as to the nature and origin of the NK cell, the genetic control of its activity, or its specificity of killing, activation or memory. Nevertheless, recent results have brought some order into a picture that at one time seemed to be degenerating into cytotoxic anarchy.

In the mouse, Herberman and his colleagues have suggested that NK cells express low amounts of surface markers characteristic of T lymphocytes (Herberman *et al. J. Immun.* 121, 304; 1978). Others, however, using even highly potent monoclonal anti-T cell antibodies, have not confirmed this. In any case, although the mouse Thy-1 antigen reportedly present on NK cells is characteristic of T and not B lymphocytes, it is also expressed in brain and other tissues, and its presence on NK cells would not necessarily link the NK effector with the T cell differ-

Killing comes naturally

from Peter Beverley & Dick Knight

tiation pathway. More persuasive in regard to the origin of the NK cells, however, are the observations that they carry a surface marker not present on T or B cells (although identified by a contaminant antibody in the anti-T cell serum anti-Lyt-1) and that the mutant *beige* mouse, which has deficient granulocyte function but normal T and B lymphocytes, has very low NK activity (Roder & Duwe *Nature* in the press).

In the human, recent work from Good's laboratory, as well as confirming the consensus that human NK cells possess receptors for the Fc portion of immunoglobulin, has suggested that both T and non-T cells are cytotoxic in NK assays (Gupta *et al. Proc. natn. Acad. Sci. U.S.A.* 75, 5137, 1978). Moreover, suggestions are emerging that different target cells are differentially susceptible to the action of different effectors. In the mouse too, Stutman has recently described an NC (natural cytotoxic) cell with slightly different phenotypic properties and distinctly different killing preference to the classic NK cell (Stutman *et al. J. Immun.* 121, 1819; 1978). If these results are confirmed, it becomes clear that both in mouse and man more than one effector cell may mediate 'natural' killing. In addition, since no convincing evidence for T-cell-mediated 'natural' killing has yet been presented for the mouse, a genuine species difference in effector compartments may exist.

Specificity can only be truly defined when the target antigens have been characterised. On the NK-susceptible mouse lymphoma target, YAC-1, three 'target structures' of molecular weights 130,000, 160,000 and 240,000, and distinct from known viral or histocompatibility antigens, have recently been identified (Roder *et al. Proc. natn. Acad. Sci. U.S.A.* in the press). On other targets, the available evidence from competition and monolayer absorption experiments suggests a broad selectivity of killing for groups of target cells as opposed to totally indiscriminate aggression. The emergence

of multiple NK effector populations, with apparent target preferences, especially in man, suggests further that not all NK cells will turn out to have the same range of specificities. However, the important question remains unresolved as to whether the observed selectivity reflects recognition of a limited number of ubiquitous antigens (and a fetal calf serum-derived antigen now seems unlikely), or the activity of many effector clones each recognising a single antigen.

What of specificity of immunisation? It is now clear that a variety of agents given *in vivo*—bacteria, viruses and tumour cells—can boost NK in mice. Interestingly, only those tumour cells themselves susceptible to NK lysis are able to augment NK activity in this way. A similarly wide range of agents boost NK *in vitro*, although the identity of the effectors of broad spectrum lysis induced after a period of culture with classical spontaneous NK, has not been proved. Recent work from Trinchieri and Santoli has shown, however, that *in vitro* inducers share the property of stimulating interferon release, and indeed, that interferon itself is a potent NK boosting agent (Trinchieri & Santoli *J. exp. Med.* 147, 1314; 1978). No data has yet been presented on whether interferon is the only final common path of NK augmentation, or whether boosting *in vivo* and *in vitro* have the same mechanism.

Irrespective of whether interferon affects NK cells directly or works through an intermediate cell, it seems that the augmentation is the expression of a higher level of activity already programmed into the NK cells. This program depends not on the environment of the cell donor, but on its genetic constitution, since transfers of bone marrow cells (containing NK cells) between different mouse strains show that the transferred cells retain the level and spectrum of activity of the donor strain.

Genes controlling NK activity have been mapped close to genes controlling rejection of parent-into-F₁ bone marrow grafts and close to H-2D. Intriguingly, products of this latter locus are critical for target cell recognition in classical T-cell cytotoxicity. However, natural killing is not confined by self or even species recognition. If T cells are confirmed as an NK effector population, it is somewhat surprising that their

activity seems to have no MHC restriction.

Does this new information on the nature, specificity, and genetic control of the NK cell allow any tentative conclusions as to its function *in vivo*? Certainly data exist correlating tumour incidence and the ability to reject tumour grafts with the level of NK activity. Moreover, there seems to be a reciprocal relationship between T cell and NK activity, as athymic nude mice and thymectomised animals have higher levels of NK than intact syngeneic mice. The data from Kiessling's group, reported in this issue of *Nature* (page 174), showing that a subset of thymo-

cytes can be killed by NK cells, and correlating the presence or absence of this subset with the level of NK activity, also suggests that regulation of NK and T cells is closely connected. Should this subset of thymocytes turn out to be particularly susceptible to reovirus-induced transformation, then the surveillance and immunoregulatory theories could be neatly fused. □

Peter Beverley is an Imperial Cancer Research Fund Staff Scientist and Dick Knight a Cancer Research Campaign Research Fellow at the ICRF Human Tumour Immunology Group, University College Hospital Medical School, London.

A two-dimensional superfluid?

from P. V. C. McClintock

Has two-dimensional superfluidity been observed? There has been a continuing controversy as to whether or not this interesting phenomenon, predicted theoretically for ideal two-dimensional systems, has been seen in real liquid helium-4 films. Dash has argued persuasively (*Phys. Rev. Lett.* **41**, 1178; 1978) that it has not; and that the experiments which had apparently demonstrated the effect were misinterpreted. Some new measurements now reported by Webster, Webster and Chester of the University of California at Los Angeles (*Phys. Rev. Lett.* **42**, 243; 1979) seem, however, to have tilted the balance of the evidence back in the affirmative direction once more, in favour of the original interpretation.

The superfluidity of the 'creeping film' which forms on surfaces dipping into liquid helium has, of course, been well known—and intensively studied—ever since it was first discovered by Rollin in 1936. It is this phenomenon which is responsible for the famous demonstration of superfluidity in which an open-topped cup of liquid helium is able to empty itself while remaining upright, using the film as a kind of syphon. The present interest centres, however, on very thin helium films: so thin, in fact, that no parameter can change within their thickness, and hence nothing can happen in the dimension at right angles to the surface on which the film is adsorbed. Such films probably constitute what are effectively two-dimensional (2-D) systems.

The theory of 2-D helium has made rapid progress in recent years under the

stimulus, particularly, of the beautiful experimental work carried out by Dash and his co-workers at the University of Washington, using exfoliated graphite as a substrate. A striking prediction made by Nelson and Kosterlitz (*Phys. Rev. Lett.* **39**, 1201; 1977) was that, if a 2-D system undergoes a superfluid transition, it will do so in a precipitous jump: the superfluid fraction ought to jump discontinuously from zero to a finite value, ρ_s^* . This is in sharp contrast to what happens in three dimensions. When ordinary bulk liquid helium undergoes the transition, the fraction of the liquid which behaves as superfluid increases continuously from zero. Nelson and Kosterlitz concluded, furthermore, that for a 2-D liquid helium-4 film the jump should be universal: that is, the ratio of ρ_s^* to the absolute temperature T_0 at onset should be quite independent of the nature of the substrate, of the coverage at which the transition takes place, or indeed, of virtually anything else.

Apparent experimental verification of this remarkable prediction was reported shortly afterwards: by Rudnick, on the basis of third sound measurements (*Phys. Rev. Lett.* **40**, 1454; 1978); and by Bishop and Reppy, based on their measurements of the superfluid fraction on a vibrating substrate (*Phys. Rev. Lett.* **40**, 1727; 1978). The latter type of measurement seems at first sight to give a particularly direct and unambiguous measure of the superfluid fraction. The period of the oscillator is dependent on its mass, and thus on the effective mass of the adsorbed helium film sticking to it; but, if some proportion of the film is superfluid then, instead of following the oscillations, this fraction will just slip, and therefore will not affect the measured

period. Values of ρ_s^*/T_0 deduced in this way were in excellent quantitative agreement with Nelson and Kosterlitz's prediction.

Dash argued, however, that the superfluid onset as measured by this technique does not represent genuine 2-D superfluidity. He suggested, first, that inhomogeneities in the Mylar substrate would cause the formation of 'puddles' of helium where the film was relatively thick, with interconnecting regions of thinner film. The observed superfluid onset therefore represented, not 2-D superfluidity in the film as a whole, but merely the occurrence of superfluidity in a sufficient proportion of the interconnecting regions. By this time the deep 'puddles' would, of course, correspond to 3-D superfluid. Second, he suggested that even if the substrate did provide an ideal atomically flat surface (which he doubted), the films themselves would be non-uniform. He envisaged that, as helium atoms were gradually added to the substrate, at a fixed temperature, they would first form a 2-D gas; but that, when a certain critical coverage was reached, interactions between the atoms would result in the formation of small patches of 2-D liquid surrounded by the 2-D vapour. As further atoms were added the connectivity of the liquid regions would eventually provide a continuous 2-D path across the whole experimental area and, if the temperature was low enough, superfluidity might then appear abruptly: the superfluid fraction at onset could clearly be non-zero, corresponding in fact to that of the liquid regions immediately before their being connected together.

In the experiment now reported from UCLA, the Websters and Chester have performed another vibrating substrate measurement, using an oscillating quartz crystal. The important difference from earlier work is that they have added an additional variable parameter to the system by mixing up to 30% of helium-3 with their helium-4: a proportion of helium-3 has a profound effect on the superfluid transition in helium-4, depressing it to lower temperatures and even eliminating it altogether if too much is added. Nonetheless, it turned out that the value of ρ_s^*/T_0 was completely unaffected by the helium-3, remaining closely equal to the universal constant predicted by Nelson and Kosterlitz—although, of course, ρ_s^* and T_0 were individually changed to a considerable degree. So, for some as yet unexplained reason, Dash's arguments do not seem to be applicable in practice.

These latest results constitute compelling evidence in support both of the theory and of the belief that the helium-4 film at onset really does behave as a two-dimensional superfluid.

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Purine insertase: a new enzyme

from Gerard O'Donovan

It is now generally believed that a large number of environmental agents cause damage to DNA. Even in the absence of environmental stimuli DNA has to cope with yet another type of damage, namely the formation of DNA apurinic/apyrimidinic sites (AP sites). These lesions are produced spontaneously by the loss of purine or pyrimidine bases from the DNA and also by the action of a specific class of enzymes known collectively as DNA N-glycosylases (previously known as N-glycosidases). Three distinct enzyme activities have been discovered to date. These N-glycosylases remove uracil (Lindahl *Proc. natn. Acad. Sci. U.S.A.* **71**, 3649; 1974), 3-methyl adenine (Laval *Nature* **269**, 829; 1977; Riazuddin & Lindahl, *Biochemistry* **17**, 2110, 1978), or hypoxanthine (Karran & Lindahl, *J. biol. Chem.* **17**, 5877, 1978) from DNA without perturbing the sugar-phosphate backbone.

Until recently these enzymes were called DNA N-glycosidases because traditionally the base-sugar bonds in nucleic acids which they cleave, were termed glycosidic bonds (Lindahl, *Proc. natn. Acad. Sci. U.S.A.* **71**, 3649; 1974). However, the current rules for carbohydrate nomenclature (*Biochemistry* **10**, 3983, 1971) state that a glycosidic bond is a linkage through oxygen obtained by replacement of the hydrogen atom of the hemiacetal hydroxyl group, while removal of the entire hydroxyl group results in a glycosyl bond (Riazuddin & Lindahl *op cit*).

Deoxyribonucleases that act on DNA containing apurinic sites have been characterised from various sources including bacteria (Gossard & Verly *Europ. J. Biochem.* **82**, 321, 1978), plants (Thibodeau & Verly *J. biol. Chem.* **252**, 3,304; 1977) and human cells (Kuhnlein *et al. Nucl. Acids Res.* **5**, 951, 1978). These enzymes are part of a duplex DNA repair pathway in which the apurinic endonuclease (AP endonuclease) makes an incision in the vicinity of the apurinic lesion, then the apurinic lesion is excised by an appropriate exonuclease and the resulting gap is filled in with a DNA polymerase before closure with DNA ligase. The apurinic lesion presumably arose spontaneously, by reaction with chemicals or as the product of a DNA N-glycosylase, one of the enzymes described above which removes ab-

normal bases from DNA by hydrolysing the glycosyl bond.

Since excision repair is a multi-enzyme process it seems reasonable that a simpler and more straightforward pathway might exist. With respect to modified DNA, N-glycosylolytic removal of abnormal bases from DNA to produce an AP site, with the concomitant reinsertion of the correct base, has been speculated on. In a recent issue of *Proc. natn. Acad. Sci. U.S.A.* (**76**, 141, 1979) Deutsch and Linn report the discovery of an enzyme activity from cultured human fibroblasts which seems to reinsert purines, but not pyrimidines, into DNA containing apurinic sites. The activity, which is partially purified (molecular weight 120,000) to be free from apurinic endonucleases, was first identified by its ability to bind specifically to partially depurinated DNA. Using radioactively labelled purines, Deutsch and Linn show that the free base is best utilised, and that the incorporation like the binding, is specific for DNA apurinic sites. Moreover, using depurinated synthetic polymers, they show that the insertion of purine bases seems to be template-specific, with guanine but not adenine being inserted into depurinated poly (dG-dC), and adenine but not guanine being inserted into poly (dA-dT).

The formation of a glycosyl bond would seem to require energy although the free energy of formation within a DNA molecule has not been measured directly. In the present study the reaction occurs in the absence of any obvious energy source. One suggestion offered by Deutsch and Linn for the free energy source in the case of a free purine substrate is the reforming of base pairs and stacking at the site of the reaction. With respect to an energy source, it is significant that another reaction in which the guanine base is utilised also occurs in the absence of energy. Here, the tRNA guanylylating enzyme replaces the Q base of certain tRNAs with guanine (Okado *et al. Nucl. Acids Res.* **3**, 2,593, 1978, Farkas & Singh, *J. biol. Chem.* **248**, 7,781; 1977).

Clearly the most likely role for the AP endonucleases is in DNA repair. Such enzymes (purine base insertases) might be extremely active in repair during normal DNA replication. But because they are present in nature in such large amounts, almost universally, it is tempting to ascribe additional roles to the AP endonucleases. As pointed out by Deutsch and Linn it could be that the utility of base insertion may not be for DNA repair but rather for situations in which the generation of genetic diversity is desirable. In addition, the eukaryotic genome contains large numbers of modified bases which

could be replaced (or inserted) as a regulatory or developmental signal. Such hypotheses are simple to formulate but difficult to prove; it will be of interest to see if future investigations unmask more exotic DNA N-glycosylases and perhaps related insertases.

A surplus of carbon dioxide

from G. Skirrow

Two recent independent contributions (Brewer *Geophys. Res. Lett.* **5**, 997, 1978, Chen & Millero *Nature* **277**, 205; 1979), both based on analysis of Geosecs data, have outlined direct experimental evidence that part of the present, continuing, atmospheric CO₂ increase consequent on fossil fuel combustion is being transferred to the sea and that this process has probably been taking place for some time. Although it has always seemed reasonable to suppose that this transfer did occur, evidence has hitherto been mainly indirect and estimates of the extent of the exchange have relied on, for example, observations of the distribution of ¹⁴C produced by nuclear bomb explosions.

The air-sea transfer of CO₂ is merely one facet, albeit an important one, of the global carbon cycle. Briefly, CO₂ is gained by the atmosphere through volcanic exhalations, by evasion from the sea and by respiration and combustion. It is lost from the atmosphere by invasion into the sea, by photosynthesis into the terrestrial and marine biospheres and by weathering processes leading to dissolution of, particularly, carbonate rocks and the transfer to the sea of HCO₃⁻-rich solutions by continental run-off. Within the oceans biogenic precipitation of CaCO₃ from the supersaturated surface waters (together with precipitation of some organic debris) transfers some carbon to the sediments, although some of the CaCO₃ dissolves during its descent in the oceans. Over geological periods of time sea-floor spreading makes the sediments available for reworking. A more detailed examination shows that the carbon cycle is intimately linked with those for other elements, notably calcium, oxygen and nutrients.

Although life forms on Earth have changed over the past few hundred million years, the general and continuous persistence of life has been taken to imply that, over this time, the various elemental cycles have never been very violently perturbed from

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their steady states and that they have an essential stability deriving from in-built negative feedback or buffering mechanisms (Garrels *et al.* *Amer. Sci.* **64**, 306; 1976). Of course, many minor perturbations have occurred in the past although these have evidently not been sufficient to destroy life totally. However, even minor fluctuations may have consequences which, if not devastating, may be distinctly uncomfortable from a 20th century human standpoint. Thus, possible climatological changes may ensue. Quite apart from academic and intellectual reasons for wishing to understand more fully the natural processes of atmospheric and marine circulation and elemental cycle stabilisation, self-interest clearly justifies investing time, effort and money in monitoring the carbon system and in developing theoretical and modelling approaches intended to have interpretative or predictive value.

It is generally accepted that fossil fuel consumption is the main cause of the atmospheric CO_2 increase over the past century, although other human activities (deforestation and changing land usage) probably play a not insignificant part (Woodwell *Scient. Amer.* **238**, 34; 1978). However, there is no doubt about the reality of the partial pressure P_{CO_2} increase; precise and detailed measurements from many sites throughout the world over the past two decades (for example, Pales & Keeling *J. geophys. Res.* **70**, 6053; 1965) have shown that since 1958 atmospheric CO_2 has increased by about 6% and that the present atmospheric partial pressure of about 340×10^{-4} atm is some $50\text{--}60 \times 10^{-4}$ atm greater than that of a century ago. Precise knowledge of the CO_2 levels before 1850 (the effective start of the Industrial Revolution) is lacking but a value of about 290×10^{-4} atm is generally assumed. It is interesting to note that the observations made by Brewer and by Chen and Millero are at least not inconsistent with this picture, although at present the levels of precision imposed by the need to correct their basic data to allow for *in situ* CO_2 generation rules out definitive pronouncements.

The rate of increase of P_{CO_2} has increased with time in a manner matching the usage of fossil fuels, although a large fraction of the output has passed into other sinks—presumably the sea. The present annual rate of increase of P_{CO_2} is almost 1×10^{-4} atm yr^{-1} , and for the purposes of projection into the future (an uncertain business because of the likely dependence on economic factors and resource depletion) a rate of increase of something like 2% per year is often assumed (Broecker & Takahashi *The Fate of Fossil Fuel CO_2 in the Oceans*, 213,

Plenum, 1977). Sometime in the 21st century atmospheric P_{CO_2} may peak at several times the pre-industrial value (Woodwell).

Pertinent questions concern the way in which the sea responds to this increase of atmospheric partial pressure of CO_2 . That is, its capacity to moderate the increase by dissolution, the consequent changes in the chemistry of the sea, and the possibility that the attack of the now more aggressive waters on the calcite and argonite fractions of the sediments will further moderate the increase. The capacity of the sea to dissolve CO_2 is considerable although the time scale for total air-sea equilibrium is measured in centuries because of the relatively slow turnover and mixing times of the various oceanic water masses. However, according to Broecker and Takahashi who are grappling with the overall problem (including interaction with the sediments) if an injection to the atmosphere of 23×10^{16} moles of CO_2 is made, then on attainment of solution equilibrium about 70% of the injection would have passed into the sea. The effect of this uptake would be to moderate the carbonate ion concentration of the water to lead to dissolution of the CaCO_3 fractions of the sediments on certain parts of the ocean floor. Formidable problems make it difficult to quantify this general picture since these investigators have to contend not only with uncertainties concerning the future rate of industrial CO_2 generation and the rate of passage into the sea, but also with the complex kinetics of calcite dissolution (for example, Morse & Berner *Am. J. Sci.* **272**, 840; 1972). The difficulties are further compounded when it is recognised that (1) carbonate sediments are not uniformly distributed over the ocean floor, and the carbonate fraction varies considerably, falling virtually to zero for deep-sea sediments below the compensation depth; (2) the effectiveness of the carbonate fraction in neutralising the CO_2 increase depends on depth; at shallow depths the water is strongly supersaturated with respect to CaCO_3 and very high P_{CO_2} values are needed to remove this supersaturation (Skirrow & Whitfield *Limnol. Oceanog.* **20**, 103; 1975). Sediments lying between the present compensation and lysocline depths would be expected to be particularly susceptible to attack; and (3) as attack on the sediments proceeds, the remaining CaCO_3 will become more deeply buried or diluted by clay and inert materials thereby reducing the rate of attack.

Notwithstanding these discouraging considerations Broecker and Takahashi have made a start on this problem. They conclude that the potentially

available CaCO_3 in those sediments likely to be susceptible to attack is equivalent to the CO_2 derivable from the world's total fossil fuel resources and that the time constant for neutralisation of the CO_2 will be 1,500 years or less.

The studies referred to in the opening paragraph may seem a long way from those of Broecker and Takahashi, but they are different aspects of a large problem which may have important ramifications for the world as a whole. There are many grey areas in which information needs still to be collected. These include the response of the biosphere to changing atmospheric CO_2 levels, and possibly less important because of the already high levels of CO_2 in soil water, the effect this increased CO_2 will have on weathering processes. □

Making molecular catalogues

from Norman Anderson

HIGH-resolution two-dimensional gel electrophoresis, as originally described by O'Farrell (*J. biol. Chem.* **250**, 4007; 1975), has proved an extremely powerful tool for the analysis of complex protein mixtures, particularly cells. It can be made to resolve and detect quantitatively virtually all the proteins expected in *E. coli* (~ 1,500) and perhaps 2,000–3,000 of the possible 4,000–7,000 thought to occur in any given mammalian cell type. So far, however, the technique has been principally used as an adjunct to studies of particular enzymes or regulational events, and has not provided the kind of global information that might be expected to emerge from a detailed analysis of what different cells do (or at least synthesise). The reason for this is the difficulty of comparing large numbers of patterns, and of remembering enough differences to draw conclusions with regard to very large numbers of protein spots. A somewhat analogous state of affairs existed in early protein crystallography when diffraction spots could be produced on X-ray film, but the structure of the proteins producing them could not be deduced. The introduction of computers, automated densitometers, and mathematical analysis ultimately allowed such structures to be determined. This process seems to be repeating itself, but at a much higher level of complexity, in the field of high resolution two-dimensional protein mapping.

What is desired is a way of reducing

2-D gel data to quantitative lists of protein components (that is, precise spot positions and integrated densities), and ways of matching and comparing lists to deduce the rules or factors governing the synthesis of each gene product. An early and relatively easy project, for example, would be to determine the number of protein gene products common to all cell types in man, or conversely, whether proteins exist which are absolutely specific to each differentiated cell type. In order to address such questions, computer-based data reduction systems are being constructed by several groups in the US and Europe. An informal meeting at the Argonne National Laboratory reviewed progress and discussed systems design with likely major users.

J. Garrels (Cold Spring Harbor Laboratory) and N. Xuong and M. Miller (University of California, San Diego) described working systems for spot detection and integration based, respectively, on slope and contour analysis. The latter seems much faster but requires interactive help in separating compact groups of spots. In each of these systems, some progress has been made towards matching pairs of gels for comparison. Two examples of second generation dedicated mini-computer systems were described by L. Anderson and J. Taylor (Argonne) and J. Garrels (CSHL). The first segment of the two-part Argonne system (called TYCHO) involves image scanning, spot detection, and quantitation by two-dimensional Gaussian fitting, matching of patterns to compensate for small differences between gels by interactive colour television graphics, and the production of spot lists containing as many human protein gene products (PGPs) as possible. The program for searching the spot lists for correlations with the type or state of the cells producing them is called KEPLER. The Cold Spring Harbor program (called QUEST) will use virtually identical hardware but is designed primarily to compare patterns as raw image data rather than spot lists to facilitate accurate ratio quantitation in double-label autoradiographic experiments. Both systems aim to accumulate a comprehensive annotated map (or list) of proteins in a particular organism (man at Argonne and mice and rats at Cold Spring Harbor) for general research use.

An important constraint in such systems (particularly when applied to large-scale genetic work) will be processing time per gel. This is currently estimated at 20 to 60 min in the second

generation systems, depending on the number of spots. A faster image processing technology based on the Sternberg-von Neumann cytocomputer (described by P. Petraitis, Environmental Research Institute, Michigan) seemed capable of reducing processing time by at least 50%, while solid array based densitometer designs (P. Spragg, University of Birmingham, UK) offered similar improvements.

The potential use of these systems for detecting possible changes in the human background mutation rate was discussed by J. V. Neel (University of Michigan). On the basis of the current Argonne throughput of approximately 10,000 2-D gels per year, and assuming that several hundred protein gene products could be examined in each person, mutation rates could be estimated on samples as small as 30,000 individuals in a reasonable time.

Preliminary results presented by E. McConkey (University of Colorado, Boulder) indicated a wide discrepancy in average heterozygosity between major cellular proteins (as observed on 2-D gels) and the proteins of plasma and red cells (measured by classical methods). This sparked a lively debate on the effects of natural selection on different groups of proteins and brought out the view that many geneticists are still convinced that charge-change variants are better detected on starch blocks than on 2-D gels. This question was settled by the development by N. L. Anderson and B. H. Hickman (Argonne) of charge shift standards prepared by progressive carbamylation of one protein. These standards stretch all the way across the gel as a series of discrete spots, one more than the number of amino groups on the original protein, and unequivocally demonstrate the detectability of unit charge shifts.

P. O'Farrell (University of California, San Francisco) described experiments to determine whether repressed mammalian genes are indeed totally silent. Using a combination of immunoaffinity and two-dimensional separations, he was able to place an upper limit of one part in 2×10^8 on the expression of a particular protein in cells carrying the corresponding gene in a repressed state. Interestingly enough, any gene products present at this level *in vivo* are less abundant than mislabeled versions of actin and other major proteins, due to the limited fidelity of translation. There is, therefore, an irreducible 'background fog' associated with high resolution protein mapping at currently achievable sensitivities as a result of thermodynamic noise in protein synthesis.

It was generally concluded that

within the year systems will exist capable of cataloguing, comparing, and performing perturbation analyses on a good fraction of the possible products of a mammalian genome. By extending the set of observable proteins from the classically assayable (presently perhaps 2% of the total) to the most abundant 50%, we will probably be afforded a new view of many biological phenomena and, perhaps, a less welcome look at how poorly controlled present experiments are at the level of gene expression. □

Saccharin: from carcinogen to promoter

from E. Boyland

THE first indications that the widely used artificial sweetener saccharin was carcinogenic were the data of 1951 in which there was "an increased incidence of the ordinarily uncommon condition of abdominal lymphosarcoma" in rats fed saccharin¹. When the technique of implantation of pellets containing suspected direct carcinogens into the bladder of mice was introduced², it was thought that, as the bladder tissue would be less able than the liver to metabolise foreign compounds, the test would distinguish compounds that required metabolic activation from those which were directly carcinogens. The implantation of cholesterol pellets containing saccharin into the urinary bladders of a few mice induced a statistically significant incidence of tumours³. At the time we suggested that "The induction of bladder tumours with saccharin suggests that the presence of the solid pellet in the bladder may have a promoting action and that the method of bladder implantation detects incomplete carcinogens". These results with saccharin were confirmed 13 years later by experiments carried out on a larger scale⁴. Previously it had been found that saccharin had some initiating action on mouse skin⁵, and so the bladder implantation experiments in the light of knowledge at that time were interpreted as showing that saccharin was a direct carcinogen and active without metabolic activation. Subsequent investigations have been unable to detect any metabolic changes of saccharin in any of several species^{6,7}.

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Following the bladder implantation experiments and results, at least eleven studies in which saccharin was fed to rats are available⁴; in eight of these urinary bladder tumours seem to have been induced, but in never more than 44% of rats and generally in a much lower proportion even when the diets contained 5% saccharin. In one study⁵ lymphomas and leukaemias occurred.

Saccharin may therefore be considered as a weak carcinogen for the rat bladder, by the following three criteria. (1) The dose needed to induce carcinogenesis was large—in some cases of the order of 1 kg per kg body weight. (2) The induction time was long; generally the bladder tumours appeared after at least 18 months continuous feeding. (3) Not all animals developed tumours. Potent carcinogens on the other hand induce cancer in all treated animals.

Later experiments may explain the mode of action of saccharin. When small 'initiating' doses of methyl nitrosourea (NMU) were instilled into the urinary bladder of Wistar rats which were then given either water or water containing saccharin, to drink, no bladder tumours were seen in rats given NMU without saccharin¹⁰. Only 2% of rats given saccharin alone developed bladder tumours in 95 weeks, but 57% of rats given NMU and saccharin developed tumours. Some of the rats with tumours had bladder stones but these were not considered a factor in the 'synergistic' effect of saccharin. The authors concluded that saccharin is cocarcinogenic and "that a promoting action of saccharin with cyclamate and/or other bladder carcinogens is an obvious possibility".

In another experiment the feeding of diets containing 2% FANF (N-(4-5 nitro-2-furyl 2-thiazoyl) formamide) to rats for 6 weeks induced bladder cancer in 4 out of 20 rats, but if this was followed by feeding 2% tryptophan or 5% saccharin 20 out of 39 and 35 out of 35 rats developed bladder cancer¹¹. In these experiments both tryptophan and saccharin seem to have acted as promoting agents. The result with tryptophan was similar to those in which rats fed 2-acetamidofluorene with either tryptophan or indole developed bladder tumours, whereas with 2-acetamidofluorene alone tumours at other sites were seen^{12,13}.

Further evidence that saccharin acts as a promoting agent was provided by the experiments in which cultures of C3H/10T1/2 cells, treated with non-transforming initiating doses of methylcholanthrene followed by saccharin, had a statistically significant number of transformations¹⁴. These authors estimated that saccharin probably functioned as a promoting agent, but was

1,000-fold less active than the potent promoter 12-O-tetradecanoyl-phorbol-13-acetate.

If saccharin is a promoter rather than a direct carcinogen, how can the earlier results be explained. The first bladder implantation experiments in animals showed that although the rat skin was resistant to the carcinogenic action of tar the epithelium of the rat bladder was sensitive¹⁵. Later experiments¹⁶ however, showed that implantation of paraffin wax pellets alone into the rat bladder produced a high incidence of cancer. Mice treated in the same way developed only a few bladder tumours and so could be used for carcinogen testing by this technique. The wax pellets therefore seem to be acting as promoters and rat urine or bladder epithelium may contain more material acting as initiators of carcinogenesis than does that of mice.

All the experiments in which bladder cancer has been induced by feeding saccharin have been in rats. The occurrence of these bladder tumours could therefore be due to the promoting action of saccharin together with endogenous initiators of carcinogenesis.

Several experiments therefore indicate that saccharin could be a promoting agent rather than a complete carcinogen. Saccharin is not mutagenic in the *Salmonella* reverse mutation assay of Ames or the cell transformation test of Styles and there is no indication that it is metabolised by animals or man or reacts with DNA. It has been suggested that as the carcinogenic action in rats and the dominant lethal effects in mice do not appear to be genetic; they are 'epigenetic'¹⁷. This term is a broad one and would include

promoting action, cocarcinogenesis, inhibition of DNA repair and suppression of immune reactions. The experimental data on saccharin indicate that it is a promoting agent and this is more precise than saying its effects are not produced by a genetic mechanism. The local effect of saccharin could be due to its being concentrated in urine and the bladder mucosa.

However this is not evidence that saccharin is 'harmless'. Carcinogenesis is a complex multistage process in which initiation, promotion or some other stage may be limiting steps. Thus although promoting agents are not complete carcinogens, in some conditions they facilitate cancer development which would not otherwise occur. In such circumstances, a promoter may therefore be as dangerous as a complete carcinogen. Saccharin is, however, unlikely to provide a serious hazard because the dose required to produce the promoting effect is so large—of the order of 1 kg per kg body weight. The very low though finite risks may be worth the benefits of its use. □

Fossil pseudo-reptiles

from Barry Cox

In Kipling's *Just So Stories*, the jaguar was dreadfully puzzled by the armadillo, for he wasn't sure whether it was a tortoise or a hedgehog. In the same way, the tiny Carboniferous tetrapods were for long a puzzle to palaeontologists, who couldn't tell whether they were amphibians or reptiles.

There was obviously no way in which their life-history could be established. It also became clear that the simple-minded assumption that any wholly terrestrial tetrapod was a reptile, while any partially aquatic tetrapod was an amphibian, wouldn't work either: there was too much ecological overlap in these early tetrapods. So palaeontologists had to look for osteological criteria on which to distinguish these animals, though their small size made this difficult—nearly all are under 30 cm long, and some are less than 10 cm long. The most reliable characteristic was found to be the structure of the vertebral centra: these seemed to have ossified in one block in the little tetrapods that were eventually called the Lepospondyls, while there were usually two pairs of ossifications in the larger forms known as Labyrinthodonts.

It eventually became clear that the lepospondyls included three distinct groups. The aquatic neotritideans had an elongate, deepened swimming tail,

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and the limbless autopods looked very like the living apodans. The bulk of the lepospondyls, which lacked these specialisations, were left in the order Microsauria. Although increasing understanding of early reptile evolution made it possible to identify several small reptilian genera and remove them from the Microsauria, the order itself remained poorly defined and lacking any comprehensive, well-illustrated descriptive and analytic review. Such a review has now been provided by Robert L. Carroll and Pamela Gaskill (*Mem. Amer. phil. Soc.*, 126, 1; 1978).

Carroll and Gaskill recognise 25 genera in the order Microsauria, including five new genera. Various characters of the skull, cranio-cervical joint and scales are diagnostic of the group, but the trunk vertebrae of some are similar to those of reptiles in having small intercentra. For many years, the best-known genus was *Microbrachis*; its clear lateral-line canals and external gills, elongate body, small limbs and three-fingered manus were therefore used as the model for the restoration of other, less complete microsauro genera. However, it is now clear that *Microbrachis* is a member of a sub-order of aquatic microsaurians that were much less varied than the remainder of the group. The members of the larger sub-order, which Carroll and Gaskill call the Tuditanomorpha, show considerable variation in the size and proportions of the body, and in the dentition.

The microsaurians occupied a number of different habitats. As well as the microbrachids, some other genera seem to have lived in ponds or streams (although not in deeper water), but the short body and massive limbs of genera such as *Tuditanus* suggest that they were wholly terrestrial. Microsaurians were covered by bony scales and heavy, double-headed ribs which may have been used in costal ventilation of the lungs. There is therefore no reason to suppose that they were as ecologically restricted as the scale-less, moist-skinned amphibians of today. Microsaurians were probably carnivorous; a number of genera have rather massive teeth, and probably preyed on molluscs or arthropods that had hard shells or carapaces.

Microsaurians are known only from the Pennsylvanian (Late Carboniferous) and Early Permian. They first appear alongside the first reptiles in the Early Pennsylvanian, but the microsaurians were at that time considerably more diverse than the reptiles, suggesting

that they had originated earlier and had had more time to diversify. But the reptiles quickly became the dominant group, and the microsaurians that survived into the Permian were mainly of the heavy-toothed, elongate type that looked less like reptiles and presumably competed less with them. The geographical distribution of microsaurians, to the adjoining continents of North America and Europe, is also notable. Carroll and Gaskill think that there is no particular significance in this fact, pointing out that Carboniferous deposits laid down on land or in shallow water are rare or unknown in Gondwanaland and Asia.

The general similarity in size and mode of life of microsaurians and modern amphibians makes it difficult to be sure

whether morphological similarities between the two groups indicate relationships or merely convergence. Although the Early Permian labyrinthodont *Doleserpeton* has been suggested as a possible ancestor of modern amphibians, Carroll and Gaskill point out that its only specific similarity is its possession of pedicellate teeth. There is still so great a gap in time between the earliest members of the modern amphibian orders and any of the Palaeozoic forms that there was ample time for their evolutionary transformation from any small Palaeozoic amphibian that had not already undergone divergent specialisations such as the loss of limbs. Only new Permian or Triassic discoveries are likely to solve this problem. □

Irreversible segregation in irradiated alloys

from R. W. Cahn

THE elementary theory of atomic diffusion shows that a solid solution, even if thermodynamically stable, cannot be in a state of complete equilibrium unless the concentration of solute attains a state of flat uniformity. The elementary theory of diffusion is always wrong, because no real solid is structurally uniform. Real pieces of alloy have free surfaces and contain grain boundaries, dislocations and other defects. It is well established that solute segregates to such singularities, and the degree of segregation in equilibrium depends in particular on the solubility of the solute: relatively insoluble species segregate more intensely. The temperature is the other major variable, and a lower temperature spells more segregation. This reversible process is known as equilibrium segregation; it has been intensively studied because of its practical implications, some (by no means all) deleterious. Increased intergranular corrosion and reduced ductility in creep are examples of consequential disadvantages, sintering of 'doped' alumina powder to give a translucent, pore-free solid is an instance of beneficial use. This scientifically most intriguing as well as practically important phenomenon has recently received a worthy review by Hondros and Seah (*Int. Metals Rev.* 22, 262; 1977). Equilibrium segregation is not, however, the sole form of segregation in solid solutions.

The alternative form is non-equilibrium or irreversible segregation, and it is associated with flows of point defects, lattice vacancies in particular. Such flows result when a solid is quenched from a high temperature so that it becomes super-saturated with vacancies, or else when it is being continuously

irradiated, with the consequential creation of a steady supply of vacancies and interstitial atoms. The flow of excess point defects is absorbed at a 'sink' (such as a free surface, grain boundary or dislocation) and the defects then disappear. If, in some way, the moving defects carry solute atoms along with them, then the resultant segregation is plainly irreversible, because it is brought about by a non-equilibrium, unidirectional process—the flux of point defects.

The study of this kind of segregation has been going on for about 10 yr and reviews are beginning to appear. An early account was by Anthony, who charmingly entitled it 'Atom Currents Generated by Vacancy Winds' (in *Diffusion in Solids* (ed. Nowick & Burton) Academic Press, 1975); a more comprehensive treatment, including the more recent irradiation studies which only began when Anthony was writing his review, was presented by Harries and Marwick of Harwell at a conference on 'Residuals, Additives and Materials Properties' in May 1978 (to be published by the Metals Society, London). With the help of some very recent papers, the true complexity as well as the practical implications, for nuclear reactors, of irreversible segregation is now becoming clear, and will be outlined here.

The experimental facts are that, under irradiation, solutes (both major and minor) segregate irreversibly to or from a free surface. The experiments are usually done with ion irradiation instead of neutrons, to hasten the

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process. This was discovered in 1974; a recent study is by Marwick and Piller (*Rad. Effects* **33**, 245; 1977) who found in a Ni-Mn-Si alloy that manganese concentrated in the vacancy-rich layer below the surface (the vacancy 'source') whereas silicon moved out of this layer towards the sinks; the segregation was very pronounced indeed. The same investigators (*J. Nucl. Mater.* **71**, 309; 1978) confirmed the segregation of nickel in a Fe-Ni-Cr alloy to the surface sink.

It has also been found that similar segregation takes place near the surface of the tiny voids that form in some metals and alloys, over a restricted temperature range, during irradiation. (These voids result from the fact that point defect sinks are apt to absorb interstitials slightly faster than vacancies, and so those vacancies which survive both recombination with interstitials and absorption at sinks, finish up in these minute, empty holes). This segregation process was first found in 1977 in aluminium (see account in *Nature* **262**, 671; 1977) and has now been confirmed by an exceedingly painstaking electron microprobe study at Harwell (Marwick *et al. Scripta Met.* **12**, 1014; 1978). Since the void diameter is mostly in the 100–200 nm range and the irradiation-induced segregation is confined to a layer adjacent to each void of about 100 nm thickness, the utmost resolution of a modern scanning electron microscope was required to demonstrate the segregation, and a number of possible errors had to be checked for. Nevertheless, the findings are quite unambiguous: in the Fe-Ni-Cr-Mo alloy used, the Ni/Fe and Ni/Cr ratios (these being the three major elements) are enhanced near the voids. This matches the earlier finding, that nickel is enriched near surface sinks.

These findings are by no means straightforward to interpret, because the theorist is faced by an embarrassment of alternative transport processes. The most obvious is by the formation of vacancy-solute and interstitial-solute associations; vacancies and self-interstitials in the host lattice become locked to solute atoms with variable binding energies, and the bound solutes are then dragged along with the point defect flux. The most comprehensive attempt to interpret in detail the recent findings in these terms is by Lam, Okamoto and Johnson (*J. Nucl. Mater.* **73**, 408; 1978), who improved a model they had been developing for several years. To make sense of the motion of various solutes either with the point defect flux (that is, to sinks) or against that flux (that is, to regions of peak radiation damage), they were forced to postulate very complex patterns of association; for instance, interstitial-solute associations were assumed to

differ so much in binding strength that some are readily mobile while others cannot move. There are too many adjustable parameters for comfort.

A novel alternative theory has been advanced by Marwick (*J. Phys. F: Metal Physics* **8**, 1849; 1978). Marwick concentrates attention on what he terms the 'inverse Kirkendall effect'. In the normal diffusional Kirkendall effect in a binary solid solution, found in the absence of irradiation, the vacancy flow is governed by the relative partial diffusion rates of the two constituents as they mingle with each other (that is, desegregate). The excess vacancies may form voids near the diffusion interface. Under irradiation, Marwick points out, the situation is stood on its head: a vacancy flow is imposed, and any disparity of partial diffusion rates leads to segregation of solute. The segregation process then creates a countervailing vacancy flux tending to cancel out that generated by irradiation—le Chatelier's Principle in operation. Marwick analyses the process in detail and proves that it is not at all necessary to postulate point defect-solute binding: segregation can result from the inverse Kirkendall effect alone. The faster diffusing species moves up the vacancy gradient (away from sinks) at a rate proportional to the difference between the two diffusivities; the slower diffusing species drifts to the sinks. This model makes sense of the observed enrichment of nickel, a slow diffuser, at surfaces and voids. More generally, Marwick postulates that the inverse Kirkendall effect largely governs the behaviour of concentrated alloy constituents while point defect-solute associations are likely to be the more important feature for dilute solutes.

Much interest has been expressed in the implications of irreversible segregation for void-swelling (Marwick *op. cit.*; Harries, *Nucl. Energy* **17**, 301; 1978). Structural materials and cladding in fuel assemblies for fast reactors are subject to slight but potentially dangerous swelling as voids form in those parts of the assemblies which are set at the relevant temperatures. Intensive research over the past few years has led to the use of cold-worked austenitic nickel-rich alloys (containing Fe+Ni+Cr+Si+Ti) which have excellent swelling resistance at all temperatures. It may now be possible to improve these alloys even further in the light of the new information on segregation, because it is clear (especially if the inverse Kirkendall effect is dominant) that the enrichment of slow-diffusing species, especially nickel, near incipient voids must impede further vacancy access, both because of the preponderance of a species in which vacancies move slowly and also because of the inverse vacancy flux (the balmy

vacancy wind) induced by the inverse Kirkendall effect. It seems likely that vacancy access to voids may be choked off almost completely, and some further accurate segregation tests with various solutes may point the way to even more effective anti-void measures. Minor solute elements may have a disproportionate effect if, as is quite possible, their presence has a large effect on the partial diffusivities of the major constituents and thus on the magnitude of the inverse Kirkendall effect. Future improvements may open up the way to fast reactor alloys containing less nickel than the present preferred alloy; nickel soaks up an undue number of neutrons and other alloys might be preferable from the viewpoint of neutron economy.

Another possibility is to prevent void formation altogether by using a non-crystalline alloy in which, presumably, no conventional vacancy flux can arise. Reichtin, Van der Sande and Baldo (*Scripta Met.* **12**, 639; 1978) have just reported that glassy Nb₄₀Ni₆₀ shows no void formation on heavy ion irradiation, and recommend that glasses like this be explored for use in nuclear reactors. □



A hundred years ago

IN the December part of the *Transunti* of the Royal Academy dei Lincei of Rome, Prof. Cossa gives an interesting account of his researches on the occurrence of the three metals cerium, didymium, and lanthanum. It appears that although these metals occur always in but minute quantities, yet their occurrence is far more frequent than is generally supposed, Prof. Cossa having been able to trace them even in bones and in the ashes of plants, not to speak of a number of minerals, such as certain apatites, Carrara marble, scheelite, &c. In Carrara marble Prof. Cossa found about two centigrammes of the mixed oxalates of cerium, lanthanum, and didymium in every kilogramme of marble; there were also traces of yttrium.

FURTHER experiments were made last Thursday in lighting the British Museum reading-room with the electric light. The result showed that by proper arrangement and at a comparatively moderate cost, there is good reason to believe that the end desired can be obtained. The Paris Société Générale d'Electricité have made the experiments at their own cost.

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review article

γ -Ray line astronomy

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γ -Ray astronomy is a valuable source of information on solar activity, supernovae and nucleosynthesis. Cosmic γ -ray lines were first observed from solar flares and more recently from the galactic centre and a transient event. The latter may give an important insight into nuclear reactions taking place near neutron stars and black holes and a measure of the gravitational redshifts of such objects.

γ -RAY lines are the most direct probe of cosmic nuclear processes. While it has been recognised^{1,2} for two decades that nuclear γ -ray spectroscopy could provide basic information on such problems as solar activity, supernova dynamics and nucleosynthesis, only recently have positive observations of celestial γ -ray lines been made.

The first cosmic γ -ray lines were observed³ from solar flares. These lines are produced in nuclear processes on time scales of several minutes by the interaction of flare-accelerated particles with the solar atmosphere. Solar γ -ray observations have provided⁴ important information on particle acceleration mechanisms, and on the flare process itself.

The strongest observed⁵ steady celestial γ -ray line is at 0.511 MeV resulting from positron annihilation. Many mechanisms can contribute to galactic positron production, and two of these, energetic particle reactions and nucleosynthesis, also produce other detectable lines. As we shall show, the bulk of the positrons responsible for this annihilation radiation may come from processes of explosive nucleosynthesis in supernovae. Such positrons result from relatively long-lived radioisotopes whose decay is accompanied by several strong de-excitation γ -ray lines. These lines have not yet been detected, but the predicted fluxes are within the range of instrumentation planned for forthcoming γ -ray observations. The measured 0.511-MeV line intensity already places constraints on the present rate of nucleosynthesis in the Galaxy.

Cosmic γ -ray lines from a transient event have recently been observed⁶. These lines seem to result from energetic particle reactions similar to those that take place in solar flares, but not in similar environments. The identification of the lines requires a redshift of a magnitude that suggests the strong gravitational fields of compact collapsed objects. Such γ -ray line observations may probe nuclear reactions taking place in the vicinity of neutron stars and black holes, and measure the gravitational redshifts of such objects⁷.

We first discuss the flare observations and their implications. The study of γ -ray lines from flares is important not only for gaining unique insights into the flare process, but also for the development of the theory of astrophysical γ -ray line production.

Solar γ rays

Solar γ -ray lines were first observed³ from the 4 and 7 August 1972 flares with a NaI(Tl) detector on the 7th Orbiting Solar Observatory (OSO 7). For the 4 August flare, the observed lines, at 2.22, 0.51, 4.44, and 6.13 MeV, and the continuum above 1 MeV, had fluxes of 0.28, 0.06, 0.03, 0.03, and 1 photons $\text{cm}^{-2} \text{s}^{-1}$ respectively. The duration of the event was about 10^3 s. Lines at 2.22 and 0.51 MeV were also detected during the decay phase of the 7 August flare, but only upper limits could be set on the 4.44 and 6.13 MeV lines and the continuum from this flare. Observations of the 2.2-MeV line have been reported^{8,9} from the flares of 22 November 1977 and 11 July 1978.

All these lines result from nuclear interactions of flare-accelerated particles most probably in the chromosphere or lower corona. The strongest line, at 2.223 MeV, is due to neutron capture on hydrogen, $^1\text{H}(n, \gamma)^2\text{H}$. The neutrons with initial energies of 1–100 MeV, are thermalised and captured in the photosphere. The width of the 2.223-MeV line, determined by the photospheric temperature, is very narrow (~ 100 eV). Neutron capture on ^1H , however, must compete⁴ with capture on ^3He , even though ^3He is only a minor constituent of the photosphere, because the cross-section for the reaction $^3\text{He}(n, p)^3\text{H}$ is about four orders of magnitude larger than that for the reaction $^1\text{H}(n, \gamma)^2\text{H}$. Observations of the 2.223-MeV line intensity set a limit on the photospheric $^3\text{He}/^4\text{He}$ ratio of several times 10^{-4} ; this limit is comparable to the measured $^3\text{He}/^4\text{He}$ ratio in the chromosphere and solar wind (see ref. 10).

Radiative neutron capture on ^{56}Fe , which produces two strong lines at 7.632 and 7.646 MeV, is also of considerable astrophysical interest⁷. These lines should be dominant in an iron-rich medium such as the surface of a neutron star.

The second strongest line from solar flares is at 0.511 MeV from positron annihilation. The principal sources of positrons in flares are π^+ mesons, short-lived radioactive positron emitters such as ^{11}C , ^{13}N , ^{14}O and ^{15}O , and the first nuclear level of ^{16}O at 6.052 MeV which decays by electron-positron pair emission. The initial energies of the positrons range from several hundred keV to tens of MeV, but only a small fraction annihilate at these high energies. The bulk of the positrons slow down to energies comparable with those of the ambient electrons, where annihilation takes place either directly or from bound states of

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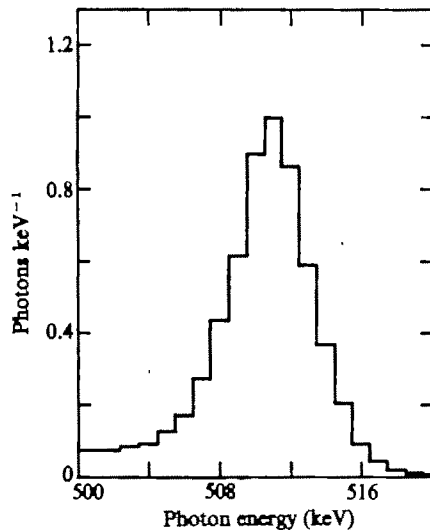


Fig. 1 Calculated¹² spectrum of positron annihilation radiation in cold atomic hydrogen.

positronium. Positronium is formed by radiative combination with free electrons and by charge exchange with neutral hydrogen; 25% of the positronium annihilates from the singlet state and 75% from the triplet state.

Direct annihilation produces two 0.511-MeV photons in the positron-electron rest frame. Singlet positronium also annihilates into two such photons, but triplet positronium decays into three photons which form a continuum at energies below 0.511 MeV. This continuum, however, is significant only if the ambient density is less than about 10^{15} cm^{-3} so that triplet positronium is not broken up by collisions before it can annihilate.

If the ambient medium is almost neutral ($n_e/n_H \leq 0.05$), about 95% of the positrons form positronium in flight. If on the other hand, the medium has a higher degree of ionisation, energy loss to the plasma thermalises¹¹ the positrons before they can annihilate directly or form positronium. In this case, direct annihilation dominates if the temperature is greater than about 10^6 K , while at lower temperatures the positrons form positronium by charge exchange and radiative combination.

If the density of the ambient medium is sufficiently low ($\leq 10^{15} \text{ cm}^{-3}$), the spectrum of the annihilation radiation in a neutral medium depends on the kinetic energy of the positronium formed in flight and on the positron-neutral atom interaction leading to direct positron annihilation. This spectrum, obtained¹² from a Monte Carlo simulation, is shown in Fig. 1. The full width at half maximum (FWHM) of the 0.511-MeV line is $\sim 5 \text{ keV}$, and the asymmetry due to triplet positronium annihilation can be clearly seen.

In an ionised medium, the width of the 0.511-MeV line depends mostly on the temperature; the FWHM is about 3 keV at 10^5 K and it varies approximately as the square root of the temperature. Note that in an ionised medium the 0.511-MeV line can be substantially narrower than in a neutral medium. For example, in a partially ionised warm gas at 10^4 K (such as in a young supernova remnant) the width of the line is $\sim 1 \text{ keV}$, while in a cold neutral medium (such as an interstellar cloud) the width is $\sim 5 \text{ keV}$.

The expected width of the 0.511-MeV line from solar flares could range¹¹ from a few keV to several tens of keV depending on whether the annihilation takes place predominantly in the cool photosphere or the hot flare plasma. Because of its low energy resolution, the NaI(Tl) detector on OSO 7 has set only an upper limit ($\sim 40 \text{ keV}$), but future high resolution observations, by measuring the line width, may determine the predominant positron annihilation site.

In addition to neutron and positron production, energetic particle reactions lead to many other lines resulting from de-excitation of nuclear levels. Figure 2 shows the spectrum from

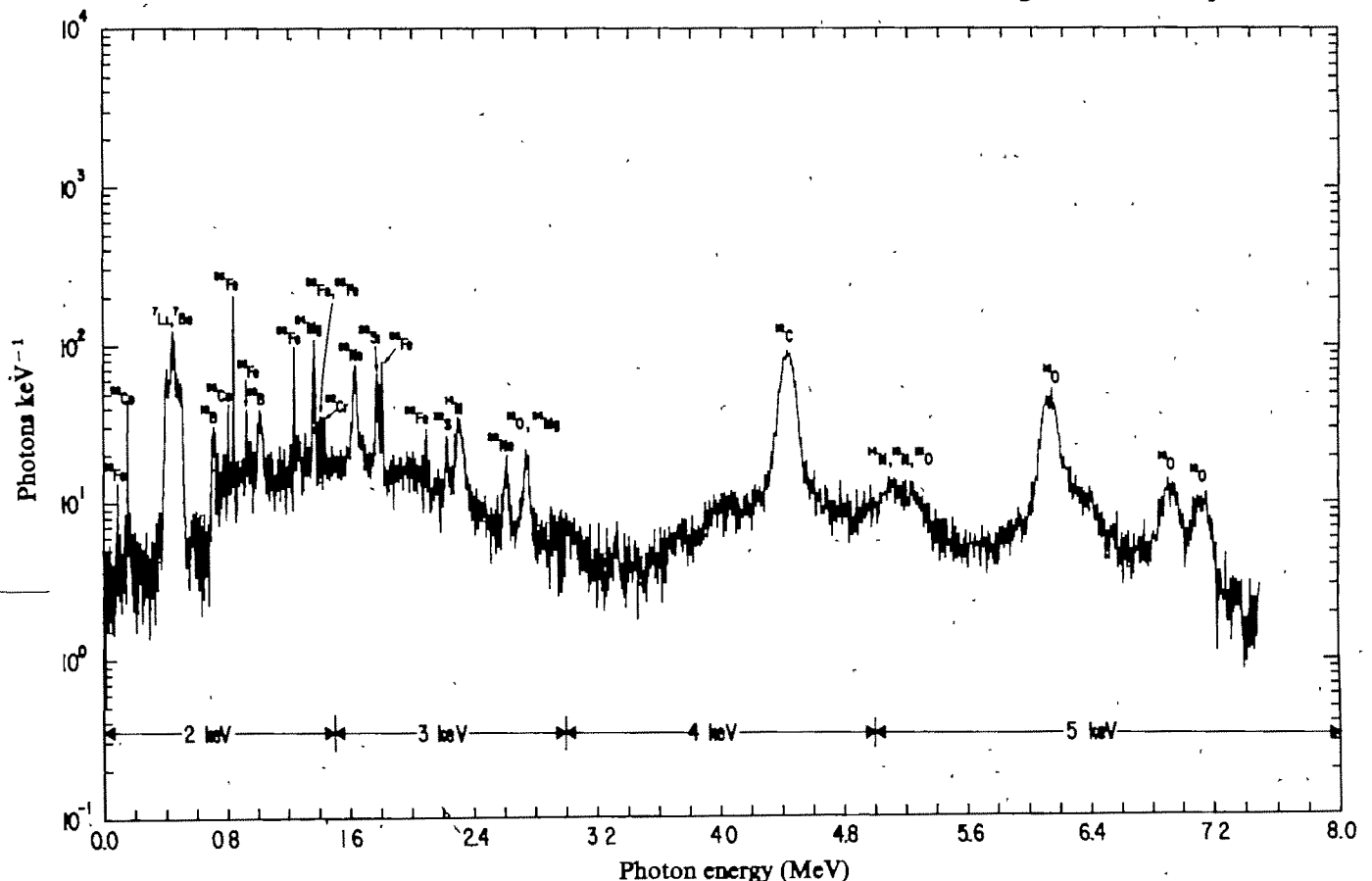


Fig. 2 Calculated¹³ nuclear de-excitation γ -ray spectrum from energetic particle reactions.

such de-excitation, calculated¹³ by a Monte Carlo simulation of an energetic particle population with spectrum proportional to E^{-2} interacting with a cold ambient medium. Here E is energy per nucleon, and both the energetic particles (at the same E) and the ambient medium are assumed to have solar composition. The narrow lines result from the de-excitation of ambient heavy nuclei excited by energetic protons and α particles, while the underlying broad component is due to de-excitation of energetic heavy nuclei interacting with ambient H and He. The strongest narrow lines, at 4.44 and 6.13 MeV, due to ^{12}C and ^{16}O de-excitation, respectively, were observed³ from the solar flare of 4 August 1972. Because the ratio of the intensities of the 4.44- and 2.223-MeV lines depends strongly on the spectrum of the energetic particles, the observed ratio provides information on this spectrum in the flare region⁴. The broad component, as well as other narrow lines which were not resolved in the 4 August 1972 observations, could account^{14,15} for all of the observed radiation above 4 MeV. Several other narrow lines could also be detected from large flares with instrumentation of higher sensitivity than that flown on OSO 7, for example, the 0.847-MeV line (Fig. 2) from ^{56}Fe and the 15.11-MeV line¹⁶ from ^{12}C .

An important result¹⁰ of solar γ -ray studies concerns the acceleration of protons, nuclei and electrons to energies greater than about 1 MeV. It seems that the mechanism for this acceleration differs from the bulk energisation of solar electrons to tens of keV where they are manifest in powerful hard X-ray emission. Acceleration to higher energies (>1 MeV), on the other hand, is probably due to a stochastic Fermi-type process which imparts $\sim 1\%$ of the flare energy to a small fraction of the ambient particles. This acceleration may be similar to galactic cosmic-ray acceleration, as several of the properties of the energetic solar particles, such as chemical composition and proton-to-electron ratio, resemble those of the cosmic rays.

Steady galactic γ -ray lines

The best observed^{5,17} nontransient galactic γ -ray line is at 0.511 MeV from the galactic centre. The observed⁵ line at 510.7 ± 0.5 keV has an intensity of 1.2×10^{-3} photons $\text{cm}^{-2} \text{s}^{-1}$ and FWHM < 3.2 keV. This observation was made in 1977 with a balloon-borne high purity Ge detector of opening angle 15° centred on the galactic centre. The line energy clearly identifies it as positron annihilation radiation; there is also some evidence for the three-photon continuum from triplet positronium annihilation.

The observed spectrum⁵ is shown in Fig. 3; the solid curve is the sum of the extrapolated low energy continuum (dashed curve) and a three-photon continuum assuming $\sim 90\%$ annihilation by positronium. This fraction is consistent with positron annihilation in a low density ($< 10^{15} \text{ cm}^{-3}$) and low temperature ($< 10^6 \text{ K}$) medium¹², and implies about 0.6 photons in the 0.511-MeV line per positron.

The 3.2-keV upper limit on the width of the 0.511-MeV line suggests¹² that the bulk of the positrons annihilate in an ionised medium ($n_e/n_H \gg 0.05$), because in cold interstellar clouds, the line width (~ 5 keV, Fig. 1) would exceed the observed upper limit. Possible sites of annihilation could be supernova remnants, H II regions at the galactic centre and the warm ionised component of the interstellar medium. Observations with higher spatial resolution could help determine the nature of source.

Turning now to the origin of the positrons, we first consider π^+ mesons produced in high energy (> 100 MeV) cosmic ray interactions in the interstellar medium. The π^+ mesons, produced in proton-proton and proton-helium interactions, decay through μ^+ mesons into positrons. An upper limit on this positron source can be set from high-energy (> 100 MeV) γ -ray observations¹⁸, as at least part of these γ rays should result from the decay of π^0 mesons which are also produced in the above interactions. From the measured π^+ and π^0 production cross

sections, we find that the observed¹⁸ flux ($\sim 3 \times 10^{-5}$ photons $\text{cm}^{-2} \text{s}^{-1}$) of > 100 MeV γ rays from a 15° cone centred on the galactic centre implies that not more than about 2% of the observed⁵ 0.511-MeV line can be from the annihilation of positrons from π^+ decay.

There is probably also a distinct low-energy (< 100 MeV) cosmic-ray component; the interstellar density of this component, however, is not known because solar modulation excludes the bulk of the low-energy particles from the inner Solar System. γ -Ray line production from these cosmic rays has been recently evaluated¹⁵ under the assumption that their local interstellar density is 1 eV cm^{-3} , and that there are substantial gradients along the line of sight towards the galactic centre in both the cosmic-ray energy density and heavy element abundances. The resultant γ -ray line emission, including the 0.511-MeV line shown in Fig. 4, is close to its maximal allowed value. If the cosmic-ray intensity were increased, X-ray line emission at ~ 6.8 keV due to charge exchange of fast Fe ions with interstellar matter would exceed the observational upper limit on this line, and if the heavy element abundances along the line of sight were increased, the absorption of X-rays would be larger than observed. The 0.511-MeV line intensity from low-energy cosmic rays, therefore, should not be larger than $\sim 7 \times 10^{-5}$ photons $\text{cm}^{-2} \text{s}^{-1} \text{ rad}^{-1}$, or also about 2% of the observed line intensity in a 15° cone around the galactic centre.

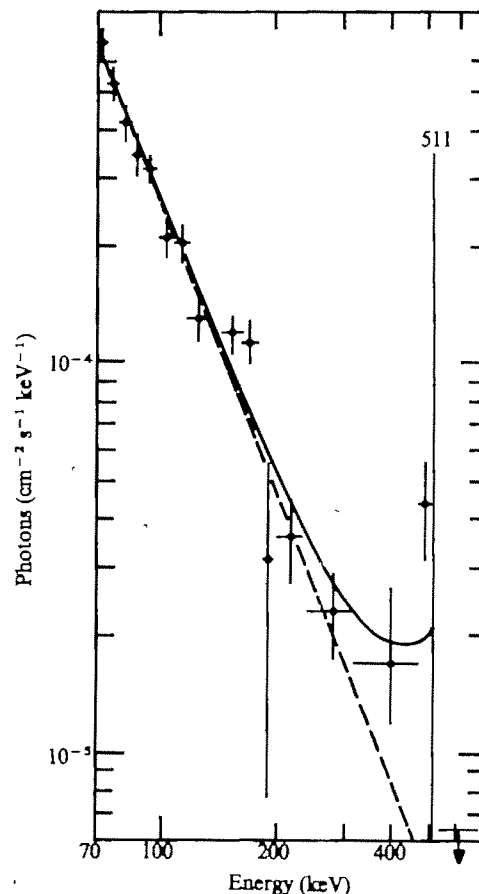


Fig. 3 Observed⁵ 0.511-MeV line and low-energy γ -ray spectrum from the galactic centre.

Low-energy cosmic-ray interactions in the interstellar medium, nevertheless, produce a rich γ -ray spectrum which includes many nuclear de-excitation lines, as can be seen in Fig. 4. Of special interest are the very narrow lines, such as that at 6.129 MeV from ^{16}O , resulting from de-excitation of nuclei in interstellar grains¹⁹. The line broadening, which in gases is caused by the recoil velocities of the excited nuclei, is greatly reduced in solids where these nuclei or their radioactive

progenitors can come to rest before de-excitation. The detection of γ -ray lines from low-energy cosmic-ray interactions in the interstellar medium with forthcoming instrumentation would not only measure the unknown interstellar density of these cosmic rays, but also provide unique information on the distribution, motion, composition and size of interstellar dust grains.

Returning to the question of the origin of the positrons, energetic particle reactions at low energies (<100 MeV) could produce positrons in discrete sources (such as in the vicinity of a massive black hole at the galactic centre⁷) without violating the constraints set by the X-ray observations¹³. If this is the case, the 0.511-MeV line should be accompanied by strong broad line emission from nuclear de-excitation, in particular the 4.44-MeV line from ^{12}C . This line may have been observed¹⁷ in 1974 with a balloon-borne NaI (TI) detector, but this measurement was not confirmed by recent observations (J. L. Matteson, personal communication) with the first High Energy Astronomical Observatory (HEAO 1). The discrepancy could be due to time variations which will be further explored with this and forthcoming satellite-borne γ -ray spectrometers.

If, however, the nuclear interaction rate is essentially constant over the positron annihilation time, then the HEAO 1 upper limit on the 4.44-MeV line intensity from the galactic centre would require that not more than about 30% of the observed 0.511-MeV line result from such energetic particle reactions. This conclusion is only weakly dependent on the assumed composition of the ambient medium and the energetic particles, since the major contributors to positron production, C, N, and O, also produce 4.44-MeV line emission by either direct excitation or spallation. The energetic particle spectrum also does not greatly affect the positron-to-4.4-MeV photon ratio¹³. Thus, some other galactic positron sources may also be required to account for the observed 0.511-MeV line.

One such source^{2,20,21} could be relatively long-lived positron-emitting radioisotopes produced by nucleosynthesis during the explosive ejection of matter in supernova explosions. The decay of these isotopes is accompanied by de-excitation γ -ray lines. The principal isotopic decay chains are shown in Table 1, together with the relevant mean lives, de-excitation line energies, and number of positrons and photons per disintegration. The isotopic yields, normalised to ^{56}Ni , are based on Solar System abundances²⁴, and on the assumption^{22,23,25} that all of the Solar System ^{56}Fe , ^{57}Fe and ^{44}Ca , and 1%, 0.5%, and 0.1% of the ^{60}Ni , ^{22}Ne and ^{26}Mg , respectively, are produced explosively through the decay chains shown in Table 1.

Even though nucleosynthesis takes place in dense regions which are opaque to γ rays, the expanding envelope can become transparent if there is sufficient delay between the synthesis of the isotopes and the emission of the photons. For the de-excitation lines, the delay is due only to the lifetime of the parent isotope. But for the 0.511-MeV line, the delay can be longer because of the finite duration of positron slowing down and annihilation. Thus, even relatively short-lived isotopes such as ^{56}Co may be important sources of positrons for annihilation radiation.

Although the lifetime of ^{56}Co may not be long enough for the supernova to become transparent to its de-excitation lines, a significant fraction of the positrons from ^{56}Co may escape from the supernova²⁰ and annihilate in regions which are transparent to the γ rays. Furthermore, even if transparency is achieved by sufficiently rapid expansion of the supernova envelope and low ejected mass, the de-excitation lines from ^{56}Co decay could only be seen for a short time (a few years) after the explosion. Therefore, these lines would be observable mostly from extragalactic supernovae from which the expected fluxes are relatively small. The slowing down and annihilation times of the positrons, however, can be longer than the interval between supernova explosions²¹, and this could lead to an essentially steady galactic emission at 0.511 MeV. The time dependences and detectability of the various other de-excitation lines listed in Table 1 have been discussed in detail elsewhere²³.

Table 1 Isotopic decay chains from nucleosynthesis in supernovae

Decay chain	Mean life (yr)	$Q/Q(^{56}\text{Ni})$	Positrons or photons per disintegration	
$^{56}\text{Ni} \rightarrow ^{56}\text{Co} \rightarrow ^{56}\text{Fe}$	0.31	1	e^+	0.2
			0.847 MeV	1
			1.238 MeV	0.7
$^{57}\text{Co} \rightarrow ^{57}\text{Fe}$	1.1	2×10^{-2}	0.122 MeV	0.88
			0.014 MeV	0.88
$^{22}\text{Na} \rightarrow ^{22}\text{Ne}$	3.8	5×10^{-3}	e^+	0.9
			1.275 MeV	1
$^{44}\text{Ti} \rightarrow ^{44}\text{Sc} \rightarrow ^{44}\text{Ca}$	68	2×10^{-3}	e^+	0.94
			1.156 MeV	1
			0.078 MeV	1
			0.068 MeV	1
$^{60}\text{Fe} \rightarrow ^{60}\text{Co} \rightarrow ^{60}\text{Ni}$	4.3×10^5	1.5×10^{-4}	1.332 MeV	1
			1.173 MeV	1
			0.059 MeV	1
$^{26}\text{Al} \rightarrow ^{26}\text{Mg}$	1.1×10^6	1.5×10^{-4}	e^+	0.85
			1.809 MeV	1

It has been estimated²⁰ that $\sim 10\%$ of the positrons from ^{56}Co decay could escape from the supernova. This escape fraction would make ^{56}Co the dominant positron source for processes of nucleosynthesis, as can be seen from Table 1. The production of $\sim 2 \times 10^{43}$ positrons s^{-1} in the Galaxy, as indicated by the 0.511-MeV line observations⁵, implies a present rate of nucleosynthesis of $\sim 10^{43}$ iron nuclei s^{-1} . This is $\sim 10\%$ of the average rate of nucleosynthesis over the age of the Galaxy, and is consistent with current models²⁶ of galactic chemical evolution. The fraction of escaping positrons from supernovae is very uncertain, however, and requires further theoretical study. If the escape fraction for ^{56}Co were much lower than 0.1, ^{22}Na decay could become the dominant positron source, as can be seen from Table 1. But the ^{22}Na yield from supernovae²⁵ is also very uncertain. ^{22}Na is also produced in novae, with a yield of about 10^{48} nuclei per nova explosion²⁷. To produce $\sim 2 \times 10^{43}$ positrons s^{-1} in the Galaxy, however, ~ 600 novae per year would be required, and this rate is higher by an order than current estimates of the galactic nova rate.

As there are other potential positron sources, a test of the explosive nucleosynthetic origin would be the detection of the associated de-excitation γ -ray lines. A good prospect is the 1.809-MeV line from ^{26}Al decay²⁸. Because of the long lifetime of this isotope, the 1.809-MeV line should be observed as steady galactic emission with an intensity of $\sim 5 \times 10^{-5}$ photons $\text{cm}^{-2} \text{s}^{-1} \text{rad}^{-1}$ from the direction of the galactic centre; the long half-life of the ^{26}Al also implies a very narrow width for this line (~ 3 keV).

Another possible positron source could be pair production in the magnetosphere of pulsars²⁹. But observations of the Crab Nebula have so far not detected line emission at 0.511 MeV from this object. Although a line at 0.400 MeV, attributed to gravitationally redshifted 0.511-MeV emission was detected³⁰ from the general direction of the Crab, this line was not seen⁶ in an earlier flight which viewed the same region. This suggests that the 0.400-MeV line from the direction of the Crab could be due to a transient event.

Transient γ -ray lines

In addition to steady γ -ray line emission exemplified by the 0.511-MeV line from the galactic centre, γ -ray lines have also been observed from transient events. γ -Ray bursts of duration up to tens of seconds are a well recognised cosmic phenomenon, but their energy spectra have not yet been measured with sufficient resolution to discern whether they contain significant line emission. There seems, however, to be another class of

γ -ray transients in which essentially all the observed radiation is in the form of lines. Such a γ -ray line transient was discovered⁶ with a balloon borne Ge(Li) detector on 10 June 1974. This event, lasting ~ 20 min was characterised by strong emission in four relatively narrow energy bands or lines with no detectable continuum. The four observed lines at energies of 0.40–0.42, 1.74–1.86, 2.18–2.26 and 5.94–5.96 MeV had average intensities of 7×10^{-3} , 3.2×10^{-2} , 1.5×10^{-2} and 1.5×10^{-2} photons $\text{cm}^{-2} \text{s}^{-1}$, respectively, during the event. These lines showed statistically significant increases in emission compared both to the intensity in the adjacent continuum and to that in the same energy bands before and after this event. A simultaneous increase was observed in the CsI(Na) shield detectors with an intensity consistent with that observed just in the lines. This transient source seems to lie within $\sim 20^\circ$ of RA 7h and dec 20° . One possible candidate for the source is the object $\gamma 195+5$ which has been observed³¹ in high-energy γ rays but not yet at other wavelengths. The Crab Nebula was also near the edge of the field of view.

There are no simple nuclear schemes that can account for all four observed lines, as there are no obvious candidate nuclei for direct emission at ~ 0.41 and 5.95 MeV. A source seems to be required⁷ which is effectively bimodal, producing both redshifted and non-redshifted lines. The observations could then be understood in terms of neutron capture and positron annihilation, which are also the strongest line producing mechanisms in solar flares. Specifically, the ~ 0.41 -MeV line could be redshifted positron annihilation radiation and the 1.79- and 5.95-MeV lines could be redshifted neutron capture on ^1H and ^{56}Fe , respectively. The required redshift Z of between 0.24 and 0.28, could arise from the gravitational field of a neutron star of mass 1.4–1.8 $M_\odot$. Episodic accretion onto such a neutron star from a close binary companion could lead to the formation of a high temperature ($>10^{10}$ K for ions) accretion disk in which

nuclear interactions could take place producing neutrons and positrons. Positron annihilation and neutron capture on iron and hydrogen at and near the surface of the neutron star would produce the observed redshifted line emission, while the same processes in the accretion disk and in the atmosphere of the companion star would produce essentially unshifted lines, of which only the 2.223-MeV line from neutron capture on hydrogen was observed. The unshifted 0.511-MeV positron annihilation line was not seen because of the large atmospheric and detector background at this energy. The double line from neutron capture on iron should be significant only in the redshifted emission from the iron-rich surface of the neutron star but not in the unshifted emission.

As mentioned above, the 0.400-MeV line observed³⁰ in May 1976 from the general direction of the Crab Nebula may also have been a γ -ray line transient as it was not detected⁶ in an earlier flight. The June 1974 and May 1976 events may in fact have the same source, as both observations included much of the same field of view; the latter event, however, was weaker by a factor of 3.

Extragalactic γ -ray lines

γ -ray lines at 4.4 and 1.6 MeV have been reported³² from the radiogalaxy Centaurus A. The 0.511-MeV line, however, which is so prominent in the spectra of solar flares and the galactic centre, was not observed from Cen A. If the γ -ray lines from Cen A are produced⁷ in quasispherical nuclear reactions in the vicinity of a massive black hole³³, then the absence of the 0.511-MeV line could be due to the large Doppler broadening in the hot accretion gas which would make the line unobservable above the continuum. γ Rays in the MeV region have also been reported³⁴ from the Seyfert galaxy MCG4151, but the energy resolution was not sufficient to resolve possible line emission.

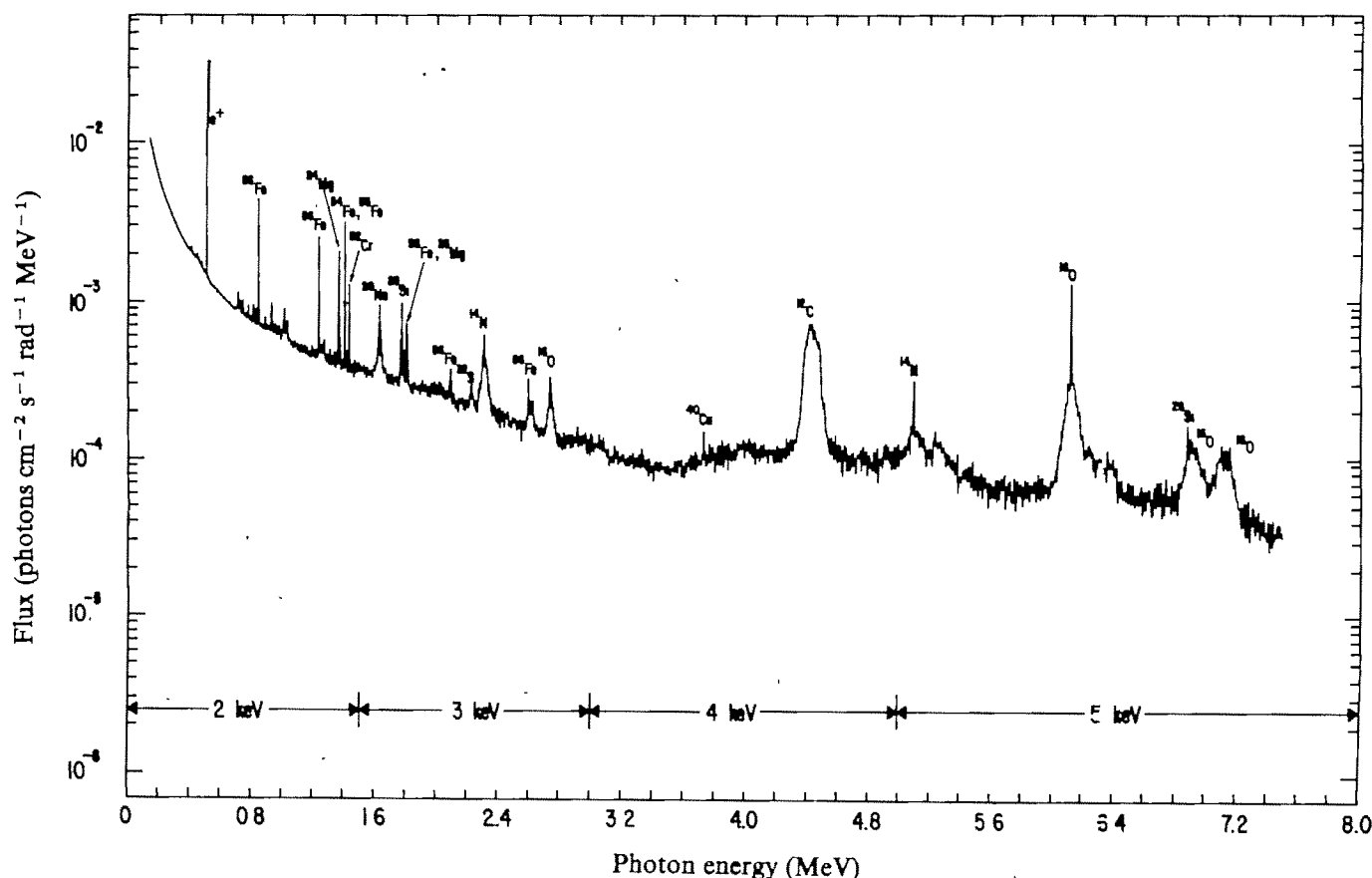


Fig. 4. Expected¹³ γ -ray spectrum from low-energy cosmic-ray interactions in the galaxy in the general direction of the galactic centre.

Outlook

Information on many astrophysical process and sites is encoded in the γ -ray line observations reviewed here. Following these exciting discoveries, γ -ray line astronomy is now moving into an era of more systematic study, made possible by the launch of HEAO 1 and the anticipated launches of HEAO C and the Solar Maximum Mission (SMM) in the autumn of 1979. The γ -ray spectrometer on HEAO 1 is already giving results. Within a year, solar flares will be explored with the γ -ray spectrometer³⁵ on SMM, and galactic and extragalactic γ -ray lines will be studied by a high resolution spectrometer³⁶ on HEAO C. At the same time, high altitude balloon-borne spectrometers should continue to provide important results on discrete sources.

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Looking further into the future, perhaps the most exciting prospect for γ -ray line astronomy will come with the launch of the Gamma Ray Observatory in 1984. This observatory is not yet an approved mission. Two of its five experiments, a Ge spectrometer and a NaI spectrometer, will observe γ -ray lines with much higher sensitivities than the instruments flown to date or planned for the immediate future. Together, these satellite and balloon-borne experiments should establish γ -ray line astronomy as a vigorous observational science.

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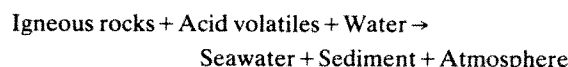
Water–rock partition coefficients and the composition of seawater and river water

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Using a simple chemical reactor model a strong correlation is established between the mean oceanic residence time of an element and the partitioning of that element between seawater and crustal rock. The ocean–rock partition coefficient ($K_{Y(sw)}$) is shown to be related to the electrostatic contribution to the element–oxygen bond energy (Q_{YO}) and a second correlation is established between these parameters. In this way the concentrations of all the elements so far identified in seawater fall in a coherent pattern. The global mean composition of river water, which represents an intermediate step in the formation of seawater from rock weathering, is shown to conform to a similar pattern.

THE concentrations of nearly 70 elements have now been determined in seawater and, although the chemistry of seawater has been frequently reviewed^{1–3} no general rules have emerged that enable the concentrations of all the elements to be incorporated in a simple yet coherent pattern. According to one hypothesis^{4–6}, seawater has been produced by the reaction,



As there have been no major changes in the mean composition of either the igneous rocks or the sediments over the past few billion years^{4,7} the composition of seawater has probably remained fairly constant over this period—in order of magnitude terms at least. Aided by the vast flux of material through the oceans^{5,7} and by the uplift and reweathering of sedimentary rock^{7,8} (sometimes following metamorphism) the ocean–rock

system has run so close to a steady state that, for more than 60 elements the rate of input to the oceans (by river transport) is almost exactly balanced by the rate of removal (by sedimentation)⁹. As the oldest marine sediments are no more than a few hundred million years old it is possible that the same material has passed through the oceans several dozen times during the Earth's history. This suggests that it should be possible to use the partitioning of the elements between the oceans and the crustal rocks to provide further insight into the nature of seawater itself.

Before we can draw meaningful conclusions from such an exercise we must consider the reactivity of the elements in seawater because, as Forchhammer¹⁰ pointed out in 1865, "the quantity of the different elements in seawater is not proportional to the quantity of the different elements which river water pours into the sea, but inversely proportional to the facility with which elements in seawater are made insoluble by general chemical or organochemical reactions in the sea". The modern approach to Forchhammer's concept of chemical reactivity in the oceans is to define a mean oceanic residence time ($\bar{t}_Y$) (ref. 11). Elements will have a short residence time if they are readily incorporated in hydrogenous mineral phases and hence rapidly removed from the oceans. Over the 2,000 Myr period that we postulate for our steady-state ocean, the water has been stirred at close to 1,000 revs Myr⁻¹. If we approximate the behaviour of this well-stirred system by a continuously stirred tank reactor (or ocean bucket) which includes the world's estuaries we can use the notation of chemical reactor theory¹² to define $\bar{t}_Y$ so that

$$\bar{t}_Y = M_Y / \bar{m}_Y \quad (1)$$

where M_Y is the total mass of Y dissolved in the oceans^{11,13} and $\bar{m}_Y$ is the mean flux of Y through the oceans in unit time. If $\bar{t}_Y$ is less than the time (t_R) required for one stirring revolution of the reactor then $\bar{t}_Y$ can not strictly be interpreted as a residence time.

The weathering cycle and oceanic residence times

To relate mean oceanic residence times to the weathering cycle we introduce a crude model. The ocean bucket will be considered as one component in a sequence of interlinked reservoirs^{14,15} where the rate of transport of material from one reservoir to the next is directly proportional to the total amount of Y in the source reservoir. The subscripts A, B and C will represent the crustal rock, river water and oceanic reservoirs respectively. If we assume that the oceans are at a steady state with respect to the flux of dissolved components and that the composition of reservoir A remains constant, it can be shown that, for first order transfer kinetics over a period that greatly exceeds the residence time of elements in reservoir B,

$$k_C \bar{Y}_C N_C = k_B \bar{Y}_B N_B = k_A \bar{Y}_A N_A$$

so that

$$\bar{t}_Y = \bar{Y}_C N_C / k_B \bar{Y}_B N_B = \bar{Y}_C N_C / k_C \bar{Y}_C N_C = \bar{Y}_C N_C / k_A \bar{Y}_A N_A \quad (2)$$

N_X is the anhydrous mass (kg) of the reservoir X and $\bar{Y}_X$ is the global mean concentration ($\mu\text{mol per kg solids}$) of the element Y

in the reservoir X so that $M_Y = N_C \bar{Y}_C$. k_X is the first order rate constant for transfer of material from reservoir X. Taking logarithms in equation (2) we find that

$$\log \bar{t}_Y = \log K_{Y(\text{ow})} - \log (k_A N_A / N_C) \quad (3)$$

where

$$K_{Y(\text{ow})} = \bar{Y}_C / \bar{Y}_A$$

Equation (3) suggests that there should be a simple relationship between the partitioning of the elements between the oceans and the crustal rocks (resulting from the long-term recycling of the system) and the mean oceanic residence times (which reflect the reactivity of the individual elements in the oceans). Using tabulated values of $\bar{Y}_A$ (ref. 16), $\bar{Y}_B$ (corrected for recycled sea spray)^{17,18} and $\bar{Y}_C$ (ref. 13) we can calculate $\log \bar{t}_Y$ and $\log K_{Y(\text{ow})}$ and produce a plot (Fig. 1) showing a significant correlation for more than 40 elements that can be summarised by the equation

$$\log \bar{t}_Y = a_0 \log K_{Y(\text{ow})} + b_0 \quad (4)$$

a_0 does not have the expected value of unity (Table 1) but this is not surprising considering the crudity of the model. The $\bar{Y}$ -values used to characterise the reservoirs are at best only order of magnitude estimates for most of the elements and no account has been taken of the individual chemical behaviour of the various elements. Despite these limitations only three elements fall clearly outside the correlation. Nitrogen enters the oceans as nitrate but, rather than accumulating in the oceanic reservoir it is converted to gaseous nitrogen under anoxic conditions and is then lost to the atmosphere. An ocean at thermodynamic equilibrium would contain 0.1 M nitrate ion²¹. To account for the behaviour of this element and also for that of carbon, a fifth reservoir (the atmosphere) should be introduced into the model. Because of this anomalous behaviour, nitrogen was omitted from the statistical analysis of Fig. 1. For silver and antimony quite large discrepancies arise between the residence times calculated from the input of dissolved solids by the world's rivers¹⁷ and those calculated from the formation of hydrogenous sediments^{22,23}. If the sedimentation values, rather than the river input values, are plotted for these elements the points fall within the correlation limits (Fig. 1, arrowed data points). Only five elements (Fe, Al, La, Zr and Pb) have residence times that are less than t_R and these elements form a tightly grouped set in Fig. 1. From tabulated values of $\bar{Y}_A$ (ref. 16) and $\bar{Y}_C$ (ref. 13) equation (4) can be used to calculate mean oceanic residence times for elements not included in Fig. 1 that should be accurate to within an order of magnitude.

The importance of the residence time concept for estimating the response of the oceans to changes in the rate of input of material has recently been re-emphasised²⁴. Because the time span of the injection must be greater than t_R for a valid application of the residence time concept, the use of $\bar{t}_Y$ -values for predicting the effects of anthropogenic inputs over the next few centuries must be treated with caution. However, these values should be useful for discussing the stability of ocean chemistry in the geological past.

Table 1 Linear regression fitting parameters for the correlations discussed in the text ($y = ax + b$)

Fig. no.	Elements considered	n^*	y	x	a	b	$Sy, x^\dagger$	$r^\ddagger$	$r_{0.1}^\S$
1	All except N	44	$\log \bar{t}_Y$	$\log K_{Y(\text{ow})}$	0.59 ± 0.05	6.25 ± 0.16	0.77	0.88	0.52
2	Na, K, Rb, Cs	4	$\log K_{Y(\text{ow})}$	Q_{YO}	-3.27 ± 0.77	23.12 ± 5.62	0.59	0.95	0.999
2	Li, Mg, Ca, Sr, Ba	5	$\log K_{Y(\text{ow})}$	Q_{YO}	-1.68 ± 0.55	9.65 ± 3.42	0.67	0.87	0.99
2	Main body of elements	60	$\log K_{Y(\text{ow})}$	Q_{YO}	-1.21 ± 0.10	0.68 ± 0.36	1.23	0.85	0.42
3	Li, Na, K, Rb, Cs, Mg, Ca, Sr, Ba	9	$\log K_{Y(\text{ow})}$	Q_{YO}	-0.59 ± 0.21	4.27 ± 1.38	0.44	0.73	0.95
3	Main body of elements	36	$\log K_{Y(\text{ow})}$	Q_{YO}	-0.77 ± 0.12	2.36 ± 0.34	0.95	0.75	0.55

* Number of data points.

† Standard error of estimate of y on x .

‡ Correlation coefficient.

§ Value of r that must be exceeded for the correlation to be significant at the 0.1% level²⁰.

|| This correlation is significant at the 5% level.

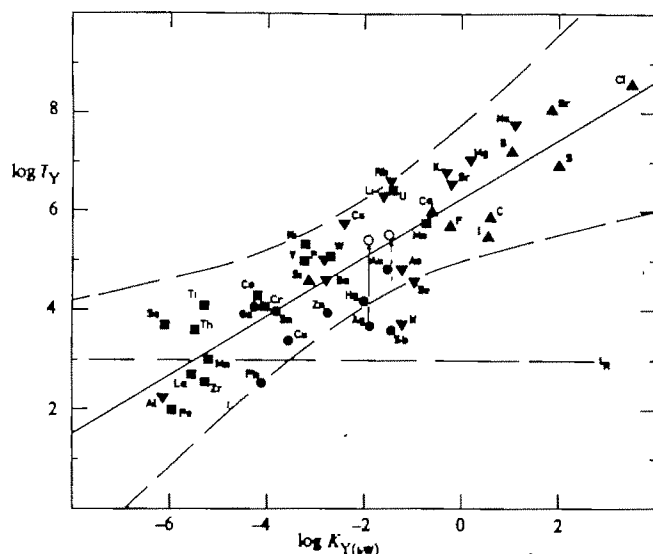


Fig. 1 The relationship between the mean oceanic residence time ($\bar{t}_Y$) and the seawater-crustal rock partition function ($K_{Y(sw)}$) of the elements. ∇ , pre-transition metals; $\blacksquare$, transition metals; $\bullet$, B-metals; $\blacktriangle$, non-metals. Open symbols indicate $\bar{t}_Y$ -values estimated from sedimentation rates. The solid line indicates the linear regression fit (Table 1) and the dashed lines the Working-Hotelling confidence band at the 0.1% significance level¹⁹. Nitrogen was omitted from the statistical analysis for reasons given in the text. The horizontal broken line indicates the time required for one stirring revolution of the ocean (t_B). (The source data used in the preparation of Figs 1 to 4 could not be included in this paper because of space limitations but they are available in tabulated form on request from the authors.)

Relationship between composition of river water and seawater

Rather than attempting to develop a more complex model to account for the observed values of a_0 and b_0 (equation (4), Table 1) we will treat equation (4) as an empirical relationship and will focus instead on the factors controlling $K_{Y(sw)}$. Substituting $\bar{t}_Y$ from equation (2) into equation (4) we can show that

$$\log(\bar{Y}_C/\bar{Y}_B) = a_0 \log(\bar{Y}_C/\bar{Y}_A) + b'_0 \quad (5)$$

where

$$b'_0 = b_0 + \log(k_B N_B / N_C)$$

This equation is an empirical relationship independent of the multiple reservoir model. Subtracting $\log K_{Y(sw)}$ from both sides of equation (5) results in

$$\log K_{Y(rw)} = (1 - a_0) \log K_{Y(sw)} + b'_0 \quad (6)$$

where $K_{Y(rw)} = \bar{Y}_B/\bar{Y}_A$. Equation (6) suggests that river water represents an intermediate stage in the formation of seawater from the weathering of rocks, and that the partitioning of elements between the solid and aqueous phases is controlled by the same factors in each case. The controlling factor cannot generally be precipitation of simple salts (such as hydroxides, and carbonates) as solubility product calculations show that the majority of the elements in seawater have lower concentrations than the limit set by their most insoluble compounds^{22,25}. Schindler²⁶ has suggested that adsorption-desorption reactions at the surfaces of minerals which are essentially oxide-dominated matrices (mainly silicates, aluminosilicates, oxides, hydroxides and carbonates) control the concentrations of many trace metals in seawater. He has produced some convincing calculations for mean oceanic residence times on the assumption that the rate of removal of trace elements from the oceans is controlled by adsorption reactions at the surface of amorphous

silica. In this and other oxide matrices the elements attach themselves to the mineral surfaces through the deprotonated surface hydroxyl groups²⁶. This is essentially an ionic interaction, the strength of which can be related to the electronegativity of the element in question²⁷.

Electronegativity and the partitioning of elements

Pauling²⁷ suggested that the nature of the chemical bond formed between any two atoms could be related to the ability of the individual atoms to attract the electrons available for bond formation. To this end he formulated an electronegativity scale that provided a crude but effective measure of the electron attracting capabilities of the elements. The measured energy of the Y-O bond is found to exceed the arithmetic mean of the Y-Y and O-O bond energies by a quantity Q_{YO} which can be related to the electronegativities of the elements (x_Y and x_O respectively) by

$$Q_{YO} = (x_Y - x_O)^2 eV \quad (7)$$

Q_{YO} is therefore a measure of the electrostatic contribution to the Y-O bond energy and we will use Q_{YO} as a measure of the attraction that an oxide-based mineral lattice will exert on the element Y. Using tabulated electronegativity values^{16,28} we can plot $\log K_{Y(sw)}$ (Fig. 2) and $\log K_{Y(rw)}$ (Fig. 3) against Q_{YO} . In accordance with equation (6) we find that $\log K_{Y(rw)}$ and $\log K_{Y(sw)}$ show similar dependence on Q_{YO} and that $\log K_{Y(rw)}$ shows the more marked dependence. In each case there are significant linear correlations between $\log K_Y$ and Q_{YO} (Table 1) summarised by the equation,

$$\log K_Y = a_1 Q_{YO} + b_1 \quad (3)$$

The smaller number of data points and greater scatter of the river water plot (Fig. 3) reflect the greater difficulty of defining and quantifying a meaningful average for river water compared

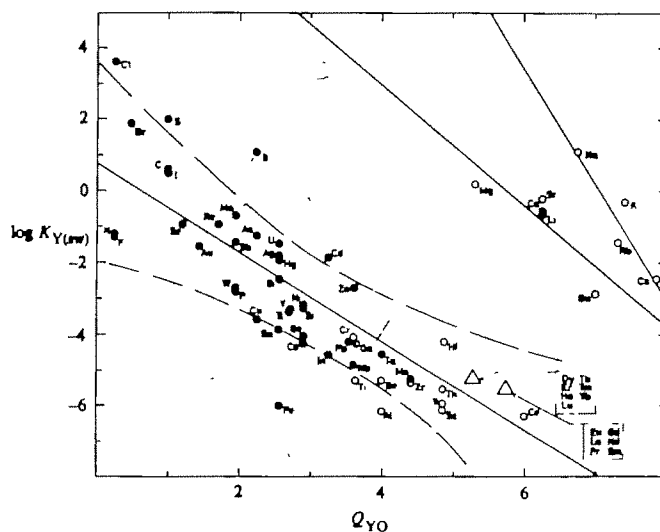


Fig. 2 The relationship between the seawater-crustal rock partition function ($K_{Y(sw)}$) and the electrostatic contribution to the Y-O bond energy (Q_{YO} , equation (7)). Classification (Table 2): $\circ$, L1 lithophiles; $\bullet$, chalcophiles and siderophiles; $\triangle$, L2 and L3 lithophiles. The solid lines indicate the linear regression fits (Table 1) and the dashed lines the Working-Hotelling confidence band at the 0.1% significance level¹⁹. Lines cutting across the main sequence of elements break the sequence into groupings discussed in the text. The oxidation states used are those considered to be thermodynamically stable in oxygenated seawater¹³ with the exception of Cr(III) and Mn(II) as there is evidence that these metastable forms are important in seawater. Thallium has been considered as Tl(III) because recalculation of the available data shows this to be the thermodynamically favoured oxidation state.

Table 2 Solid-state classification of the elements*

Geochemical classification	Preferential occurrence	Chemical characteristics	Q_{YO} range
Siderophile (S)	Metallic phase with Fe	More noble than Fe. Weak O and S affinity	1-4
Chalcophile (C)	Sulphide phase	Strong S affinity	1-4
		Cationic (L1)	>3.5
		Metal oxyanions or oxocations (L2)	2-4
Lithophile (L)	Silicate phase	Strong O affinity	<3†
		Non-metal oxyanions or halide ions (L3)	

* Adapted from the geochemical classification given in ref. 35.

† For halide ions $Q_{YO} < 1$.

with seawater. The anomalous positions of Ag and Sb in this plot probably arise from an overestimate of $\bar{Y}_B$ in each case particularly as the oceanic residence times of the elements calculated using river inputs are also anomalous (Fig. 1). This plot should be reconsidered when more accurate data become available for the global mean composition of river water. It should also be possible to construct analogous plots for particular fresh water systems (rivers or lakes) provided that the mean composition of the rocks in the appropriate drainage basins are used instead of $\bar{Y}_A$.

In the seawater plot (Fig. 2) we find that three groups of elements fall outside the main correlation. The elements Cl, S, B are normally considered to be 'excess volatiles'²⁹, that is they have a significant source in volcanic emissions which could result in an enhanced partition coefficient. Cd is fairly volatile (boiling point 765 °C) and may be enriched in the oceans in a similar way. In river water (Fig. 3) where the residence time is too short (<1 yr) for 'excess volatile' inputs to be significant these elements all fall on the main correlation. The other divergences from the main linear correlation are related to hydroxide solubilities. To achieve the expected value of $K_{Y(rw)}$, Fe would need to exceed its hydroxide solubility product by several orders of magnitude²¹. Iron therefore falls below the main correlation because of a combination of high abundance in crustal rock and low solubility in seawater. Although the data are more equivocal, a similar explanation probably applies to Ti and Al which are also very abundant in the Earth's crust³⁰. In river water (Fig. 3) where the lower pH and lower ionic strength make hydroxide precipitation less favourable these three elements also fall in the main correlation. The final group of anomalous elements are the Group IA and IIA metals (less beryllium). These elements differ from the rest of the electropositive elements in that they form soluble oxides and hydroxides. This arises from the relatively low charge/radius ratio of these cations, and as a result they show only a weak affinity for oxide lattices in aqueous media despite their low electronegativity. The extent to which the Group IA cations are extracted, for example, depends largely on the hydration energies that are released when the cations are incorporated into the water structure so that their $K_{Y(rw)}$ -values follow the Hoffmeister affinity sequence for ion-exchange³¹. These elements are only slowly removed from seawater so that they have long residence times (Fig. 1), and anomalously high K_Y values (Fig. 2). In river water (Fig. 3) where the timescale is much shorter the anomaly is much less marked.

The elements on the main correlation lines of Figs 2 and 3 are arranged according to their ionic affinity for oxygen, and the resulting sequence correlates well with the approximate geochemical classification of the elements first introduced by Goldschmidt³² (see Table 2). The lithophile/chalcophile division is a solid-state analogue of Ahrlund's classification of (a) and (b) cations in aqueous solutions^{33,34}: (a) cations prefer F, O, N as ligands while (b) cations prefer Cl, S, P. Both the solution and solid-state classifications show coherent groupings in the periodic table^{34,35}, but only the solid-state (geochemical) classification shows a clear correlation with the sequence of elements in Figs 2 and 3. We will redefine C, N, P, S as lithophiles as they occur as oxyanions in seawater. The litho-

philes can then be divided into three groups according to the nature of their interaction with oxygen (Table 2). The main correlation in Figs 2 and 3 has been divided into three parts. The middle portion contains virtually all the siderophiles and chalcophiles, which interact only weakly with oxygen. The ionic (L1, Table 2) lithophiles are grouped on the right of the main correlation (high Q_{YO} values) and the covalent (L3) lithophiles on the left (low Q_{YO} values). This clear correlation with the geochemical classification strongly suggests that solid-state chemistry controls the fractionation of the elements between the crustal rocks and seawater. Complexation of the elements in solution seems to be of lesser importance as the sequence of elements in Fig. 2 shows no correlation with the (a)/(b) classification of cations^{33,34}. In particular the typical (b) cations³⁴ (Ag, Au, Hg and Tl) which alone among the cations considered interact very strongly with chloride (the dominant ligand in seawater) show no anomalous behaviour. The solution chemistry of the elements is significant only where it directly affects hydroxide solubilities (Fe, Ti, Al and the Group IA and IIA metals).

The main correlation line in Fig. 2 can be used to predict $\log K_{Y(rw)}$ for the stable elements which have not yet been determined in seawater, that is Te and the platinum metals (Ru, Rh, Pd, Os, Ir, Pt). Using tabulated electronegativity values (ref. 16, p 73) we find $\log K_Y = -1.4$ for the platinum metals and -1.7

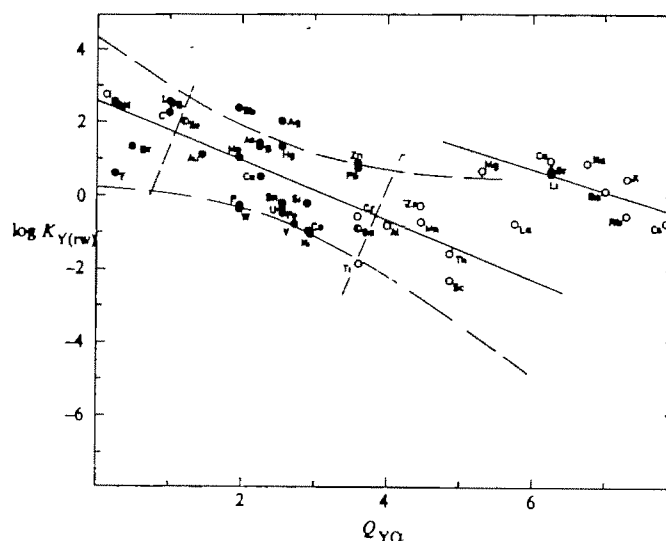


Fig. 3 The relationship between the river water-crustal rock partition function ($K_{Y(rw)}$) and the electrostatic contribution to the Y-O bond energy (Q_{YO} , equation (7)). Symbols as in Fig. 2. The solid lines indicate the linear regression fits (Table 1) and the dashed lines indicate the Working-Hotelling confidence band at the 1% significance level¹⁹. Lines cutting across the main sequence of elements break the sequence into groupings discussed in the text.

for Te. The crustal rock analyses for these elements are too scattered¹⁶ for an estimate of dissolved seawater concentration to be derived from K_Y . As expected Te (chalcophile) and the platinum metals (siderophiles) fall in the middle portion of the plot.

Weathering model revisited

We will now use a plot (Fig. 4) of the average seawater concentrations of the elements ($\bar{y}_C$, mol l⁻¹) against their average river water concentrations ($\bar{y}_B$, mol l⁻¹) to look again at the overall consequences of the weathering process. During the rapid passage through the fresh water reservoir, material is dissolved from the crustal rocks into water with a relatively low pH and ionic strength. As most of the elements are grouped within two orders of magnitude of $\log K_{Y(w)} = 0$ in Fig. 3, the composition of the solids dissolved in the fresh water reservoir bears a reasonable resemblance to that of the parent rocks. As this water runs into the ocean bucket it enters a reservoir with a much longer holding time containing water which has (after many turns of the weathering cycle) a relatively high pH and ionic strength. Here material can accumulate up to the age limit

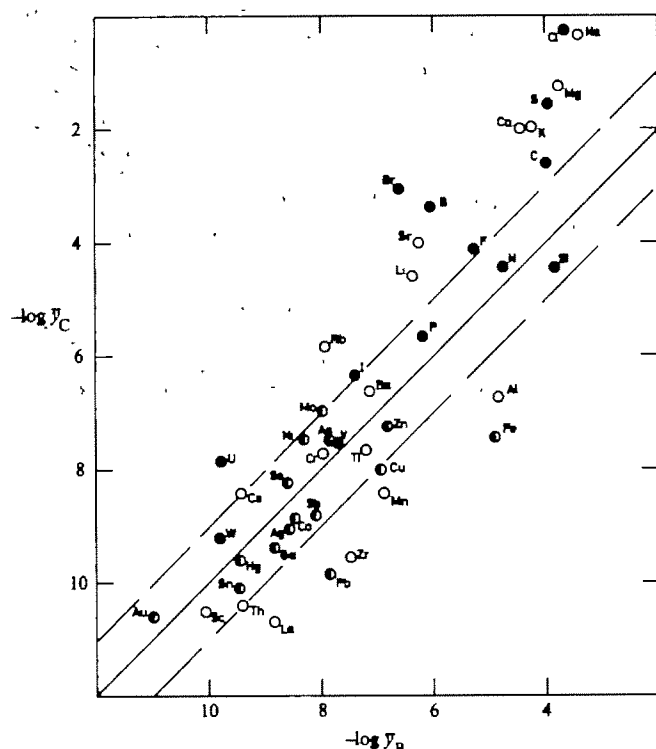


Fig. 4 The relationship between the mean concentrations of the elements in seawater ($\bar{y}_C$, mol l⁻¹)¹³ and in river water ($\bar{y}_B$, mol l⁻¹)¹³. To convert $\log \bar{y}_N$ to $\log \bar{y}_C$ subtract 4.43 units and 1.45 units respectively for river water and seawater. The solid line follows the 1:1 correlation and the dashed lines encompass points that fall within an order of magnitude of this line. Symbols as in Fig. 2.

of the reservoir itself unless its concentration is controlled at some intermediate level by interactions with the suspended matter. The residence times of bromine and chlorine (which are concentrated to the greatest extent in the oceans relative to the rivers) are approximately equal to the age of the ocean basins themselves (Fig. 1). The elements which accumulate in the oceans (Fig. 4, elements more than an order of magnitude above the 1:1 correlation) generally show significant positive deviations from the main sequence of elements in Fig. 2 indicating

either that their concentrations are controlled by their solution chemistry rather than by their solid state chemistry (Group IA and IIA elements) or that they might have a significant auxiliary source within the oceans (Cl, S, B).

More than half of the elements are already within an order of magnitude of their final oceanic concentration (Fig. 4) before they enter the ocean bucket. The bulk of these elements fall within the main correlation bands for both seawater (Fig. 2) and river water (Fig. 3). This suggests that the controlling processes are the same in both river water and seawater and are in geological terms kinetically fast.

All the elements that show a significant depletion in the oceans relative to the river water reservoir (Fig. 4; elements more than an order of magnitude below the 1:1 correlation) have oceanic residence times that are appreciably less than the time required for a single stirring revolution of the oceans (t_R , Fig. 1). In river water all of these elements fall within the main sequence of elements (Fig. 3) suggesting that, in the initial stages at least, their concentrations are controlled by their solid-state chemistry as outlined earlier. In seawater only iron and aluminium out of this set of elements fall significantly below the main sequence of elements (Fig. 2) and their oceanic concentrations are probably controlled by the solubility of their hydroxides. The other elements (La, Pb, Zr) fall clearly within the main sequence in Fig. 2 suggesting that in the oceans, too, their concentrations are controlled by their solid-state chemistry.

The non-equilibrium behaviour of nitrogen in the oceans was mentioned in connection with Fig. 1. Comparing Figs 2 and 3 we see that in Fig. 3 (river water) nitrogen is very close to chlorine, but that in Fig. 2 nitrogen falls almost five orders of magnitude below chlorine on the $\log K_Y$ axis. If, however, we include the elemental nitrogen present in the atmosphere and dissolved in seawater (that is the nitrogen which has been lost from the ocean reservoir to the atmosphere reservoir²¹), the value of $\log K_{N(w)}$ falls only a short way below the value for $\log K_{Cl(w)}$.

Conclusions

By considering that the oceans are in a steady state with respect to the flux of weathered components from the crustal rocks and that the whole weathering cycle has been processed many times during the Earth's history, we can use the partition function $K_{Y(w)}$ to rationalise the observed composition of seawater. There is a strong correlation between $K_{Y(w)}$ and the mean oceanic residence time ($\bar{t}_Y$) as both these parameters are controlled by the ease with which the various elements are exchanged between the rocks and the aqueous phase. The ionic contribution to the Y-O bond exerts an overall control over the partitioning of the elements between the aqueous phase and the solid phase and the correlation between $\log K_{Y(w)}$ and Q_{YO} provides a rational explanation, in order of magnitude terms at least, for the seawater recipe. A similar correlation also exists for world average river water. The combination of these correlations provide a quantitative and general basis for Forchhammer's postulate¹⁰ and interrelate the concepts of reactivity and crustal abundance that have been frequently proposed as controlling factors in determining the chemistry of seawater^{13,22,36}.

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Transcription and processing of cloned yeast tyrosine tRNA genes microinjected into frog oocytes

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Xenopus oocytes transcribe cloned yeast tyrosine tRNA genes and process the RNA. The processing includes base modifications, addition of the CCA end, and splicing of the intervening sequence. The primary transcript has a 5' leader extension which varies considerably in sequence and in length between different tRNA^{Tyr} loci, but is nevertheless present in all of them.

METHODOLOGICAL advances have made possible the cloning and growth in *Escherichia coli* of segments of DNA coding for various individual eukaryotic genes and these genes can be sequenced using the rapid gel methods that have greatly simplified nucleotide sequence determination. However, the function of these nucleotide sequences is not always readily apparent from the primary structure. For example, it is difficult to recognise from the DNA sequence the location of promoters and the precise start of the primary transcript. Thus there is a need for a functional assay for cloned eukaryotic genes. Here, we analyse the expression of cloned yeast tRNA^{Tyr} genes after microinjection into living *Xenopus laevis* oocytes.

Saccharomyces cerevisiae has eight unlinked tRNA^{Tyr} loci¹, each of which can be mutated into an ochre, amber or UGA suppressor^{2,3}. All eight genes code for the same mature tRNA^{Tyr} sequence. Seven of these genes have been cloned⁴ and four have been sequenced (ref. 5 and Phillipsen, Cameron and Davis, personal communication). An intervening sequence of 14 base pairs was found inserted at the 3' end of the anticodon. The sequence of the intervening sequence is also conserved between the various genes, with the exception of one base pair which has been observed to vary. However, at the flanking region at the 5' (starting) side of the tRNA sequence, the DNA sequences are almost totally dissimilar between the different genes⁵.

The intervening sequence is normally transcribed in yeast, then excised and the RNA molecule re-ligated to produce the mature tRNA^{6,7} (a process hereafter referred to as 'splicing'). This is known from the existence of a yeast temperature-sensitive mutant⁸ that accumulates a 92-nucleotide tRNA^{Tyr} precursor with the same 5' and 3' ends as the mature tRNA and

containing the intervening sequence. This work did not indicate whether this precursor represented the primary transcript, or if there is an even longer initial transcript (see below).

DNA microinjected into the nucleus of *Xenopus* oocytes is transcribed⁹ and translated¹⁰. Genes transcribed by RNA polymerase III are expressed very efficiently and with high fidelity in oocytes, as shown initially for 5S ribosomal RNA genes^{11,12} and subsequently for tRNA genes¹³⁻¹⁵. Recently, the transcription of tRNA genes from *Drosophila* and the fission yeast *Schizosaccharomyces pombe* has also been reported¹⁷ in a cell-free system derived from *Xenopus* oocytes¹⁶.

In this study we use plasmids containing yeast tRNA^{Tyr} genes for a detailed analysis of the primary transcript of a eukaryotic tRNA gene, and its processing in living cells. Our analysis was greatly facilitated by the use of genes of known nucleotide sequence, and of several members of the same gene family. We find that the initial transcript contains, in addition to the intervening sequence, an extra segment at the 5' end. This 5' leader sequence is not conserved, and varies considerably in length and nucleotide sequence between the various members of the tRNA^{Tyr} gene family. In addition, we show that many of the frog enzymes involved in tRNA production are not species specific, as they can accurately recognise yeast genes. These enzymes include RNA polymerase III, RNase P, RNA methylases, tRNA nucleotidyl transferase (CCase) and the splicing enzyme(s).

Frog oocytes transcribe yeast tRNA genes

Figure 1 shows the result of an experiment in which oocytes were injected into the nucleus with DNA of plasmid pYT-C and [α -³²P]GTP. Plasmid pTY-C (ref. 5) contains a 3-kilobase fragment of yeast DNA that includes a tRNA^{Tyr} gene and its flanking sequences cloned in the plasmid pBR 322 (ref. 18). This tRNA^{Tyr} gene corresponds to the known nonsense suppressor locus SUP 8 (M.V.O., Longhey and Hall, in preparation). Several newly synthesized RNA bands are detectable after injection of the plasmid, and their approximate lengths can be estimated using appropriate RNA markers (Fig. 1, lanes f, j). One faint band of 108 nucleotides and three main bands of 104, 92 and 78 nucleotides can be distinguished.

Thirty minutes after injection (Fig. 1, lane *b*) only the 108 and 104 nucleotide bands are detectable. The weak 108 band is always found, and although not seen very clearly in Fig. 1, is clearly visible in Fig. 4, lanes *a* and *b*, and in Fig. 5, lanes *a* and *b*. At longer incubation times the 92-nucleotide precursor is the most abundant product (Fig. 1, lanes *c*–*e*). The 78-nucleotide band co-migrates with purified yeast tyrosine tRNA (compare

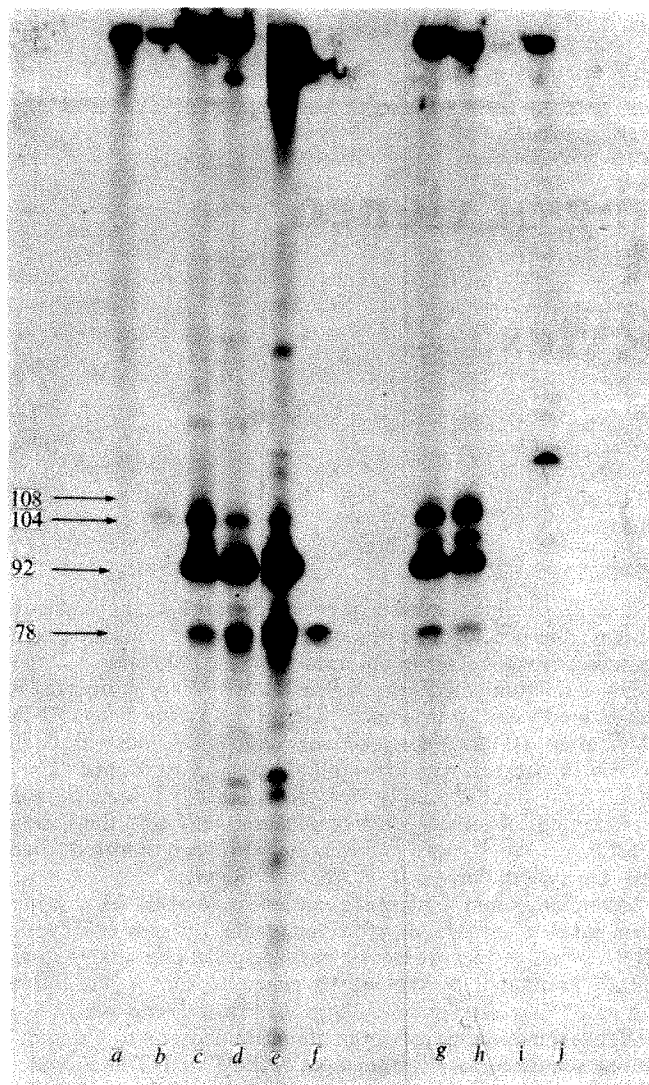


Fig. 1 Autoradiogram of ^{32}P -RNAs synthesised by *Xenopus* oocytes injected into the nucleus with a plasmid containing a yeast tRNA^{Tyr} gene and cultured in saline solution for various times³⁶. Each oocyte was injected with 30 nl of a solution containing 400 $\mu\text{g ml}^{-1}$ pYT-C DNA and 10 mCi ml^{-1} [α - ^{32}P]GTP (350 Ci mmol^{-1} , Amersham). After incubation the RNA was extracted³⁷ and electrophoresed in a 7 M urea–12% polyacrylamide gel using thin (0.35 mm) spacers³⁸. About 1 oocyte worth of RNA was loaded into each slot, and the gel exposed for 2 d using an Ilford fast tungstate intensifying screen and pre-exposed Fuji Rx film³⁹. Samples *e* and *f* were run in adjacent lanes in a different 12% gel. Lane *a*, oocytes injected with pBR 322 DNA and incubated for 6 h (no yeast DNA); *b*, pYT-C injected and incubated for 30 min; *c*, pYT-C, 6 h; *d*, pYT-C, 12 h; *e*, pYT-C, 24 h. Lane *f*, purified yeast tyrosine tRNA marker¹, which was end-labelled with polynucleotide kinase and [γ - ^{32}P]ATP⁴⁰. Lane *g*, oocytes injected with pYT-C and incubated for 6 h; *h*, same as *g* but injected with 1 $\mu\text{g ml}^{-1}$ α -amanitin; *i*, same as *g* but with 100 $\mu\text{g ml}^{-1}$ α -amanitin. Lane *j*, oocytes injected with pXlo-8, a plasmid containing *Xenopus* 5S genes¹² included here as a size marker (the 5S band has 120 nucleotides, and the pre-5S one is 135 nucleotides long).

lanes *e* and *f*, Fig. 1). The proportion of the 78-nucleotide material increases with time, but is never more than the amount in the 92-precursor band (lanes *c*, *d*, *e* in Fig. 1 show 6, 12 and 24 h incubations, respectively). Even after 4 d in culture (data not shown), the 78 band represents only 30% of the RNA induced by the injection of yeast DNA. (Note, however, that oocytes transcribe the injected DNA continuously throughout this period.)

In addition to these bands, other minor products are detectable. A minor band of about 97 nucleotides is consistently observed (Fig. 1, lane *g*), and probably represents a true intermediate step in the processing of the longer initial transcript into the 92 band, as it is quite prominent after 2 h of incubation (Fig. 4, lane *a*).

Yeast tRNA^{Tyr} genes are transcribed by the oocyte RNA polymerase III, as shown by α -amanitin inhibition (Fig. 1, lanes *g*, *h*, *i*). α -Amanitin, 1 $\mu\text{g ml}^{-1}$, injected with the DNA did not significantly affect tRNA transcription. At this concentration α -amanitin abolishes RNA polymerase II transcription in oocytes injected with SV40 DNA^{10,12} or prokaryotic plasmids.¹⁹

We conclude that yeast tRNA^{Tyr} genes are transcribed in oocytes by the correct RNA polymerase (type III). We can detect a weak band 108 nucleotides long, which is then presumably processed into three main RNAs of about 104, 92 and 78 nucleotides.

Initial transcripts have a 5' leader extension

Knowledge of the DNA sequence (Fig. 2) has greatly facilitated analysis of the composition of the RNA bands; this was done using the classical RNA sequencing techniques of Sanger *et al.*^{20,21}

As the main band accumulated is about 92 nucleotides long, it seemed possible that it could be the same kind of precursor as that accumulated in the yeast mutant^{6,7}, which has the same ends as the mature tRNA but contains the intervening sequence (tRNA^{Tyr} has 78 and the intervening sequence 14 nucleotides; 78 + 14 = 92). Figure 2 shows the ribonuclease T1 oligonucleotides that can be predicted for such a molecule (this enzyme cuts RNA after G residues). Analysis of the T1 fingerprint shown in Fig. 3, left panel, confirmed that the 92-nucleotide material synthesised in oocytes contains the intervening sequence.

The spots in this GTP-labelled fingerprint were identified by elution and secondary digestion with pancreatic ribonuclease (which cuts after C and U). As the oocyte does not exchange the ^{32}P label of one injected labelled nucleotide into other nucleotides¹⁵, the spots can be recognised by the nearest neighbour to the G residue (underlined in Fig. 3, central panel). For example, the T1 oligonucleotide ApCpU $\overline{\text{G}}$ p would be cut by pancreatic ribonuclease into ApCp/U $\overline{\text{G}}$ p, giving U as the nearest neighbour to G. The identifications were confirmed by fingerprints of ATP or CTP labelled materials, in which certain spots are not labelled and others vary in intensity according to the number of labelled phosphates present. For the purposes of this study the most important spots are numbers 15 and 16, which contain the intervening sequence (and which will disappear in the 78 material), and spot 14, which contains the 5' end of the molecule.

Figure 3, right panel, shows the T1 fingerprint of the 104-nucleotide band. The oligonucleotides of this precursor are the same as for the 92 material, except that spot 14 (which was the 5' end of this RNA) no longer appears in its usual position. Two new large oligonucleotides appear (hatched tracing in centre panel, Fig. 3), and the nearest-neighbour data (Fig. 3) are consistent with their being UUAAUUG and ACUCUCG, which originate from the region of the pYT-C DNA at the 5' side of the mature tRNA.

We conclude from this that the larger precursor differs from the 92-nucleotide band at its 5' end, having a 5' leader whose sequence is consistent with that predicted from the DNA sequence of the gene.

Fig. 2 The nucleotide sequence of four tRNA^{Tyr} genes. All four genes share the same sequence between positions 1 and 92 (except for one base pair in the intervening sequence (boxed), which is CG in pYT-C and G, and TA in pYT-A and pSU4-A). The construction of these plasmids and their sequencing were described in ref. 5; all have a segment of yeast DNA cloned in plasmid pBR 322. The sequence to the left of position 1 (negative numbers) differs for the various genes, and is indicated in the upper part of the figure. The sequence of yeast tRNA^{Tyr} is indicated, as well as the sequence of the 92-nucleotide precursor RNA that accumulates in a yeast mutant^{6,7}. The predicted T1 oligonucleotides for the latter molecule are numbered in the same way as in ref. 7. pSU4-A is an ochre suppressor mutant of gene A, which was isolated from a yeast strain of different genetic background (thus explaining the nucleotide that changes on the 5' side of the start of the mature tRNA).

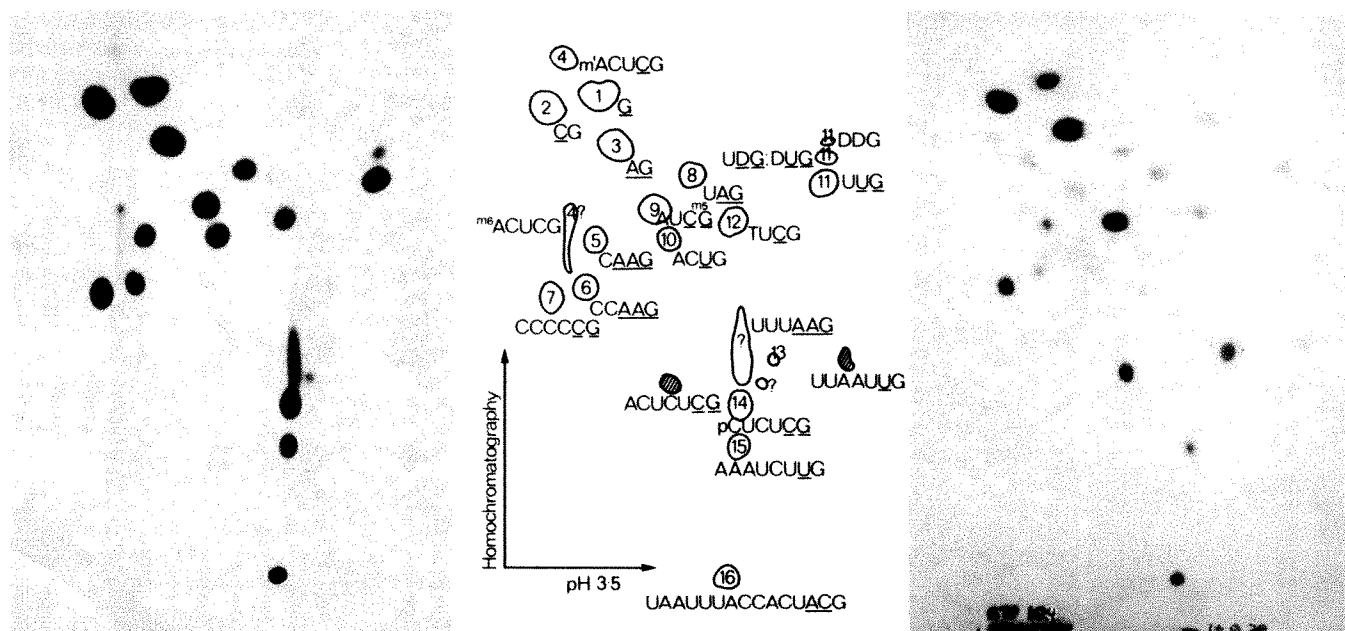
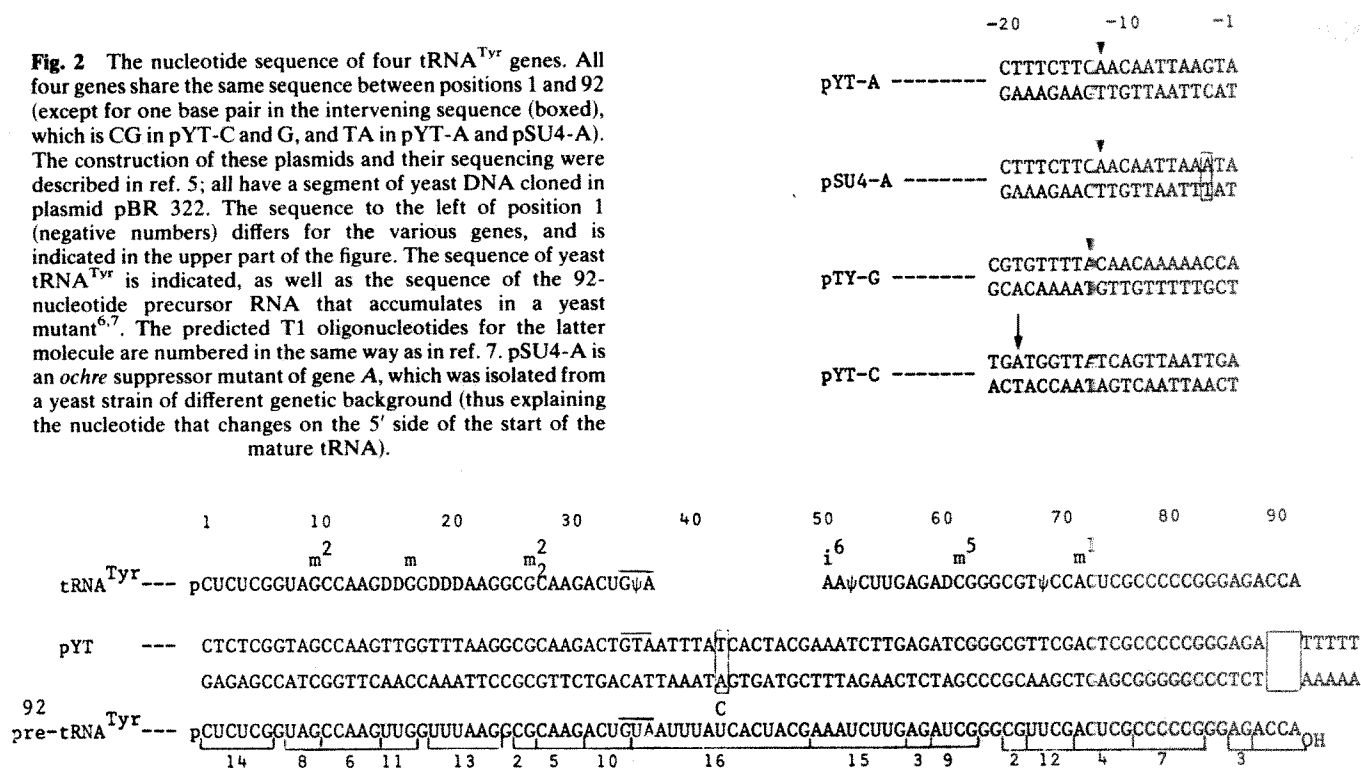


Fig. 3 Fingerprints of ribonuclease T1 oligonucleotides of tRNA^{Tyr} precursors synthesised by oocytes injected with pYT-C and [α -³²P]GTP. Left panel, 92-nucleotide precursor; right panel, 104-nucleotide precursor; centre panel, tracings indicating the composition of each spot, with spots numbered as in Fig. 2; the oligonucleotides that were found to be labelled after nearest-neighbour analysis (secondary digestion with pancreatic RNase) are underlined. The two new long oligonucleotides that appear in the 104 material are hatched. The 104-nucleotide material has some short oligonucleotides (paler spots on the upper part of the fingerprint) which come from the background oocyte RNA. The background of endogenous transcripts is not negligible in this case because the 104 band is relatively weak, and the fingerprint has to be exposed for longer periods. We cannot exclude the possibility that the 104 band may contain an additional short oligonucleotide, because it could be masked under one of these background spots. The RNA bands were eluted⁴⁰ from preparative gels and then digested, fingerprinted and further analysed as described by Barrell²¹. The pancreatic RNase secondary digestion products were analysed by electrophoresis in 40 cm DEAE-cellulose thin layer plates (Cel. 300 DEAE, Polygram, Macherley-Nagel) at pH 3.5 (1,000 V for 3½ h). The oligonucleotides migrate in the same way as in the usual DEAE-paper electrophoresis method²¹, but we find that thin layers are easier to handle.

Mapping the primary transcript

The primary transcript is the weak 108-nucleotide band. This was established by injecting ATP or GTP labelled in the γ -phosphate. As shown in Fig. 4, [γ - ^{32} P]ATP was incorporated only into the 108 band, whereas all other bands lack the terminal triphosphate. According to the length of this molecule, this should start at about position -19. (There is evidence that the large precursors do not have the CCA end (see below) but a short row of U residues at the 3' end of the primary transcript would have gone unnoticed in our fingerprints.) The only A residue in this region is in position -19 of pYT-C (Fig. 2), which is therefore the most likely starting place.

The presence of this 5' leader extension was confirmed by comparing the transcription of tRNA^{Tyr} genes from different loci. We mentioned earlier that the sequence of the region flanking the 5' side of the tRNA sequence varies considerably among the different yeast tRNA^{Tyr} loci. From the work described in the previous section, we now know that this region is transcribed into RNA in the initial transcripts. The plasmids pYT-C and pYT-A, for example, differ in the position of all their

A residues (except for the one at position -5) in this region (Fig. 2). Therefore, the initial transcripts of these two genes should be of different lengths, and Fig. 5 shows that this is indeed the case.

Thirty minutes after injection, pYT-C induces the 108 and 104 bands (Fig. 5, lane *a*), whereas the equivalent bands induced by pYT-A are about 5 to 6 nucleotides shorter (Fig. 5, lane *c*). Assuming that transcription starts with A or G, the most likely start for the primary transcript of pYT-A is in one of the A nucleotides in positions -12 or -11 (Fig. 2). The plasmid pSU4-A, which contains an *ochre* suppressor mutant of gene *A*, induces the same bands as pYT-A (Fig. 5, lanes *e, f*), as expected from its DNA sequence (Fig. 2). Plasmid pYT-G (Fig. 5, lanes *g, h*) produces several bands, the longest of which has about the same length (or, in some gels, slightly more) as in pYT-A and

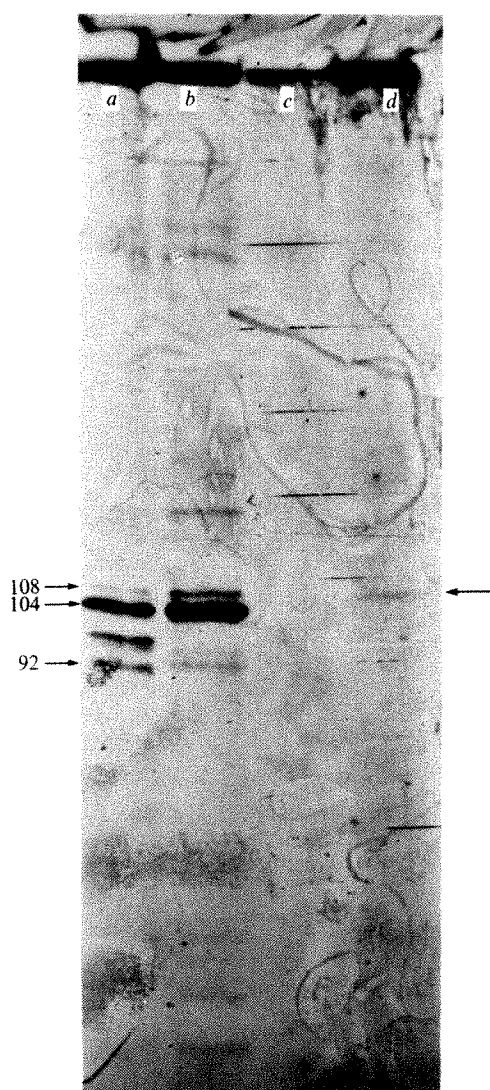


Fig. 4 The 108 band is the primary transcript. Gel electrophoresis of the RNAs synthesised in *Xenopus* oocytes injected with pYT-C DNA and with: lane *a*, [α - ^{32} P]GTP, 2 h incubation; *b*, [α - ^{32} P]GTP, 30 min incubation; *c*, [γ - ^{32} P]GTP, 2 h incubation; *d*, [γ - ^{32} P]ATP, 2 h incubation. The position of the various precursor bands is indicated. The γ -labelled nucleotides were injected at 50 mCi ml⁻¹. The band of the primary transcript is indicated in slot *d*.

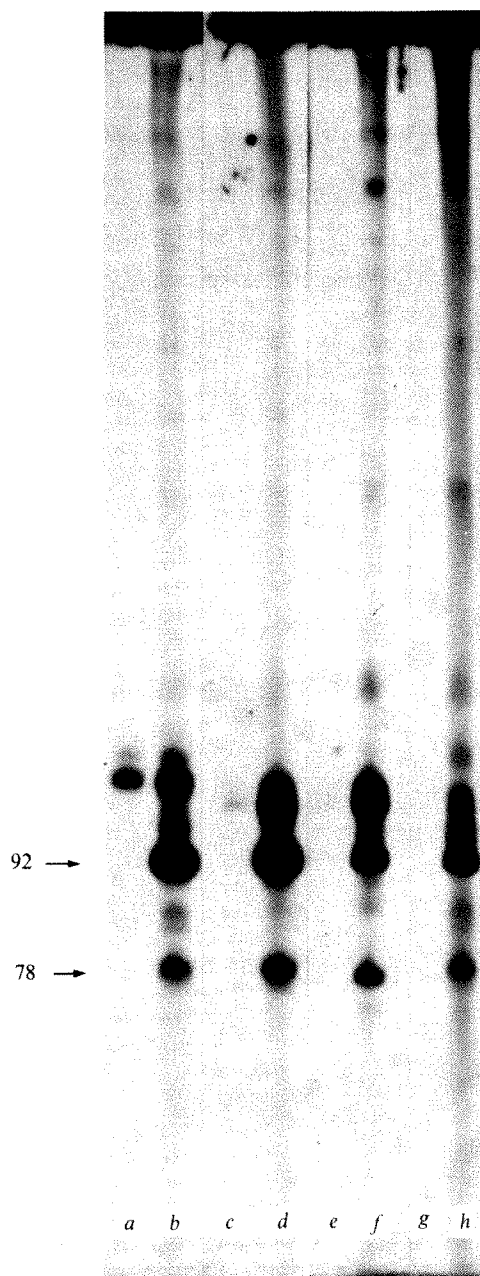


Fig. 5 The initial transcripts vary in length in different tRNA^{Tyr} loci. Gel electrophoresis of RNAs synthesised by oocytes injected with [α - ^{32} P]GTP and: lane *a*, pYT-C DNA, 30 min incubation; *b*, pYT-C, 6 h incubation; *c*, pYT-A, 30 min incubation; *d*, pYT-A, 6 h incubation; *e*, pSU4-A, 30 min incubation; *f*, pSU4-A, 6 h incubation; *g*, pYT-G, 30 min incubation; *h*, pYT-G, 6 h incubation.

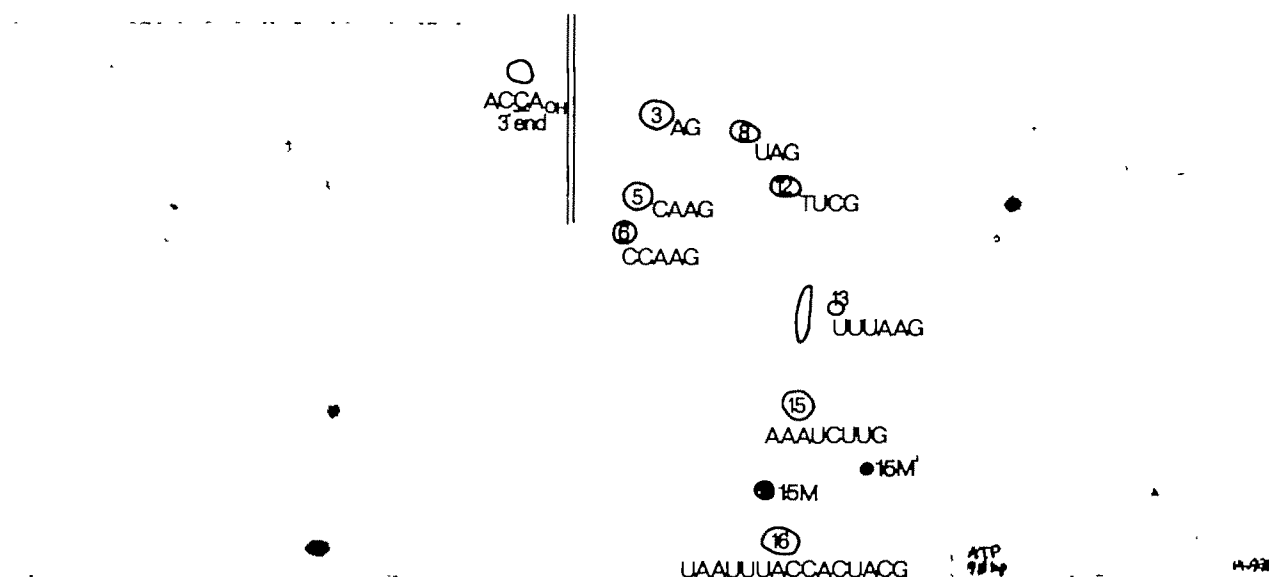


Fig. 6 T1 oligonucleotides of the 92-nucleotide material (left panel) and the 78 nucleotide band (right panel) induced by the injection of pYT-C DNA into *Xenopus* oocytes. Separation was as in Fig. 3. The oocytes were labelled with [α - ^{32} P]ATP, and for this reason many of the spots present in Fig. 3 are unlabelled here. Spot 12 does not contain A but is still labelled because in the RNA its G residue has an A as nearest neighbour (sequence T $\nrightarrow$ CGA). The CCA end oligonucleotide runs very slowly in the first dimension, and was therefore run in a separate second dimension plate. Spots 15 and 16 are absent in the 78-nucleotide band, and a new spot (15 M, hatched symbols) appears.

pSU4-A. All four plasmids produce the same 92 and 78 nucleotide bands at longer incubation times (Fig. 5, lanes b, d, f, h); this is as expected, as the four genes have a very similar nucleotide sequence within this region (Fig. 2).

We conclude that the primary transcript of pYT-C starts in pppA, and that it has a 5' leader sequence most probably 19 nucleotides long. We believe this to be the first time that the transcription initiation site of an eukaryotic tRNA gene has been mapped within a region of known DNA sequence. Frog oocytes injected with DNA and γ - ^{32} P-triphosphates should be useful for identifying primary transcripts in other cloned genes.

Base modifications and the CCA end are added post-transcriptionally

Oocyte enzymes can modify at least some bases of tRNA^{Tyr} transcripts correctly. For example, of the six labelled cytosines that we examined in the nearest-neighbour analysis of the fingerprint shown in Fig. 3, left panel (92-nucleotide band labelled with GTP), only one cytosine was found to be methylated. The 5-methylcytosine was found in oligonucleotide 9 (data not shown) and corresponds to the only cytosine normally methylated in yeast tyrosine tRNA^{Tyr}^{22,23}. Other bases are probably also modified as judged from mobility changes in the fingerprints (for example, spots 4 and 11 in Fig. 3, left panel), but they were not studied in detail.

The various processing steps occur in a precise order. For example, oligonucleotide 9 is completely modified by the 92-precursor stage, but it is not modified in the 104 band. As seen in Fig. 3, spot 9 (AUCG) is displaced in the 104 fingerprint, where it co-migrates (confirmed by nearest-neighbour analysis) with spot 10 (ACUG). This is expected if spot 9 is not modified, because oligonucleotides 9 and 10 have the same length and base composition (one of each of the four nucleotides), and therefore the two spots should not be resolved by the fingerprinting technique in their unmodified form. (In yeast tRNA^{Tyr} oligonucleotide 9 is modified into AD $\overline{C}$ G; and the dihydrouridine is the modification most likely to produce such a displacement in the fingerprint.)

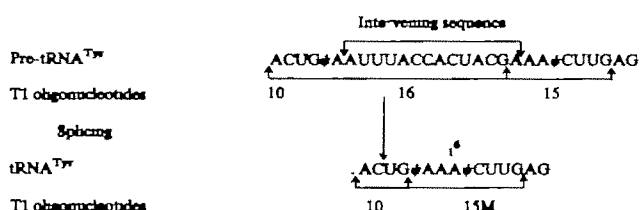
The CCA 3' end of the tRNA^{Tyr} is not encoded in the DNA, but is added post-transcriptionally by a tRNA nucleotidyl transferase (CCase). A spot corresponding to the oligonucleotide ApCpCpAOH was found in ATP (Fig. 6) and CTP labelled

fingerprints of the 78 and 92 nucleotide species, but not in GTP-labelled ones. The sequence of this spot was confirmed by nearest-neighbour analysis after pancreatic ribonuclease digestion (C with [α - ^{32} P]ATP and AC with [α - ^{32} P]CTP). A nucleotidyl transferase activity capable of adding a terminal A residue to tRNA and viral RNAs has been previously described in injected oocytes^{24,25}. The CCA end is present in the 92 and 78 nucleotide bands, but is absent from the 104 precursor (data not shown).

The intervening sequence is excised and the molecule re-ligated

The final product of the *Xenopus* oocyte processing seems to be a covalently intact 78-nucleotide species that does not contain the intervening sequence. Our data do not unequivocally demonstrate that the *Xenopus* splicing enzymes make the identical splice that occurs in yeast, but the splicing point can be located at or within two or three nucleotides of the correct site.

The 78 and 92 nucleotide bands differ only in the region of the intervening sequence. Figure 6 shows that T1 oligonucleotides 15 and 16 (which contain the intervening sequence) are absent in the 78 material, whereas all the other spots are conserved (this is known for GTP, ATP and CTP labelled fingerprints). In addition, a new spot appears in the tRNA-size material (15M in Fig. 6). In yeast, the splicing of the intervening sequence normally involves:



As in yeast, spot 15M made in oocytes is a larger derivative of oligonucleotide 15. This was identified by nearest-neighbour analysis of spot 15M which gave U using α GTP, and AAAU using α CTP. (There is also a second spot, much less intense, which appears reproducibly in the 78 material and is indicated as 15M' in Fig. 6; this minor component is probably also derived

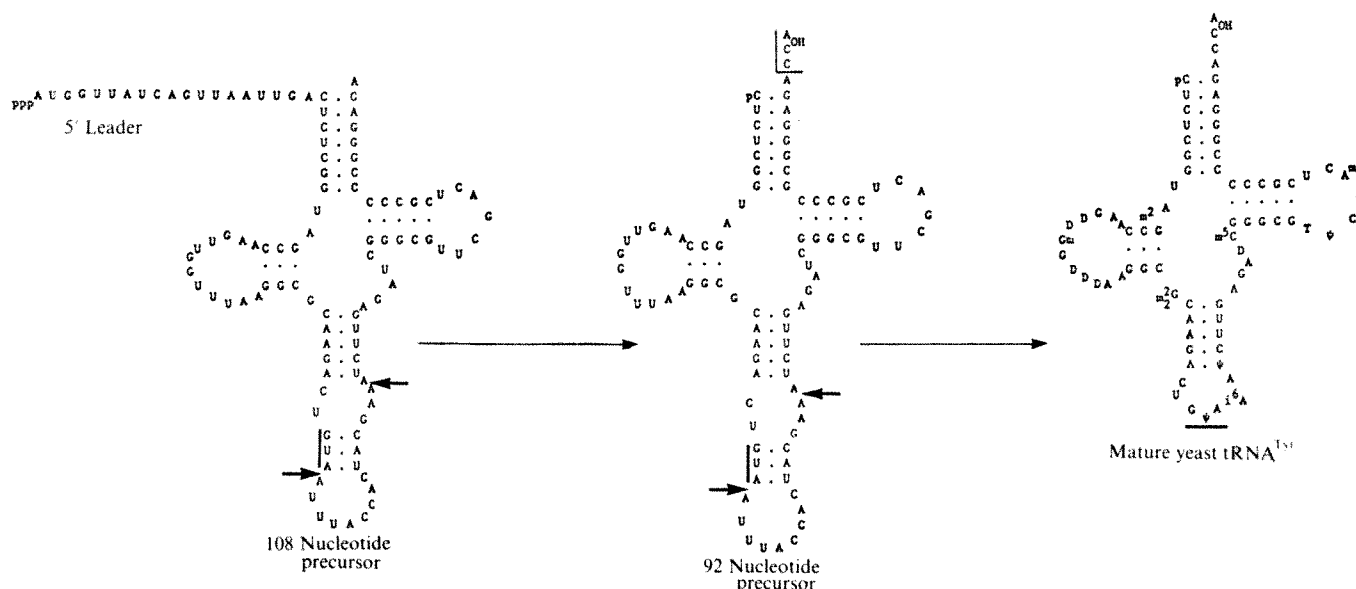


Fig. 7 Main processing stages in the expression of *Saccharomyces cerevisiae* tyrosine tRNA gene C. The primary transcript has a 5' leader sequence 19 nucleotides long, and an intervening sequence 14 nucleotides long. The 5' leader segment differs in length and composition in transcripts of different tRNA^{Tyr} genes. The 5' leader is removed in at least two steps and the molecule is converted into a 92-nucleotide precursor that has been previously identified in yeast^{6,7}. The 92-nucleotide molecule contains several base modifications (not shown here) and the CCA end that is added post-transcriptionally. The intervening sequence is then excised and the molecule re-ligated to produce mature tRNA^{Tyr} (shown here with the base modifications that occur in yeast^{22,23}). The position of the anticodon in the various precursors is underlined. The secondary structure shown here for the 92-nucleotide precursor is the one preferred in ref. 7.

from spot 15, because its nearest neighbour to G is U.) Spot 15M from oocytes, however, moves more slowly in the first dimension than does its yeast counterpart⁷. This mobility difference could be due to a difference in base modification (oligonucleotide 15M from yeast contains an isopentenyl adenosine). However, we cannot rule out the alternative explanation that oligonucleotide 15M has an anomalous mobility because the *Xenopus* processing system splices the yeast precursor incorrectly, although this is unlikely. The splicing site must not lie within oligonucleotide 10 because this spot is found in α -CTP and α -GTP labelled fingerprints of the 78-nucleotide material (data not shown). The nearest-neighbour analysis of spot 15M requires that this oligonucleotide contains the sequence AAAUC, and UG in its 3' end. These data, nonetheless, do not exclude the possibility of a splice giving an oligonucleotide 15M at least 3 nucleotides longer than in yeast (for example, UAAUAAUCUUG). We consider this possibility unlikely in view of the co-migration of the mature yeast tRNA^{Tyr} and the oocyte product in polyacrylamide gels (Fig. 1).

After the intervening sequence is excised, the molecule is re-ligated. This is known because the 78 band maintains the same mobility after heating the RNA at 95 °C for 3 min in 2 volumes of formamide before loading directly on an acrylamide-7 M urea gel. In these denaturing conditions²⁶ this band would have migrated as two half-molecules if not covalently closed. Splicing of yeast tRNAs by *Xenopus* cells has also been found for the tryptophan tRNA genes using *in vitro* nuclear extracts (Ogden, Beckmann, Abelson, Söll and Schmidt, manuscript in preparation).

Conclusions and prospects

Figure 7 outlines the sequence of processing steps for yeast tRNA^{Tyr} that we have inferred from the analysis of the major RNAs that accumulate in oocytes in response to the injection of DNA from the plasmid pYT-C. Rigorous demonstration of precursor-product relationships by means of pulse-chase experiments is not feasible in this experimental system, but the time course with which the different RNA species accumulate is consistent with the view that mature tRNA^{Tyr} is formed by an orderly series of steps in which the RNAs represented in Fig. 7 are sequentially formed. In this interpretation, the 5' leader is

removed in at least two stages, resulting in a molecule with the same 5' end as the mature tRNA, but still containing the intervening sequence. Several base modifications and the addition of the CCA end are detectable for the first time at this stage. This 92-nucleotide precursor has been identified *in vivo* in a yeast mutant^{6,7}, but none of the larger RNAs has been previously described. Yeast therefore has tRNA precursors with 5' leader extensions similar to those described in bacteria²⁷.

The possible significance of the 5' leader sequence is intriguing. This transcribed region not only varies considerably in nucleotide sequence⁵ between the different tRNA^{Tyr} loci, but also in length (Fig. 5). However, all four tRNA^{Tyr} genes examined so far have a 5' leader sequence. One is then left with the question of why these segments of RNA with no obvious sequence or length constraints are always present, and not eliminated altogether. Although this cannot be answered, one possibility is that they introduce order in the intricate processing that the primary transcript must undergo before becoming tRNA, that is, some modifications could occur while the 5' leader is still present, but others cannot occur until this segment is removed.

The final major processing step—the removal of the intervening sequence and re-ligation of the RNA—is not as efficient (30% maximum) in *Xenopus* oocytes as in wild-type yeast cells⁸, but in some ways it is remarkable that it is conserved at all in such a highly heterologous system. Clearly, the enzymes involved can tolerate extensive changes in primary sequence, as the yeast and *Xenopus* tRNA^{Tyr} genes have diverged markedly (S. Clarkson and F. Müller, personal communication).

The specificity with which *Xenopus* RNA polymerase III recognises these transcriptional units is particularly surprising in view of the major biochemical differences that have been observed between the yeast enzyme and those from higher eukaryotes. The yeast enzyme differs from vertebrate RNA polymerase III in both its α -amanitin insensitivity²⁸⁻³⁰ and subunit composition³¹. We have no direct evidence that the *Xenopus* enzyme initiates transcription at the same site as in yeast. The demonstration of transcription initiation in the immediate proximity of the tRNA coding sequence strongly suggests, however, that the *Xenopus* enzyme is correctly recognising the true yeast transcription unit. For example, the plasmid

pYT-C contains 7 kilobases of DNA, but the only discrete transcripts originate from the tRNA^{Tyr} gene region, which represents <2% of the plasmid. This also applies to the other plasmids used in this study. Our results suggest that although *Xenopus* and yeast polymerase III differ in their biochemical properties, they still recognise the same initiation signals in DNA.

More extensive DNA sequencing will be required in the region preceding the transcription initiation sites within the different tRNA^{Tyr} genes before any conclusions can be drawn about the nature of polymerase III 'promoter' sequences³². The yeast tRNA^{Tyr} gene system has the major advantage that it should be possible to identify base pairs essential to the initiation of transcription by obtaining mutants that affect this process. All eight yeast tRNA^{Tyr} genes correspond to known nonsense suppressor loci^{2,3} and the set of cloned restriction fragments that contain these genes has been cross-correlated with the set of genetic loci (M.V.O., Longhey and Hall, in preparation). Methods are also well established for obtaining second-site mutations within these genes by starting with a functional suppressor and selecting for the loss of suppression^{33,34}. The system described here should provide an effective technique for

screening mutant genes for defects in transcription and in the different processing steps.

The finding that *Xenopus* enzymes can carry out several key steps in the processing of yeast tRNA^{Tyr} transcripts supports the view that yeast suppressor genes might provide nonsense suppressors if introduced into the cells of higher organisms³⁵. However, we do not yet know whether a fully functional gene product is synthesised.

We hope that in the future some of the prospects outlined above might be achieved. In the meantime, however, it is already clear that very precise information about cloned genes and their primary transcripts can now be obtained using the most crude (but most physiological) assay system of all—their expression in an intact living cell.

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letters

Angular dimensions of accreting young stars

ALTHOUGH every star passes through a pre-main sequence stage during its formation, only a few astronomical objects have been considered as likely candidates for the early protostellar evolutionary stage¹. The bulk of the energy observed as radiation emitted by a compact source in the near IR, from 2 to 10 μm , is attributed to thermal emission from a dust shell, where the opacity is a rapidly varying function of the wavelength. To study radiative transfer models, the knowledge of the spatial intensity distribution on the object surface in the near IR is crucial. We have developed a method of IR speckle interferometry² which allows the spatial spectrum of an object in a given direction in the modulus sense to be determined up to the frequency cut-off of the telescope. As usual in speckle interferometry techniques the phase is lost unless sophisticated phase-restoration techniques are used. We applied this method

to several protostellar candidates: the BN object, W3IRS5, R MonIRS3, CRL2591 and S140IR at wavelengths 4.8 and 3.5 μm . We present here the results of dealing with BN and W3IRS5, and a more detailed interpretation of the whole set will be given elsewhere.

The specklograph has the following configuration²: the image is formed at the Coudé focus ($f/181$) of the Mayall 3.81-m telescope from Kitt Peak National Observatory. A scanning mirror driven by a saw-tooth wave generator displaces this image on a slit: the motion is perpendicular to the slit and linear with time. The slit is of the order of the seeing disk size, and its width smaller than the Airy radius. The flux is collected through a cold filter ($\lambda = 4.8$ or 3.45 μm ; see Table 1) onto an InSb detector. Alternate scans were made on the object and on adjacent sky to measure in similar conditions the object power and the system- and sky-noise power. One-dimensional power spectra of the source were obtained through the following process: (1) the power spectrum of the source and of the noise were averaged over a given integration time T_0 amounting to N_B

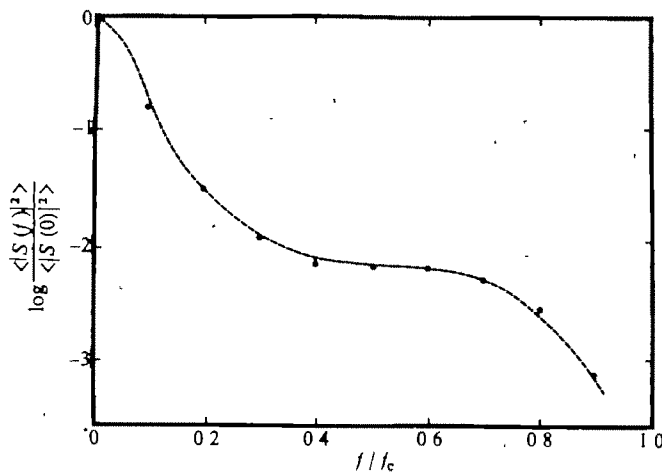


Fig. 1 The atmosphere and telescope normalized MTF, $\langle |S(f)|^2 \rangle / \langle |S(0)|^2 \rangle$ as measured at $4.8 \mu\text{m}$ on a point source (μGem). The observations (●) are compared to the best available model of atmospheric MTF (log normal model). The telescope MTF is the theoretical diffraction limited pupil. The resulting MTF is given by the dashed line, and the best fit with the data is obtained for $r_0(4.8 \mu\text{m}) = 78 \text{ cm}$.

scans with individual duration τ ; (2) the noise power spectrum was subtracted from the source power spectrum; (3) the resulting spectrum was divided by the atmosphere plus telescope modulation transfer function (MTF), in the modulus sense.

The latter MTF is obtained on a bright point-like star, the observation of which is alternated with the observation of the object. Figure 1 shows a typical MTF including the atmosphere and the telescope transfer functions, as determined on a point source star (μGem). A more detailed analysis of the MTF properties is given in refs 2, 3. We obtain the power spectrum data points from

$$\langle |S(f)|^2 \rangle = \langle |I_A(f)|^2 - |b_A(f)|^2 \rangle_{\text{reference star}}$$

This process is summarised by

$$\text{estimation of } |o_A(f)|^2 = \frac{\langle |I_A(f)|^2 - |b_A(f)|^2 \rangle_{\text{object}}}{\langle |I_A(f)|^2 - |b_A(f)|^2 \rangle_{\text{reference star}}}$$

for $f \leq f_c$ with $f_c = D/\lambda$ the frequency cut-off of the telescope. $|o_A(f)|^2$ is a cross-section of the object two-dimensional power spectrum in the scan direction, versus spatial frequency f . $I_A(f)$ and $b_A(f)$ are respectively source and noise spectra. Averaging is made over the observation time. The estimated $|o_A(f)|$ is an average over the band pass $\Delta\lambda$.

A phase-demodulation synchronised with the scan provided a real-time detection of the object and allowed fine guiding to an accuracy well within the dimensions of the seeing disk.

Fluctuations of the wave front coherence length, that is, the Fried parameter, r_0 , with time, limit the above restoration process at least in the current experimental set-up. Most of the data presented here were obtained in stable observing conditions, where this latter effect is small or negligible. Table 1 gives

the observing parameters for the two sources discussed below. Figs 2–4 present the quantity $|o_A(f)|/|o_A(0)|$ as determined by the reduction process for either source, versus spatial frequency in the direction ϕ_0 . Error bars represent one standard deviation determined from the signal-to-noise ratio on individual scans and the number of scans N_B .

Both sources present a low frequency peak which at $4.8 \mu\text{m}$ is more conspicuous on BN than on W3IRS5. The fraction of the total source power in this peak is small: biasing effects in the reduction process⁴ do produce such peaks but we have corrected the spectra for this effect². We will, however, only assign it tentatively to an extended component within the BN object and refrain from discussing at length its possible significance.

At the time of observation the $4.8 \mu\text{m}$ spectra were degraded by spurious 60 Hz system noise beyond $f \approx 2 \text{ arc s}^{-1}$. This was not the case for the $3.45 \mu\text{m}$ spectrum on Fig. 4, which shows a very good signal-to-noise ratio up to $0.95 f_c$. This particular spectrum illustrates well the capability of the speckle method to fully exploit the MTF of a large telescope. The oscillations appearing at high frequency in Fig. 4 are expected as the process (3) above divides noise by noise for $f \gg f_c$.

W3IRS5: this source is clearly resolved. We fit the spectrum with a function

$$\left| \frac{2J_1(\pi\alpha_0 f)}{\pi\alpha_0 f} \right|$$

corresponding to a source of uniform brightness with a diameter α_0 and find $\alpha_0 = 0.28 \pm 0.08 \text{ arc s}$. This large error bar is due to rather poor atmospheric conditions during this observation and is determined from the comparison of independent spectra. If the source had radial symmetry its radial profile $O(\alpha)$ would be completely restored.

If there is any flattening the configuration is less determined and would need scans at various angles ϕ . The above angular value α_0 refers only to the direction ϕ_0 .

A single size measurement for W3IRS5 is quoted by Werner *et al.*¹ to be 3,000 AU, or 1.08 arc s . As they gave no wavelength no comparison was possible.

Wynn-Williams *et al.*⁵ derive a 350 K black body energy curve between 2 and $5 \mu\text{m}$, which corresponds to a diameter of 0.2 arc s , in agreement with our measurement in the absence of flattening. Aitken and Jones⁶ have computed the extinction to the central object from the depth of the silicate feature and derive a corrected 600 K spectrum, to which corresponds a diameter of 0.24 arc s .

Bedijn *et al.*⁷ quoted an unpublished evidence for a double source in this object, for which we find no obvious evidence. Unfortunately, this result prevents them computing a model for W3IRS5. A less elaborate model is made by Finn and Simon⁸ which fits W3IRS5 spectrum with a central cold object having a black body temperature of 375 K. The corresponding $4.8 \mu\text{m}$ diameter is 0.33 arc s , which again agrees with the present measurement.

BN object: The $4.8 \mu\text{m}$ spectrum for the BN object is significantly different from the W3IRS5 spectrum. The central peak is slightly wider but again its power is a small fraction of the total. The main difference lies in the compact source which

Table 1 Observational parameters

Wavelength (μm)	Object	Date (1978)	Magnitude m_λ	Flux (Jy)	T_0 (S)	Scan angle* (ϕ_0)	Reference star	$r_0^\dagger$ (cm)
4.78 μm	W3IRS5	Sept 11	0.8	80	552	150°	β Peg	110
$\Delta\lambda = 0.21 \mu\text{m}$	BN	Sept 11	-0.1	190	312	40°	μ Gem	240
(L)								
3.45 μm	BN	Sept 10	2.2	40	61	40°	μ Gem	165
$\Delta\lambda = 0.57 \mu\text{m}$								

Scan duration is $\tau = 38.4 \text{ ms}$.

* ϕ_0 is the angle between the scan direction and north, counted counterclockwise.

† r_0 is estimated using the low-frequency Korff asymptotic expression for the atmospheric MTF.

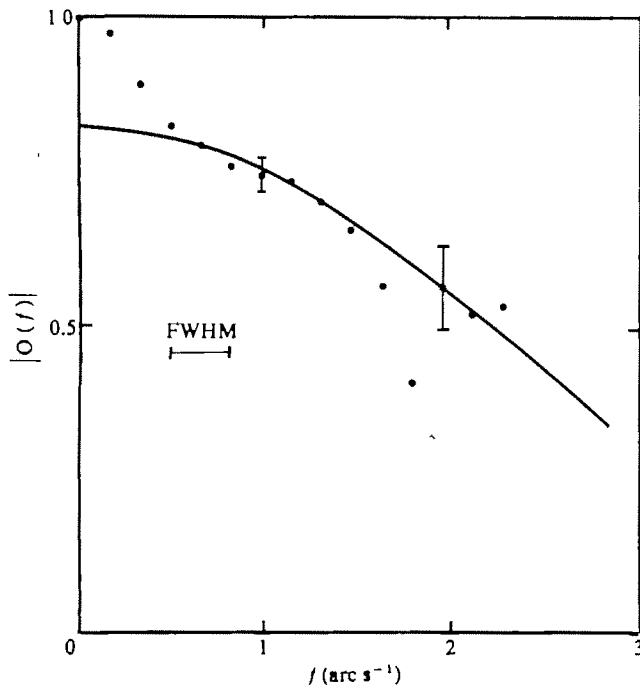


Fig. 2 W3IRS5 power spectrum $|O_\lambda(f)|/|O_\lambda(0)|$ versus spatial frequency in the direction $\phi_0 = 150^\circ$. Total observation time $T_0 = 552$ s. The full curve is the spectrum of a disk with uniform brightness and diameter $\alpha_0 = 0.28$ arc s. $\lambda = 4.8$ μ m. FWHM is the frequency resolution.

seems barely resolved in this particular direction ϕ_0 . The diameter of a source of uniform brightness as above would be $\alpha_0(4.8 \mu\text{m}) \leq 0.1$ arc s if we assume the measured spectrum to depart from the spectrum of an unresolved source by less than 5% on the explored frequency range.

The central peak is fitted with a gaussian component corresponding to an extended gaussian emission with FWHM 1.5 arc s.

The 3.45 μ m BN spectrum is easier to interpret. The central peak is present but is very dim and broader. It would again correspond to an extended emission with a gaussian intensity

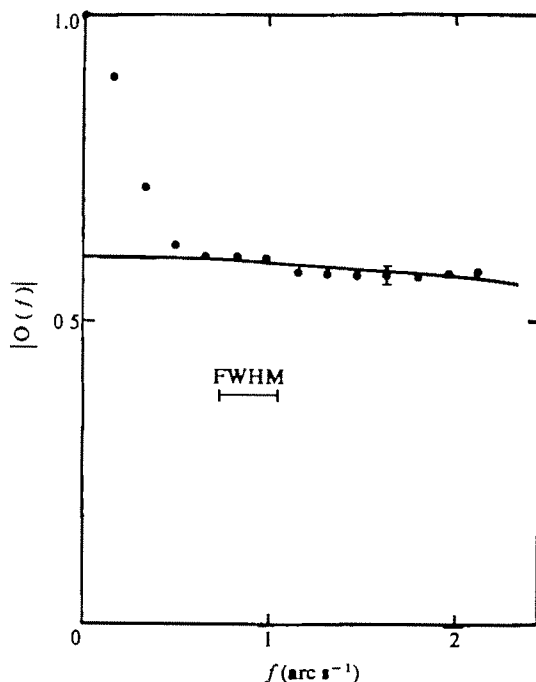


Fig. 3 BN power spectrum $|O_\lambda(f)|/|O_\lambda(0)|$ versus spatial frequency in the direction $\phi_0 = 40^\circ$. Total observation time $T_0 = 312$ s. The full curve is the spectrum of a disk with uniform brightness and diameter $\alpha_0 = 0.1$ arc s. $\lambda = 4.8$ μ m.

profile and a FWHM of 0.9 arc s. The decrease of $|O_\lambda(f)|$ towards high frequencies is noticeable: Fig. 4 shows the spectrum of a disk having a uniform brightness and a diameter $\alpha_0(3.5 \mu\text{m}) = 0.1$ arc s. The telescope resolution in the Rayleigh criterion sense is given by $1.22 \lambda/D = 0.23$ arc s: the measured diameter is below this limit, because the high signal-to-noise ratio in the spectrum allows us to determine where it departs from being a point source spectrum.

Low⁹ has observed BN with spatial interferometry techniques and presents it as "unresolved" but does not specify the observation angle nor the wavelength nor the upper limit of the diameter. Becklin *et al.*¹⁰ fit the observed spectral distribution with a 530 K black body curve corresponding to a 0.1 arc s diameter at 4.8 μ m. This agrees with our data if there is no flattening.

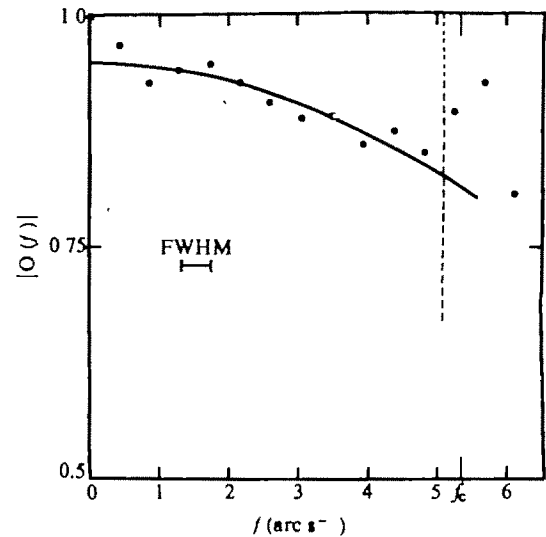


Fig. 4 BN power spectrum $|O_\lambda(f)|/|O_\lambda(0)|$ versus spatial frequency in the direction $\phi_0 = 40^\circ$. Total observation time $T_0 = 61$ s. The full curve is the spectrum of a disk with uniform brightness and diameter $\alpha_0 = 0.1$ arc s. The vertical dotted line at $f = 0.95 f_c$ sets the estimated confidence limit for the restoration process. $\lambda = 3.45$ μ m.

A more elaborate treatment is given by Bedijn *et al.*⁷ who built a series of models for accreting stars. Applying these models to BN, these authors considered four possible cases, where a satisfactory fit of the observed spectrum was obtained, with a mass ratio of three between silicates and graphite grains.

The predicted 5- μ m diameter—90% of the energy within it—varies from 0.42 to 0.12 arc s. The two first models are eliminated for dynamical reasons, our data only comply with the latter one. It corresponds to the lowest luminosity $2.9 \times 10^3 L_\odot$ for the embedded object, to a foreground extinction $A_V = 12.3$, and to a spectral type B3. The large model uncertainty on the luminosity—a factor six—is greatly reduced by measurement of the angular diameter.

As Bedijn *et al.*⁷ do not give the wavelength dependence of the size for $\lambda < 5$ μ m, it is difficult to compare the 3.5 μ m data. Nevertheless, one expects the diameter of the halo, if real, to slightly decrease, as observed, owing to the contribution of inner

Table 2 Measured angular diameter (in direction ϕ_0)

	Wavelength (μ m)	ϕ_0	Extended* halo envelope (FWHM, arc s)	Central source diameter (uniform disk) arc s
W3IRS-5	4.8	150°	1.5	0.28 $\pm$ 0.08
			Very weak	
	4.8	40°	1.5	$\leq$ 0.10
BN	3.45	40°	0.9	0.10
			Very weak	

*Interpret with caution.

layers at a higher temperature and Yorke (personal communication) predicts the existence of a halo surrounding the central object for $\lambda > 2.5 \mu\text{m}$. More elaborate models are now needed, especially to compare the wavelength—and radial—dependence of the emerging intensity in the region of possible accretion.

These measurements confirm that two likely candidates for pre-main-sequence objects in the solar neighbourhood are high luminosity sources embedded in a very compact heated shell. Following Bedijn *et al.*⁷ it is unlikely that such a phase can be sustained for longer than 10^5 yr. Other evidence for a transient expansion phenomenon around the BN object was recently pointed out by Hall *et al.*¹¹ in the discovery of an expanding shell observed in the lines of the CO molecule.

Although these first measurements are restricted to a single radial direction, and do not yet answer the crucial question of the flattening in the accretion disk and its possible rotation, the method proves that sizes of the order of 40 AU are within reach in the thermal IR: radiative transfer problems and inner dynamics of accretion cores can therefore be studied observationally.

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Quasi-biennial variation of the solar neutrino flux and solar activity

THE observed flux of the solar neutrinos is lower by a factor of five than that theoretically estimated from the study of the standard model of the solar interior¹. To explain this discrepancy various theories have been proposed which disregard some of the assumptions inherent in this model. However, no theory has been able to reduce the theoretical fluxes to the observed ones^{2,3}. The experimental data obtained by Davis *et al.*¹ indicate that this discrepancy is real, so that these data should be seriously considered in any future research on the solar neutrinos. They have published these data for each period that they have observed since 1968, (Fig. 1)^{4,5}. We have used their data on the solar neutrino fluxes since 1968 to study whether the neutrino flux has any tendency to vary periodically during the solar cycle of 11 yr. The dependence of the neutrino flux on the solar activity, namely, the phase of the solar cycle, was suggested by Sheldon⁶. He explained that, in the late 1960s, the neutrino production rate in the solar core seemed to be dependent on the degree of the solar activity, as he could only refer to the early results on the neutrino flux observations. It is

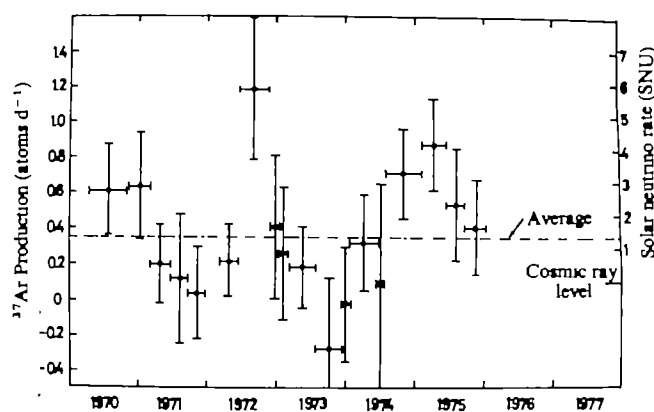


Fig. 1 The observed fluxes of the solar neutrinos for the years 1970–76 (Davis *et al.* 1976).

shown here that this idea is not supported by the data on the neutrino flux obtained since 1968.

The neutrino flux may have been varying quasi-biennially throughout the solar cycle since 1968 (ref. 7), and this flux variation seems to be independent of the mean trend of the variation of the solar activity for 11 yr. Therefore, this 'quasi-biennial' variation of the neutrino flux must be related to some feature of the solar phenomena, because such a variation with a period as short as 2 yr, may produce some phenomena on the solar photosphere which are causally connected with those taking place in the interior of the Sun. One of these must be the variation of the relative sunspot numbers because they are

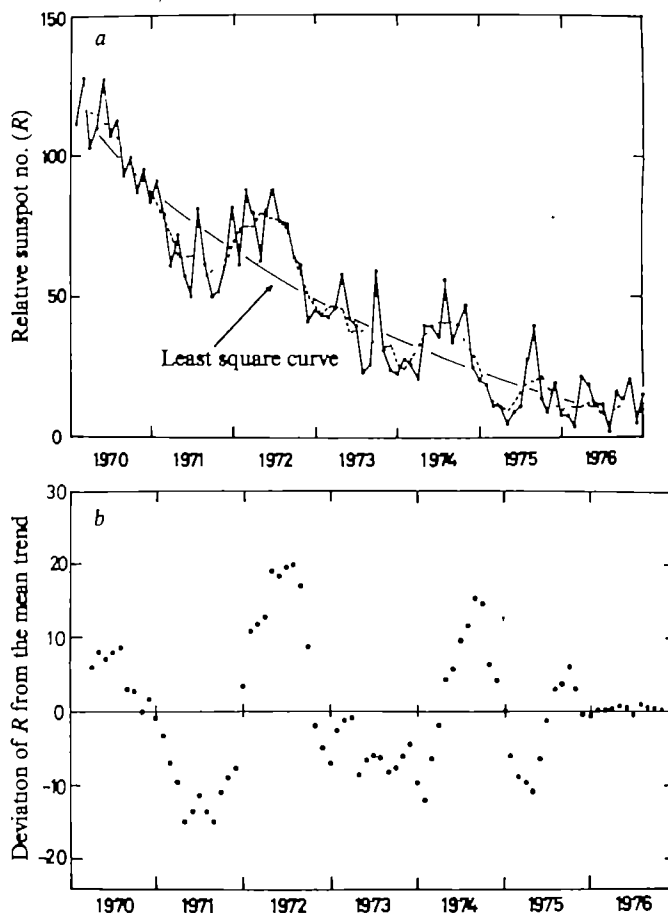


Fig. 2 a, The monthly mean values of the relative sunspot numbers for the years 1970–76. The moving average for 5 months is indicated by the dashed line. The mean trend of the variation of the sunspot numbers is shown by the solid line; b, The deviation of the relative sunspot numbers from the mean trend shown in (a).

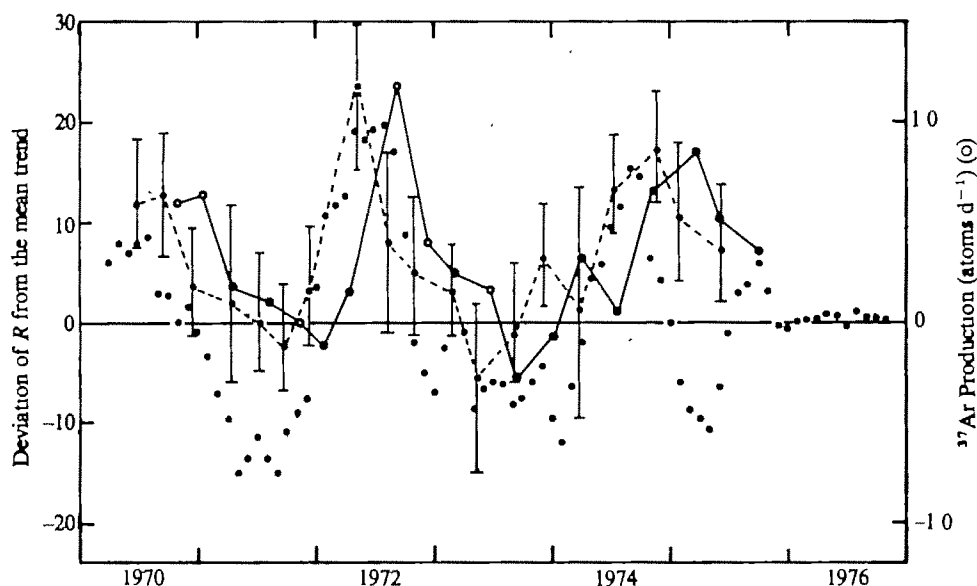


Fig. 3 The relationship between the variation of the solar neutrino flux and the deviation of the solar activity from the mean trend for the years 1970–76. The open circles indicate the mean observed fluxes of the solar neutrinos shown in Fig. 1. The dashed lines give those fluxes shifted earlier by 4 months.

produced by some instability associated with hydromagnetic convective motion beneath the photosphere.

According to the power spectral analyses by Shapiro *et al.*⁸, the relative sunspot numbers vary with a period of 25.7 months. Compared with the result shown in Fig. 1, it turns out that this period is very similar to that for the quasi-biennial variation of the solar neutrino flux.

To determine whether a quasi-biennial variation for the relative sunspot numbers exists between 1970 and 1976, the moving average for 5 months was first obtained for the monthly mean relative sunspot numbers as shown in Fig. 2a. This shows that the relative sunspot numbers deduced from this average have a clear tendency to vary quasi-biennially while superimposing the mean trend, shown by the thick line, which represents the pattern for the 11-yr variation of these sunspot numbers. From the result shown in Fig. 2a, the deviation of these numbers from this mean trend can be evaluated by subtracting the numbers for the mean trend from the observed sunspot numbers averaged over 5 months with respect to each month.

The relative sunspot numbers deviation from the mean trend is shown in Fig. 2b. This shows that these numbers vary quasi-biennially, although the data for 1976 do not show such tendency clearly because this year was at the solar activity minimum. The time-series analytical method has then been applied to estimate the length of an apparent period for this quasi-biennial variation as shown in Fig. 2b. The result clearly indicates that the relative sunspot numbers for these years also vary with a period of 25.3 months, which is almost the same as obtained for other years⁸. This suggests that the solar activity always varies with a period of about 25 months, which is independent of the phase of the solar activity cycle. Furthermore, the geomagnetic disturbances are controlled by the quasi-biennial variation of the solar activity⁹.

Comparing the result of Fig. 2b with the observed data on the neutrino flux shown in Fig. 1, it follows that when the deviation is positive the observed flux of the solar neutrinos seems to be generally very high and vice versa, if we neglect small time difference.

To examine this relationship quantitatively, the observed fluxes of the solar neutrinos have been superimposed on the result shown in Fig. 2b. The result is shown in Fig. 3, where the open circles are the observed values of the solar neutrino fluxes, which are connected with the solid lines, although the error bars have been omitted. The filled circles connected with the chain lines indicate the observed fluxes shifted earlier by 4 months, for which the error bars are added to show a correlation between these fluxes and the deviation of the relative sunspot numbers. Figure 3 shows that although the statistical variation of the solar

neutrino fluxes is quite large, these fluxes, except for those of 1976, vary in phase with the variation of the sunspot numbers deviated from their mean trend. This result is, however, unexpected when we use the standard model of the Sun to investigate the production process of the solar neutrinos. This result suggests that this process is causally related to some unknown process taking place in the interior of the Sun, which must also be responsible for the quasi-biennial variation of the solar activity. As the cause of the solar activity is associated with large-scale internal motion associated with some unstable convective modes, this process may be accompanied by some form of the motion. Furthermore, the result shown here suggests that this motion can penetrate into the region where the thermonuclear fusion is taking place and thus disturb the stable burning processes of its hydrogen and the helium-3. No theory on such a motion has yet been proposed but our result means that further investigation is needed on the large-scale internal motion of the Sun, which causally connects the fusion processes with the quasi-biennial variation of the solar activity.

The standard model does not provide any means to investigate such convective motion penetrable to the nuclear-burning central core of the Sun. However, some oscillatory modes of motions may exist with longer periods not previously observed. One of these may vary with a period of about 2 yr if the quasi-biennial variation of the relative sunspot numbers has to be produced from some modes of the convective motion beneath the solar photosphere. Thus the continued observation of the solar neutrino flux for several solar cycles would give us an important clue when examining the accuracy of the standard model and its limitations in investigating the interior of the Sun in relation to the nuclear-burning processes.

There is evidence, in fact, that the azimuthal component of the convective motion, measured as the angular velocity, seems to be varying quasi-biennially during the last solar cycle no. 20 (1965–76) (ref. 7). This motion, therefore, seems to be associated with the convective motion mentioned above. However, such motion can never be derived from the study of the standard model of the Sun.

These results indicate that the production rate of the neutrinos in the solar interior varies quasi-biennially and may be causally associated with the convective motions responsible for the quasi-biennial variation of the relative sunspot numbers.

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Tensile strength of water

THE measured tensile strength of water has long been known to be significantly less than theoretical predictions and the reduced strength is normally attributed to the presence of solid impurities that serve as nucleation sites for rupture of the liquid¹. A favourite method of measuring tensile strength is through the dynamic stressing of a liquid by an acoustic field. At sufficiently large values of the peak negative acoustic-pressure amplitude the liquid ruptures and forms a rapidly growing vapour cavity that collapses violently during the positive portion of the cycle. This cavity collapse is normally violent enough to be observed easily with the unaided eye or ear. Such an event is termed the acoustic cavitation threshold and is a measure of the tensile strength of a liquid. Although some successes have been obtained in achieving measured values of the tensile strength comparable to theoretical predictions for extremely small samples² or after extensive filtration³, I know of no current theory for the prediction of values of the dynamic tensile strength for ordinary distilled water. Using a modified version of a recent theory by Apfel⁴, an equation is presented here that correctly predicts the variation of the acoustic cavitation threshold of water with surface tension, dissolved gas content and temperature.

It is assumed that there exists in the body of the liquid numerous nonpolar solid impurities that contain cracks and crevices in which gas pockets may be stabilised against dissolution. Scanning electron micrographs have been made of filtered solid impurities and show innumerable sites for possible gas-pocket stabilisation⁵. Figure 1a is a typical micrograph of a filtered particle. Following a theory of Apfel⁴, and others^{1,6}, it is possible to determine requirements on the threshold acoustic pressure required to nucleate a free gas bubble that will serve as a preferential site for liquid rupture. Apfel⁴ expresses the threshold pressure P_A as

$$P_A \geq P_H - \gamma P_G - P_V + (P_H - P_G - P_V) \left| \frac{\cos(\alpha_R - \beta)}{\cos(\alpha_A - \beta)} \right| \quad (1)$$

for conditions such that $\alpha_R > \beta$. In equation (1), P_H is the hydrostatic pressure, P_G the equilibrium pressure of the gas dissolved in the liquid, P_V the vapour pressure, γ a constant with value between 0 and 1 which measures the ability of the nucleating gas pocket to maintain the equilibrium gas pressure, β the half-angle of the conical crevice, and α_A and α_R are the advancing and receding contact angles of the liquid–gas interface in the crevice. This equation has been modified as follows to apply it to the experimental situation. It is assumed that during the stabilisation process the interface advances to a specified position and stops. This position determines the diameter of the crevice at the position where the interface meets the crevice wall. It is further assumed that the crevices stabilise in water at normal surface tension and later reduction of surface tension by addition of surfactant does not affect the position of the stabilised interface. The shape of the interface may change but its position in the crevice will not. This assumption allows us to express the quantity $|\cos(\alpha_A - \beta)|$ as a constant, dependent only on the initial value of the surface tension. A second modification of the equation involves the nucleation process. The receding contact angle is affected by addition of a surfactant and its dependence

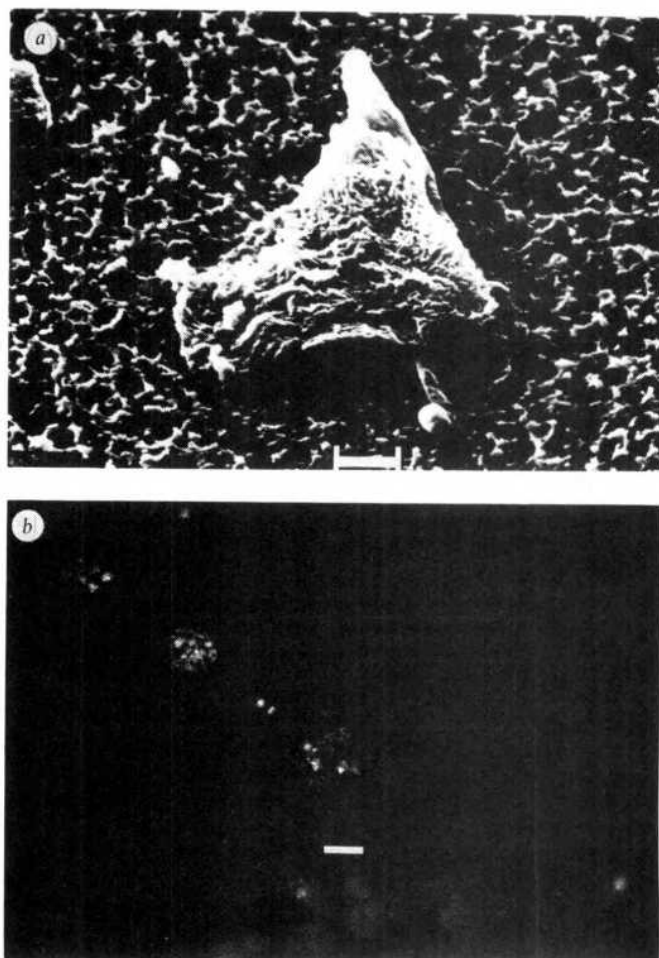


Fig. 1 a, Scanning electron photomicrograph of a solid impurity. The particle was removed by filtration of distilled water with a 0.45- μm pore size filter, shown as the mottled area in the background. Note the ragged appearance of the surface showing the numerous possible nucleation sites. Scale bar, 5 μm . b, Photograph showing creation and resultant motion of a transient cavity. The cavity was initiated at the centre of a resonating piezoelectric cylinder and was propelled outwards by radiation-pressure forces. Here the flash interval was 2.4 ms, the acoustic pressure amplitude at the antinode was 4.7 bar, and the maximum velocity achieved was about 100 cm s^{-1} . Scale bar, 1 mm.

on surface tension must be specified. This is done by writing $\cos(\alpha_R - \beta) = \cos[\alpha_E - (\alpha_H + \beta)]$ where α_E is the equilibrium contact angle and α_H is the hysteresis between the equilibrium and the receding contact angle. Such a modification allows us to express the receding contact angle in terms of the surface tension. Bargeman and Van Voorst Vader⁷ have found that α_E can be expressed as

$$\cos \alpha_E = -1 + C/\sigma \quad (2)$$

where σ is the surface tension and C is a constant that depends on the surface properties of the solid. For nonpolar solids such as paraffin, and beeswax, $C \approx 50$.

The acoustic cavitation threshold can thus be expressed in terms of measurable parameters as

$$P_A = (P_H - P_V - \gamma P_G) + \frac{(P_H - P_V - P_G)}{\delta} \times \{\cos \phi (C/\sigma - 1) + \sin \phi [1 - (C/\sigma - 1)^2]^{1/2}\} \quad (3)$$

To apply this theory to ordinary water, the unknown parameters, γ , δ and ϕ must be specified. γ is 1.0 for situations in which the gas pressure in the nucleating cavity is maintained at

equilibrium with that in the fluid. As a precise measurement of this constant is impossible, it is assumed that equilibrium will be maintained and thus $\gamma = 1.0$. δ is a parameter which measures the initial value of the advancing contact angle, and is given by $\delta = |\cos(\alpha_A - \beta)|$. Values for the advancing contact angle of

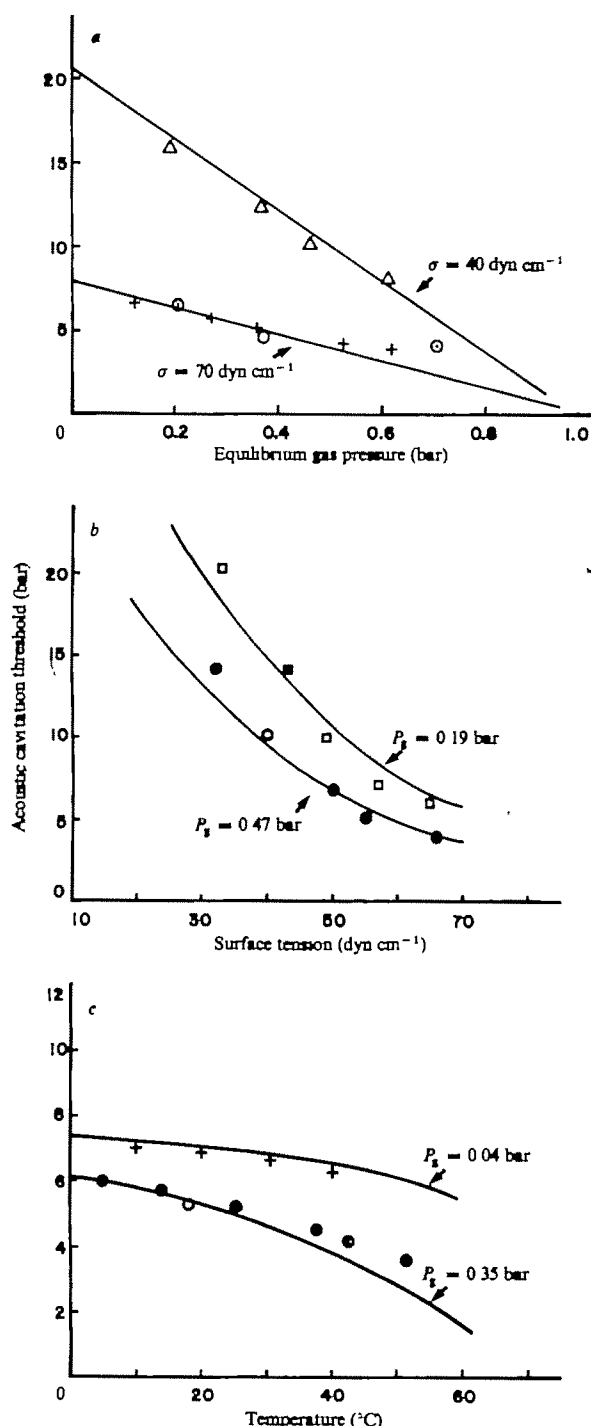


Fig. 2 Variation of the acoustic cavitation threshold of water with (a) dissolved gas content for two values of the liquid surface tension; (b) with surface tension for two values of the equilibrium gas pressure; and (c) with temperature for two values of the equilibrium gas pressure. The curves are for equation (3) with $\gamma = 1.0$, $\delta = 0.035$ and $\phi = 32^\circ$. In (a) Δ and + are experimental measurements from this study and $\circ$ points are values obtained by Strasberg⁶; the frequency in (a) and (b) was 36 kHz and the temperature 25 °C. In (c) the frequency was 22 kHz and the surface tension was 70 dyn cm⁻¹; + points are the normalised values of Galloway¹¹; $\circ$ are measurements from this study. The temperature dependence of the vapour pressure P_v and the equilibrium gas pressure P_g have been inserted into equation (3).

water on nonpolar solids are of the order of 106° . Bargeman⁹ lists values of α_A for four nonpolar solids with an average of 106° , standard deviation 6° ; water on paraffin wax is known to be approximately 106° (ref. 9). α_A was thus selected to be 106° . The crevice angle β is assumed to be small; crevices need small angles for stabilisation; a value of $\beta = 14^\circ$ was selected to give $\delta = 0.035$. Values of the hysteresis angle of water plus surfactant on nonpolar solids were measured by Furmidge¹⁰. He obtained values ranging from 14° to 44° for water plus surfactants on white beeswax. To obtain a good fit to the data, α_H was chosen to be 18° ; hence $\phi = \alpha_H + \beta = 32^\circ$.

The incipient cavitation threshold was measured for acoustic cavitation in distilled water. A hollow piezoelectric cylindrical transducer was used that was open at one end and closed at the other with a thin glass window (thickness 0.01 cm). The thin glass window approximated a pressure-release boundary and the cylinder was driven in a $(r, \theta, z) = (3, 0, 3)$ resonant mode that generated cavitation only along the axis of the cylinder and only at the middle antinode. The height of the cylinder was ~ 7.5 cm, the inside diameter was 7.0 cm, the thickness was 0.5 cm. Figure 1b shows a photograph of a cavitation event that was obtained under stroboscopic illumination. The cavity was created along the axis of the cylinder, approximate centre of photograph, and was propelled outwards by radiation pressure forces¹².

The variation of the cavitation thresholds is shown in Fig. 2a-c respectively: note the excellent agreement with theory. The selection of the parameters γ , δ and ϕ was varied slightly to obtain a best fit but was restricted to lie within the range of measured values. The theory shows no variation with frequency; measurements were made in the low kilohertz region where the measured threshold does not depend upon frequency¹³. For higher frequencies, cavity dynamics are important in determining the threshold^{4,14}.

It is seen that the dynamic tensile strength of distilled water can be accurately predicted if proper account is made of the solid impurity content of the liquid.

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Simulated lidar return from a one-dimensional stratospheric aerosol model

RESULTS of theoretical calculations on lidar backscatter from the stratospheric aerosol are presented here. The calculations use the size distribution, particle number density and particle composition predicted by a one-dimensional model of the stratospheric aerosol layer. The theoretically calculated backscatter profile is compared with experimental results obtained from actual lidar observations of the layer.

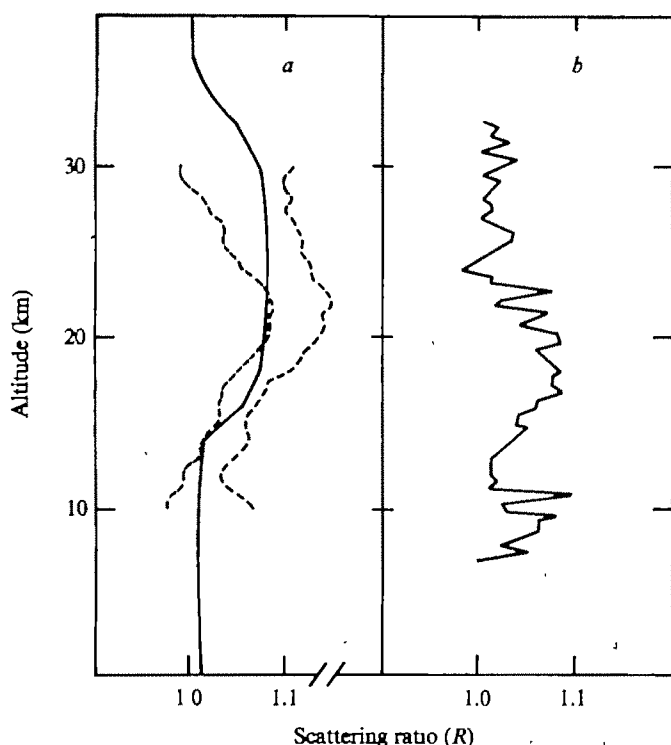


Fig. 1 a, Scattering ratio (R) as a function of altitude calculated from aerosol model. Dotted lines represent the range of experimental uncertainty (from ref. 11). b, Lidar backscattering ratio obtained 20 September, 1978 with Langley Research Center 48° lidar system.

The one-dimensional stratospheric aerosol model used here is described fully elsewhere¹; therefore we shall give only a few pertinent details. The model assumes that the primary source of sulphur to the stratosphere is biogenic OCS released at ground level². This OCS diffuses into the stratosphere where it is photodissociated, leading to the production of gas phase sulphuric acid. Aerosol droplets are formed by heteromolecular-heterogeneous nucleation of gaseous sulphuric acid and water onto pre-existing solid particles of tropospheric origin. The aerosol particles are spherical, liquid $\text{H}_2\text{SO}_4\text{-H}_2\text{O}$ solution droplets with solid cores. The particles undergo the physical processes of condensation, evaporation, coagulation, sedimentation and vertical eddy mixing³. The model uses these chemical and physical mechanisms to predict altitude profiles for all important aerosol parameters, including the size distribution, number concentration and amount of sulphuric acid in the drops.

Model predictions have been compared with *in situ* measurements of the stratospheric aerosol¹, and agreement is good. Consequently, the model should predict aerosol backscatter ratios reasonably well. However, *in situ* measurements are quite rare above 25 km so they cannot be used to check model results at high altitudes. Therefore, we wish to show that reasonable lidar backscatter profiles can be predicted theoretically using the aerosol model. We also use model results to perform a few simple theoretical 'experiments' to determine how lidar observations will be affected by changes in certain properties of the aerosol layer.

The model simulates a 'background' aerosol; that is, sudden injections of sulphuric gases and particles were not incorporated in the model. Thus, the model results represent the stratospheric aerosol during volcanically quiescent periods. Fortunately, there is a fairly comprehensive lidar record of the aerosol layer over the past decade^{4,5} and much of the data have been obtained during periods of little or no volcanic activity. The last volcano which injected a significant amount of material into the stratosphere was Volcán de Fuego which erupted in October 1974. As

shown by McCormick *et al.*⁶ the volcanic debris in the stratosphere which could be attributed to Fuego decayed away with a $1/e$ time constant of about one year. Before Fuego, a large amount of volcanic matter was injected into the stratosphere by Agung (March 1963). However, just before Fuego, and at present (November 1978), lidar backscatter studies show an aerosol layer close to 'background' levels. Therefore, our theoretical results are compared with data taken either shortly before Fuego or near the present date.

The aerosol parameters used in our calculations are the following: the lidar backscatter intensity was calculated using a Mie model to evaluate the aerosol backscatter coefficient (f_a) for wavelengths $\lambda = 0.6943 \mu\text{m}$ (lidar wavelength) and $\lambda = 1.06 \mu\text{m}$. Both the Mie model and the aerosol model use standard atmosphere⁷ mid-latitude profiles for temperature, density and water vapour. Using the index of refraction data given by Palmer and Williamson⁸ and model results, we determined the altitude dependent index of refraction of the sulphuric acid aerosols. The values varied from 1.41 at 10 km to 1.44 at 38 km. We assumed that the index of refraction of the particles was that of the sulphuric acid-water shell, an assumption which is valid for particles with small cores⁹. The model distinguishes between sulphuric acid aerosol droplets and solid 'condensation nuclei' of tropospheric origin. For the condensation nuclei the real part of the index of refraction was taken to be 1.50, independent of altitude¹⁰.

The results of our calculations are shown in Figs 1-4. These are profiles of the lidar scattering ratio, R , defined by

$$R = \frac{f_a + f_m}{f_m}$$

where f_m is the molecular (Rayleigh) backscattering coefficient.

Figure 1 shows the calculated lidar backscatter ratio at $\lambda = 0.6943 \mu\text{m}$ from aerosol droplets and condensation nuclei between 10 and 40 km altitude. On the same plot we give the range of $\pm$ one standard deviation about the mean measured in 16 lidar observations taken in the period June 1973 to March 1974 by Russell *et al.*¹¹. This was shortly before Fuego so presumably the stratospheric aerosol was primarily non-volcanic in origin. As Fig. 1 shows, the model prediction is in good agreement with experimental results. It should be noted, however, that individual lidar returns show a great deal of structure which is not present in the model. To illustrate this, a typical lidar backscatter ratio profile is shown in Fig. 1b. The structure is probably a result of small scale temperature and

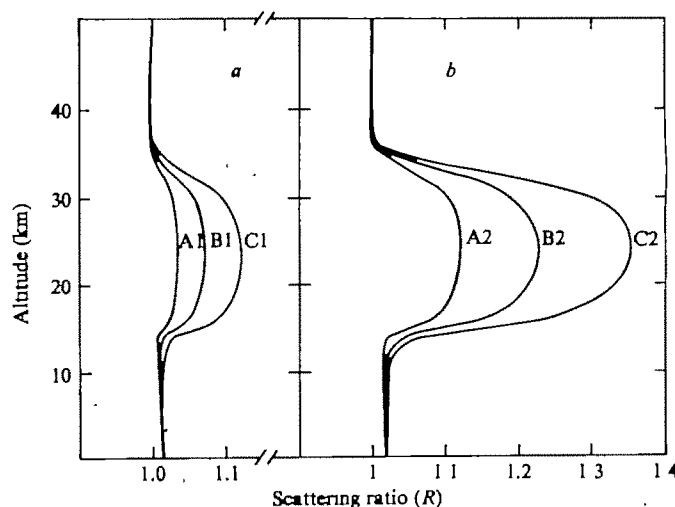


Fig. 2 a, Calculated scattering ratio profiles for different values of real part of index of refraction (η) for wavelength $\lambda = 0.6943 \mu\text{m}$. Curve A1, B1, C1 are for $\eta = 1.3, 1.4, 1.5$, respectively. b, Scattering ratio profiles for wavelength $\lambda = 1.06 \mu\text{m}$. Curves A2, B2, C2 are for $\eta = 1.3, 1.4, 1.5$, respectively.

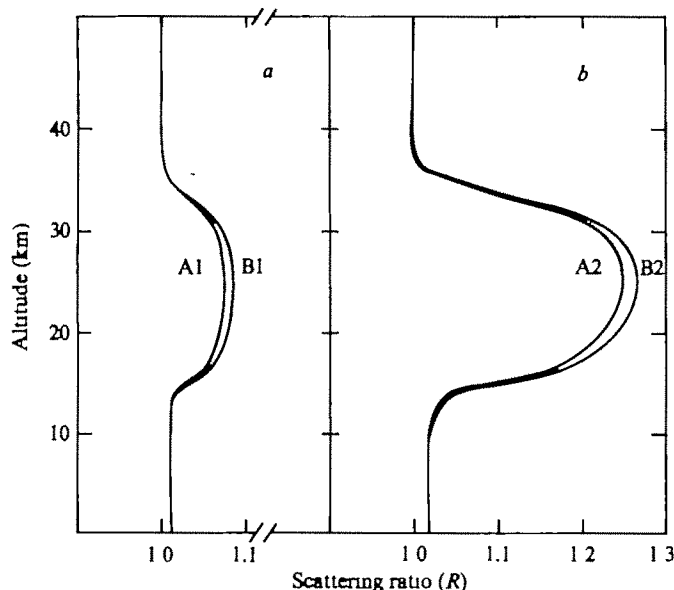


Fig. 3 a, Calculated scattering ratio profiles for different values of imaginary index of refraction (k) for $\lambda = 0.6943 \mu\text{m}$. Curves A1 and B1 are for $k = 0.00$ and 0.1 , respectively. b, Scattering ratio profiles for $\lambda = 1.06 \mu\text{m}$. A2 and B2 are for $k = 0.00$ and 0.1 , respectively.

water vapour fluctuation and the advection of particles due to stratospheric dynamics. These phenomena are not included in the model. Consequently we can expect agreement between the model and the average of a number of observations, but not for a particular lidar return.

The agreement between model and observation shown in Fig. 1 is particularly striking when one considers the effect of volcanic activity on the lidar returns. For example, post Fuego observations^{3,6} gave scattering ratio (R) profiles that were very sharply peaked and reached maximum values >4 . The pre-Fuego data shown in Fig. 1 (and present day observations of the layer) give maximum values of R of about 1.08.

The calculated lidar signal in Fig. 1 indicates the top of the layer around 35 km. The microphysical processes incorporated in the model result in the evaporation of the sulphuric acid droplets at about 34 km. However, there are some droplets above that altitude due to mixing. Also, the evaporation of the droplets is a source of condensation nuclei (cores). Therefore, the predicted lidar return does not drop off until somewhat above the evaporation level. Lazrus and Gandrud¹² have reported the existence of sulphates up to 36 km, which is slightly higher than indicated by model results or by measurements made with lidar and other methods.

To determine the sensitivity of lidar observations to the index of refraction (η) we carried out calculations varying η for both the aerosol particles and the condensation nuclei. Changes in η for the condensation nuclei did not affect the results because they are so small that they do not contribute significantly to the backscatter. Even the presence of highly absorbing condensation nuclei will hardly affect the lidar return. To verify this we varied the imaginary part of the index of refraction (k) of the condensation nuclei from 0.0 to 1.2 and obtained backscatter profiles which were nearly identical.

However, changes in η for the aerosol particles do affect the backscatter ratio profile. Figure 2a shows calculated profiles assuming η to be 1.3, 1.4 and 1.5. The curves obtained for $\eta = 1.3$ and 1.5 fall outside the range of values given in Fig. 1. This result is significant as it indicates that the index of refraction of the stratospheric particles could be evaluated from lidar returns. (Such an analysis should only be undertaken if the results of many lidar returns are averaged and the atmospheric conditions are comparable to those used in the model. Also one

should have independent information on the size distribution and number density of the particles as these also affect the return). Note that η for ammonium sulphate is slightly greater than 1.5 at $0.6943 \mu\text{m}$. Thus, the results given in Fig. 2a suggest that the aerosol particles are unlikely to be composed of ammonium sulphate, as was believed by earlier investigators of the stratospheric layer.

The value of k for sulphuric acid-water solutions at $0.6943 \mu\text{m}$ is of the order of 10^{-8} and in most of our calculations we used a zero imaginary index. However, to investigate the effect of a large k on lidar backscatter we performed some calculations with $k = 0.1$. It must be emphasised that such a value is many orders of magnitude larger than expected. Nevertheless, as shown in Fig. 3a, the effect on the backscatter ratio profile is not large.

It has been suggested that multi-wavelength lidar studies of the aerosol layer should be carried out. Figures 2b and 3b show the effect of observing the stratospheric aerosol with a $1.06 \mu\text{m}$ wavelength lidar. The most salient characteristic of these figures is the fact that the scattering ratio at $1.06 \mu\text{m}$ is much larger than at $0.6943 \mu\text{m}$. But a comparison of Fig. 2a and b shows that the relative change in scattering ratio with changes in η is nearly the same for both wavelengths. Thus lidar observations at these two wavelengths would apparently give little additional information on the index of refraction. Similarly, comparing Fig. 3a and b we note that a change in λ gives a larger absolute difference between the absorbing and non-absorbing cases, but the relative difference between the two cases is only slightly greater for the larger wavelength.

Meteoritic ablation in the mesosphere might be a significant source of small particles in the stratosphere. To determine the effect of increasing the number of small particles on the lidar return, we incremented the number of condensation nuclei of size $0.01 \mu\text{m}$ radius to a mixing ratio of 800 particles mg^{-1} air above 30 km, and to follow the profile given by Kaselau *et al.*¹³ below 30 km. This enormous increase in small particles had no discernible effect on plotted backscatter ratio profiles. The reason is, of course, that the $0.6943 \mu\text{m}$ backscatter is quite insensitive to small particles. Consequently, one cannot expect lidar studies to determine whether meteoritic ablation contributes significantly to the particulate loading in the stratosphere.

Finally, to evaluate changes in temperature on the lidar return, we calculated the backscatter profile using aerosol model results

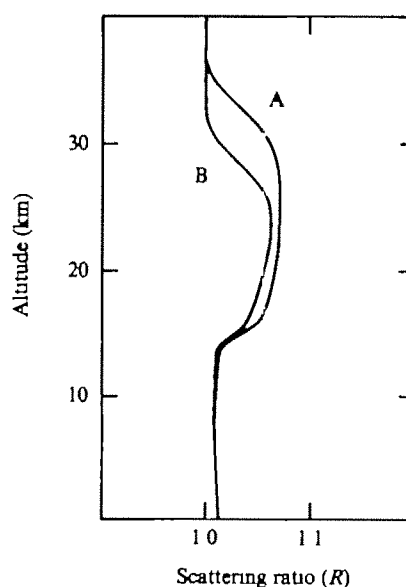


Fig. 4 Calculated scattering ratio for different stratospheric temperature profiles. Curve A is for spring/autumn 45° latitude standard atmosphere. Curve B is for July 75° north latitude standard atmosphere.

obtained with a 'warm stratosphere' temperature profile (USA Standard Atmosphere, July, 75° N latitude). The results, presented in Fig. 4, indicate that the lidar returns drop off at lower altitudes, and there is a small decrease in maximum values of the scattering ratio. Calculations using a 'cold stratosphere' (high latitude winter) show a profile extending to higher altitudes, as expected. The model assumes a smoothly varying temperature profile and does not include advection so the sharp peaks in scattering ratio at low temperatures which were noted by McCormick, *et al.*⁶ do not show up in our calculations.

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On the detection of lunar volatile emissions

ONE of the most interesting scientific results of the Apollo programme was the discovery that the Moon sporadically releases radiogenic gases¹⁻⁴. It is likely (although not yet proved) that these emissions are correlated with other detectable lunar activity such as shallow moonquakes^{5,8} and the elusive visible events known as 'lunar transient phenomena' or LTPs^{6,7}. Runcorn has argued⁸ that these three types of lunar activity are linked through tidal triggering of cylindrical fault systems around the circular mare, which results in gradual settling of the mascons and venting from the cracks. Considerable evidence exists which favours such a model, including seismic⁹, photogeologic¹⁰ and electromagnetic induction¹¹ data. However, a search for sporadic venting using the Apollo lunar surface Suprathermal Ion Detector Experiments (SIDEs) was unsuccessful¹². This note points out that the SIDE instruments lack the sensitivity to detect even large gas emissions if the venting occurs primarily at a few well-defined LTP sites, and suggests specific flight instruments for the proposed ESA Polar Orbiting Lunar Observatory (POLO) mission^{13,14} which could detect active venting, would help to determine the energy source for LTPs, and would increase our knowledge of lunar geophysics.

It is known that ⁴⁰Ar, ⁴He, and ²²²Rn are outgassed from the lunar interior^{2,4}, where they are formed from the decay of ²³⁸U, ⁴⁰K and ²³²Th. Based on the Rn data, Hodges argues² that the characteristic time for the radiogenic gas release process is ~70 d, with the Rn carried to the lunar surface by the more plentiful Ar and He in an abundance ratio of approximately 137,500:37,500:1 (He:Ar:Rn). The total loss rate for these species from the Moon is about 1.6 × 10³⁰ atoms yr⁻¹ or 1.4 × 10⁴ kg yr⁻¹ with a mean atomic weight of 11.6 AMU. Vondrak³⁰ has calculated the maximum column density ρ of vented gas at an arbitrary distance, R , from a source on the lunar surface. For both direct flux and diffusive transport models, he finds $\rho = S/\epsilon\pi R^2$ provided the gases are not strongly absorbed by the surface, where S is the number of particles released from the (assumed) impulsive point source. Vondrak argues that a gas release would be readily detectable if it increased the column density at one of the three SIDE sites by about a factor of five above the normal lunar dayside density of 10¹² atoms cm⁻². If it is assumed that the vented gases are primarily the He and Ar which are known to be released, rather than CO, CO₂, H₂O and so on as in Vondrak's study (mean atomic weight 32 AMU), his results are modified slightly. Table 1 shows the minimum venting required from one of the six most prominent LTP sites^{6,7} to be detected at a SIDE location. These results indicate that if the lunar He, Ar and Rn vented from the interior are released sporadically at one or more of these LTP sites, it is unlikely that the gas release would have been detected by a SIDE instrument. For example, if half of the released lunar radiogenic He and Ar were to be vented sporadically from the crater Aristarchus and its neighbourhood (where more than one-third of all reported LTPs have been detected)⁷, these events would go unnoticed by the SIDEs. Of course, it is expected that the gases produced in the interior are vented from the Moon at several locations, so that a very large fractional release of the yearly He-Ar-Rn loss is required at one site close to a SIDE to be detectable (for example, one-sixth of the yearly loss vented at the crater Alphonsus in one brief event would be observable at the Apollo 14 site). Therefore the SIDE data are compatible with the other Apollo measurements which indicate sporadic He, Ar and Rn releases.

If the LTP are related to sporadic venting of these radiogenic gases, the detection of such events should be one of the mission objectives of the proposed POLO spacecraft. Previous studies have demonstrated that great difficulties arise in attempts to find energy sources at the lunar surface which could adequately explain the LTP^{7,15}, which in some cases must exhibit a specific lunar surface brightness of at least 1-10 W m⁻² (optical) to be observable from the Earth¹⁶. One process¹⁶ which may be capable of exciting local clouds of He and Ar to the required brightness levels is the Alfvén critical velocity effect^{17,18}. This effect is a nonthermal ionisation process which occurs when a plasma moves through a neutral gas in the presence of a magnetic field at a velocity greater than $\sim(2e\psi/m)^{1/2}$ where ψ and m are the ionisation potential and mass of the neutral atom respectively. The exact mechanisms which transform the relative ion-neutral kinetic energy into heating of the free electrons which must do the ionisation are not fully understood^{19,21}, but the existence of critical velocity phenomena has been

Table 1 Minimum He-Ar release from LTP sites which would be detectable at SIDE locations, after Vondrak³⁰

Vent location	Gas release (kg)		
	Apollo 12	Apollo 14	Apollo 15
Aristarchus	1 × 10 ⁴	1.3 × 10 ⁴	1.8 × 10 ⁴
Alphonsus	4 × 10 ³	2.4 × 10 ³	1.4 × 10 ⁴
Plato	2.6 × 10 ⁴	2.5 × 10 ⁴	5.8 × 10 ³
Gasendi	3.6 × 10 ³	5.4 × 10 ³	3.0 × 10 ⁴
Agrippa	1 × 10 ⁴	7.3 × 10 ³	4.0 × 10 ³
Proclus	4.4 × 10 ⁴	3.6 × 10 ⁴	1.4 × 10 ⁴

established in laboratory experiments²⁰ and possibly on the lunar surface²². The interaction between the solar wind and an emitted He-Ar cloud should exhibit this effect, although there are uncertainties due to scaling from the laboratory experiments to the lunar situation¹⁶. The supermagnetosonic plasma-neutral interaction should be characterised by the emission of radiation, primarily at infrared and longer wavelengths, which is partially plane polarised relative to the ambient magnetic field²³. This polarisation is a result of the nonlinear plasma processes in the interaction which create a population of nonthermal electrons in the interaction region with velocities primarily along the magnetic field direction. Unlike other proposed energy sources for LTP, this critical velocity model relies on internal lunar energy for the phenomena. The thermal energy of the escaping neutral gas is converted into free electron energy by the plasma interaction, and then into radiation by coulomb excitation of neutrals. That is, the magnetised solar wind acts like an electromagnetic catalyst which converts the expansion energy of the gas cloud into visible photons.

In addition to the other experiments¹³ which should be included on a lunar survey mission, a POLO spacecraft should carry instruments to detect and analyse the sporadic venting of lunar radiogenic gases. These should include: an α -particle spectrometer² to measure the ²²²Rn and ²¹⁰Po distributions on the lunar surface²³ and thus indicate regions where recent venting has taken place⁴; a combined infrared spectrometer/polarimeter to determine the surface mineralogy by reflectance spectra^{24,25} and to search for the He and Ar emission lines expected in the plasma-neutral interactions^{16,26,27}; and a low-to-medium energy charged particle detector capable of both high spatial (pitch angle) and energy resolution. The last instrument could map the lunar surface remanent magnetic fields using the electron reflection technique^{28,29}, and could search for bursts of low energy (80–100 eV)^{26,27}, low pitch-angle (relative to the solar wind magnetic field) nonthermal electrons generated in gas clouds vented from the Moon by the proposed critical velocity effect.

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Palaeomagnetic field strengths from sediments baked by lava flows of the Chaîne des Puys, France

MEASUREMENTS of ancient geomagnetic strength are of great interest but are much less common than measurements of direction. This is due partly to the scarcity of materials which are both well dated and suitable for study, and partly to the amount of time required to make measurements using presently available techniques. We report here three new results from baked sediments which have been dated by thermoluminescence (TL) or radiocarbon methods. One of them is early Holocene and the others are late Pleistocene (Table 1). The localities have been described by Huxtable *et al.*¹, from whom we obtained samples.

Laboratory measurements were performed on cubic specimens (23 mm edges, cut from the samples), using a 'Digico' magnetometer with an 80-mm diameter balanced fluxgate detector². Directions of natural remanent magnetisation (NRM) from those samples which were orientated have been discussed elsewhere¹.

Ancient field strengths were determined using the modified Thelliers' technique^{3–5}, which involves reheating specimens to progressively higher temperatures and comparing the ancient magnetisation with a new one acquired in a known laboratory magnetic field. Two heatings were performed at each temperature, using a furnace of the type described by Barbetti⁶ inside the Rubens coil system constructed by Weaver¹⁰. The first heating and cooling cycle at each temperature was performed in zero magnetic field, and the second in a magnetic field of 48.5 μ T. Measurements of remanent magnetisation were made at room temperature after every heating; values of the partial NRM (PNRM) remaining were obtained from the first of each pair of measurements, and values for the newly acquired partial thermoremanent magnetisation (PTRM) were obtained by vector subtraction between the pairs of measurements for each heating temperature.

The measurements were analysed in the normal way by constructing an NRM-TRM diagram, in which PNRM values are plotted against PTRM values with heating temperature as a parameter^{7,8}. Lines were fitted to appropriate sets of points using the 'maximum likelihood' method¹¹, assuming that errors in the PNRM and PTRM are normally and identically distributed but uncorrelated. The slopes of the fitted lines are a measure of the ratio of NRM to TRM, and estimates of ancient field strength are derived on the basis that the magnetisations are proportional to the magnetic fields in which they were acquired.

The results are summarised in Table 1, and representative NRM-TRM diagrams are shown in Fig. 1. Decisions about which points to include in the line-fitting analyses were made in an empirical fashion. Trial analyses were performed for each specimen and the high-temperature points progressively excluded until the width of the calculated confidence limits approached a minimum; final choices were made for each site after inspection of the results from the appropriate group of specimens.

Five of the St Saturnin specimens (from three black-coloured samples) gave consistent (though somewhat variable) results with a mean of $79 \pm 14 \mu$ T. Two specimens from sample i15 gave much lower values (Table 1 and Fig. 1), and have therefore been excluded from the mean. The low values might be explained by deformation or the introduction of small quantities of fine-grained material after the ancient baking, or they may be related to some peculiarity in the magnetic mineralogy.

Five red-coloured specimens from Royat gave highly consistent results (Table 1). The NRM-TRM diagrams (see Fig. 1) exhibited linearity over wide temperature ranges, with small departures (decreases in PTRM-carrying capacity) above 600 °C; they rank among the best we have ever obtained from

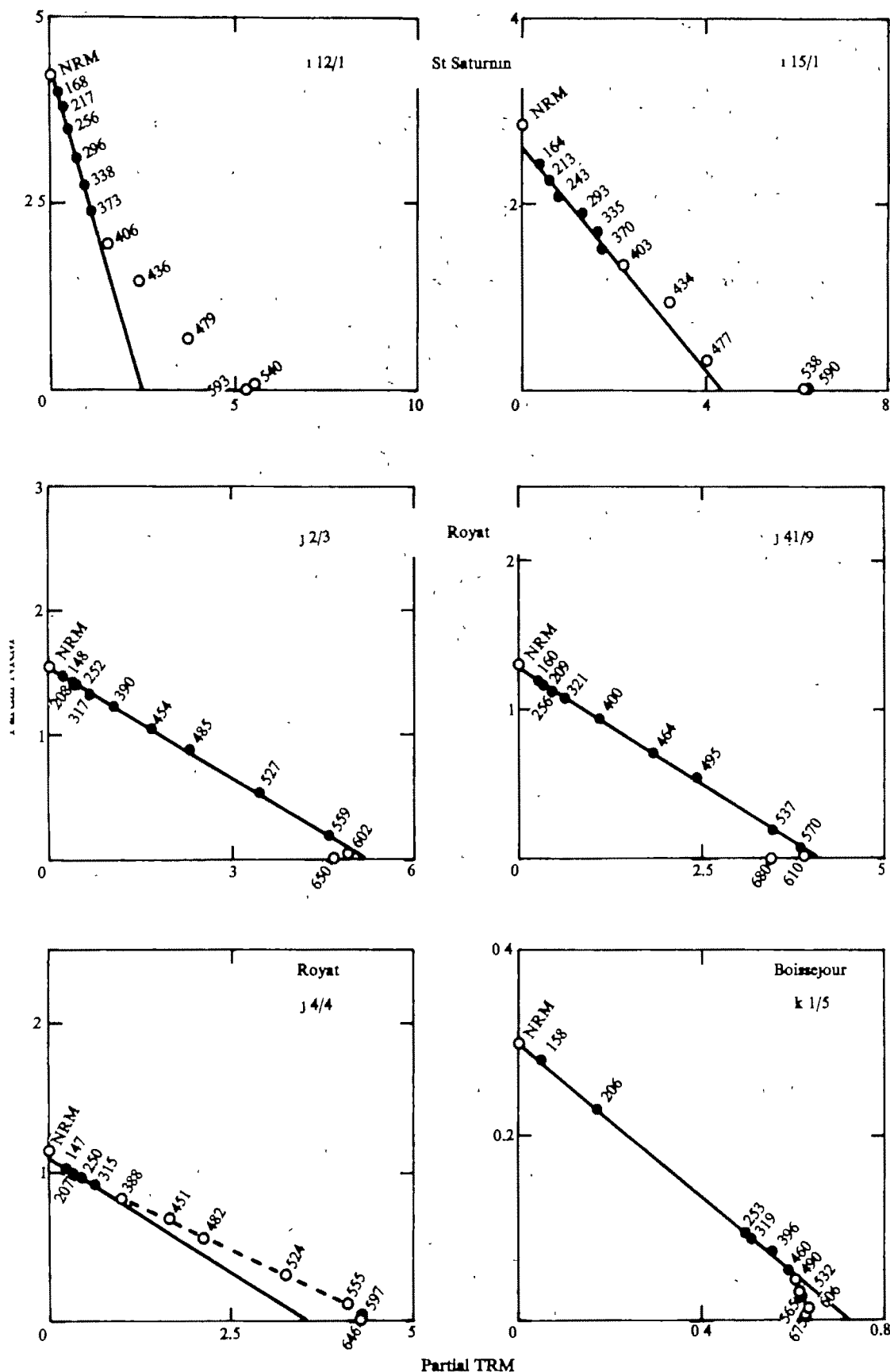


Fig. 1 Representative NRM-TRM diagrams. The units are $\text{mA m}^2 \text{kg}^{-1}$, the horizontal scales are twice the vertical ones, and numbers near the points give the laboratory heating temperatures in $^{\circ}\text{C}$. Lines have been fitted to the sets of points represented by solid symbols. The dashed line for Royat specimen j4/4 has been fitted to the points from 388°C to 555°C ; it has a slope $\sim 25\%$ lower than the solid line and is thought to result from the introduction of new sediment after the ancient baking.

ancient materials. We also analysed specimen j4/4, from a separate sample which contained a small ($\sim 1\%$) but clearly visible proportion of grey-green clay distributed along internal fracture surfaces. Four points on the NRM-TRM diagram (Fig. 1) between 147°C and 315°C gave a result (Table 1) identical to the mean value of the other specimens, whereas the points from 388 to 555°C lie on a well defined line with significantly lower

slope. These observations agree with what one might reasonably expect; that the introduction of sediment after the ancient baking should make little difference to the PNRM but a great deal of difference to the PTRM, particularly at temperatures above $\sim 300^{\circ}\text{C}$ when minerals such as goethite may be transformed to haematite¹².

The four red-coloured specimens from Boissejour gave very

Table 1 Summary of ages and palaeomagnetic field strength measurements

Site	Age (yr BP)	Specimen	Temperature range (°C) (n points)	Ancient field strength (μT)	Dipole moment (10 ²² A m ²)
St Saturnin	8100 ± 800 OxTL 132i Ref. 1 7650 ± 350 Conventional ¹⁴ C Sa-90, Ref. 2	i12/1	168–373 (6)	85 ± 6	12.8 ± 4.2
		i12/2	162–356 (6)	83 ± 21	
		i17/1	162–368 (6)	91 ± 39	
		i19/1	162–368 (6)	71 ± 11	
		i19/2	166–456 (6)	63 ± 23	
			Mean	79 ± 14	
		i15/1	164–370 (6)	29 ± 7	
Royat	25,800 ± 2,200 OxTL132j Ref. 1	j2/3	148–559 (9)	14 ± 1	2.4 ± 0.2
		j2/4	146–544 (9)	16 ± 1	
		j41/5	134–558 (10)	15 ± 1	
		j41/9	160–570 (9)	15 ± 1	
		j41/24	146–551 (9)	15 ± 1	
			Mean	15 ± 1	
		j4/4	147–315 (4)	15 ± 4	
Boissejour	~50,000 OxTL132k Ref. 1	k1/5	158–460 (6)	20 ± 1	3.3 ± 0.9
		k1/6	156–457 (6)	20 ± 1	
		k1/9	156–455 (6)	20 ± 1	
		k1/14	156–449 (6)	21 ± 1	
			Mean	20 ± 1	

Provenance of the samples is given in ref. 1, specimens are identified by sample code and individual numerals after the slash. Column 4 gives the temperature range and number of points used in the final line-fitting analyses on the NRM-TRM diagrams. Estimates of ancient field strength are given with 95% confidence limits for each specimen, and site mean estimates are listed. Dipole moments have been calculated using the palaeomagnetic direction for Royat³ (to give a virtual dipole moment; ref. 4) and the axial dipole inclination for the other two sites (to give virtual axial dipole moments); the 95% confidence limits include contributions from the uncertainties in both strength and direction (α_{95} taken as 20° for St Saturnin and Boissejour).

similar results. An unusually large proportion of the total magnetisation (both NRM and TRM) was associated with temperatures below ~250 °C, suggesting the presence of a mineral (such as titanomagnetite) with a low Curie temperature in addition to the usual magnetic minerals with Curie temperatures above 550 °C. The specimens exhibited a noticeable decrease in PTRM-carrying capacity at temperatures above 460 °C (see Fig. 1), and the higher-temperature points have been excluded from the final line-fitting analysis.

Mean values of ancient field strength, and corresponding values for the geomagnetic dipole moment, are given in Table 1. These results have several implications. First, the value for St Saturnin is higher than the present-day dipole moment (8.0×10^{22} A m²). That confirms previous results obtained by Bucha^{13–15} from Çatal Hüyük in Turkey (mean 10.0×10^{22} A m²), but is somewhat higher than results from Japan¹⁶ (mean 6.1×10^{22} A m²). An average of those and the St Saturnin result yields a dipole moment of 9.6×10^{22} A m² for the period 8,200–7,000 conventional radiocarbon yr ago.

The virtual dipole moment for Royat (Table 1) is low and the palaeomagnetic direction (295.5°, +66.5° which is 26° from the axial dipole direction for the site) is also unusual³. The TL ages for Royat¹ and the Laschamp event (33,000 ± 4,000 yr; refs 17, 18) are not significantly different, and they may be linked as previously suggested³. Also the Laschamp event may have occurred at the same time as the Lake Mungo geomagnetic excursion in Australia¹⁹ (which has a very similar TL age of 33,500 ± 4,300 yrs; ref. 20), and that the Royat flow may have occurred up to a few thousand years later (perhaps at the same time as the younger excursion at Lake Mungo when low values were recorded for dipole moment¹⁹). It is also interesting that a double-excursion occurred at around the same time at Mono Lake in California²¹.

The result for Boissejour (Table 1) indicates a low geomagnetic dipole moment at an earlier date. The possible effects of

geomagnetic strength variation on the radiocarbon time-scale for the late Pleistocene will be discussed in a forthcoming paper.

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Preferential formation of the atmosphere-sialic crust system from the upper mantle

IN the past decade, extensive studies of the rare gas content of mantle derived rocks have led to several models of Earth degassing, all of which assume that the argon of the atmosphere degassed from the entire mantle. We give evidence here to support the concept of concurrent transfer of elements to the atmosphere and sialic crust preferentially from the upper mantle. This concept accounts for the following observations: (1) the ^{40}Ar concentrations in the rapidly quenched glassy margins of deep sea pillow basalts are too low for many estimates of the K concentration of the mantle^{1,2}; (2) the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of rocks derived from the deep mantle is similar to the atmospheric $^{40}\text{Ar}/^{36}\text{Ar}$ ratio³⁻⁷; (3) the upper mantle under oceanic ridges is depleted in K and other large-ion-lithophile elements. A schematic representation of the upper mantle depletion process is given in Fig. 1 which shows that partial melting associated with spreading ridges and Benioff zones exclusively in the upper mantle is responsible for the accretion of continents and associated degassing to the atmosphere.

The concentration of argon in glassy margins of submarine basalts provides evidence for depletion of argon in the upper mantle. Several factors can affect the composition and concentration of argon isotopes in submarine basalts, but degassing and contamination by atmospheric argon probably dominate⁸. The effect of atmospheric mixing is demonstrated in Fig. 2. In this type of plot, mixtures of atmospheric argon with a uniform source of mantle argon will form a line with a slope equal to ^{40}Ar content of the mantle and which connects the atmospheric and mantle $^{40}\text{Ar}/^{36}\text{Ar}$ values. Figure 2 shows basalt samples forming a broad mixing band between $^{40}\text{Ar}/^{36}\text{Ar}$ ratios above 10,000 and the atmospheric value of 295.5. The scatter in the data may be attributed to degassing which affects the $1/^{36}\text{Ar}$ values but

not the $^{40}\text{Ar}/^{36}\text{Ar}$ value. Concentration of argon into the melt during partial melting may contribute to the scatter, but probably to a lesser extent⁸. A maximum of $3.22 \times 10^{-6} \text{ ml g}^{-1}$ is derived for the ^{40}Ar concentration of the mantle source from the slope of the steepest mixing line in Fig. 2. We consider this value to be a maximum, not only because of the rather striking upper limit exhibited by the mixing line, but also because the ^{40}Ar concentration measured on what may be the least degassed oceanic ridge sample available, a 'popping rock' recovered from 5,750 m in the Cayman Trough, is $2.0 \times 10^{-6} \text{ ml g}^{-1}$, well below this value. Furthermore, 65% of all the ^{40}Ar concentrations measured form a distinctive peak between 0.9×10^{-6} and $3.0 \times 10^{-6} \text{ ml g}^{-1}$ with the other 35% of the data scattered between 0.9×10^{-7} and $0.9 \times 10^{-6} \text{ ml g}^{-1}$ (ref. 2).

The concentration of ^{40}Ar in the oceanic crust can be used to compute the original K content of the mantle by calculating the 'reconstituted' concentration of ^{40}Ar , that concentration of ^{40}Ar that would now exist in the mantle if it had never degassed ^{40}Ar to the atmosphere. Previously published estimates of reconstituted ^{40}Ar have assumed the atmospheric ^{40}Ar degassed from the entire mantle. In the preferential degassing model presented here, the mass of the mantle from which the atmosphere-sialic crust ^{40}Ar was derived, is allowed to vary from 0 to 100% of the total mantle and the reconstituted ^{40}Ar is calculated according to the formula

$$\Sigma^{40}\text{Ar} = [^{40}\text{Ar}_m] + \frac{^{40}\text{Ar}_{at} + ^{40}\text{Ar}_s}{xM} \quad (1)$$

where; $\Sigma^{40}\text{Ar}$ = the reconstituted ^{40}Ar , the concentration of ^{40}Ar that would exist in the mantle today after returning the ^{40}Ar from the atmosphere and sialic crust to the portion of the mantle from which it degassed; $[^{40}\text{Ar}_m]$ = the concentration of ^{40}Ar now in the depleted mantle, here represented by the ^{40}Ar in glassy submarine basalts; $^{40}\text{Ar}_{at}$ = the mass of ^{40}Ar in the atmosphere; $^{40}\text{Ar}_s$ = the mass of ^{40}Ar in the sialic crust, estimated by assuming that the K content of the sialic crust is 2.5% and the average argon retention age 2,000 Myr; x = the fraction of the mantle from which the atmosphere and sialic crust formed; M = the mass of the mantle ($42.56 \times 10^{26} \text{ g}$).

The original K content of the mantle can be calculated from ^{40}Ar assuming an age 4,500 Myr for the Earth. The results are depicted in Fig. 3, which plots $[^{40}\text{Ar}_m]$ versus the per cent of the mantle degassed. As shown in Fig. 3, if the ^{40}Ar content of glassy submarine basalts represents the maximum ^{40}Ar of the depleted mantle today, and the atmosphere degassed from the entire mantle, then the original K content of the mantle was <200 p.p.m. An original K content of the Earth this low is

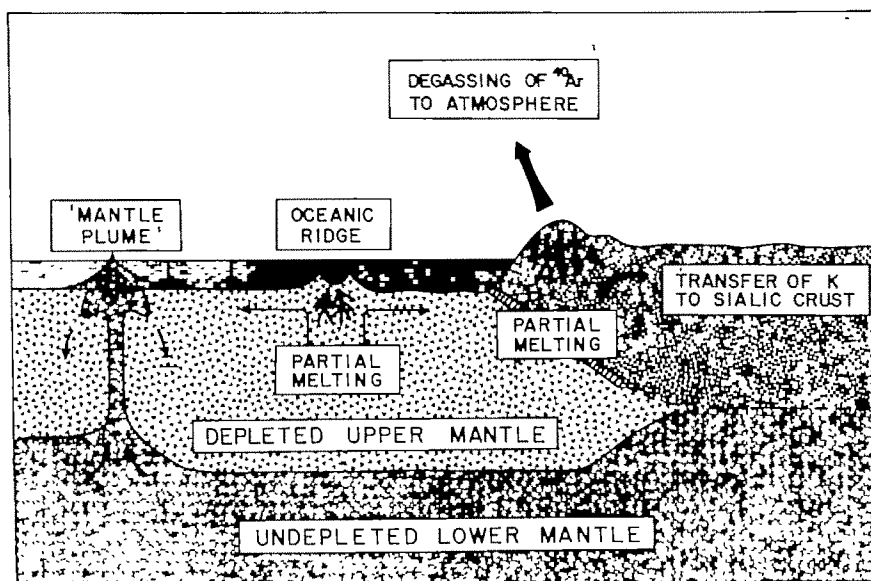


Fig. 1 Diagrammatic representation of preferential formation of the atmosphere and sialic crust from the upper mantle.

incompatible with many whole-Earth heat flow models. Higher values for the original K content are allowed if the atmosphere and sialic crust degassed preferentially from the upper mantle.

Further implications of the concept of preferential degassing of the atmosphere can be demonstrated by constructing a model of Ar and K distribution in the Earth based on the following constraints: (1) the atmosphere-sialic crust system formed from the upper 415 km of the mantle. (2) Degassing of ^{40}Ar took place, with simultaneous transfer of K to the sialic crust⁹. (3) The maximum ^{40}Ar of the depleted upper mantle is equivalent to the maximum ^{40}Ar in glassy submarine basalts (see Fig. 2).

The first constraint limits the zone of preferential depletion to the upper 415 km, or about 25% of the total mantle. The 415-km seismic step depth was chosen because of a seismic velocity increase which corresponds to a transition from a zone of low density to a zone of high density and may represent a transition from depleted to non-depleted mantle. If it is assumed that all the atmospheric ^{36}Ar degassed from the depleted zone, it is possible to calculate that the ^{36}Ar of the non-depleted mantle is $\sim 10^{-7} \text{ ml g}^{-1}$. The second constraint specifying coherency between argon degassing and K transfer is justified because all of the ^{40}Ar of the atmosphere can be produced from a sialic crust containing a 2.5% K (ref. 9). The actual value of K may be as low as 1.25%, therefore the amount of K which must be depleted from the upper 415 km of the mantle to produce the sialic crust ranges from 240 to 475 p.p.m. Constraint (3) permits calculation of ^{40}Ar of the non-depleted lower mantle (see equation (1)), which for preferential degassing to 415 km is calculated to be $4.5 \times 10^{-5} \text{ ml g}^{-1}$. The $^{40}\text{Ar}/^{36}\text{Ar}$ of the non-depleted mantle ($4.5 \times 10^{-5}/1.0 \times 10^{-7}$) is 450. The original K content of the non-depleted mantle is calculated from $\Sigma^{40}\text{Ar}$ and the stipulation of degassing to 415 km and is found to be 660 p.p.m. (Fig. 3). By subtracting the K removed to the sialic crust, the K content of the depleted mantle is calculated to be 125–422 p.p.m.

All the results of the model calculations are summarised in Table 1. This model is not presented as a unique solution for the preferentially degassed mantle, but rather as a starting matrix

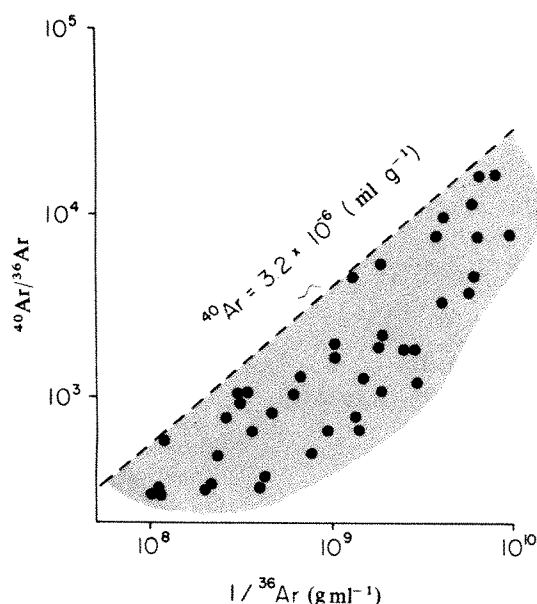


Fig. 2 The $^{40}\text{Ar}/^{36}\text{Ar}$ ratio compared with the $1/^{36}\text{Ar}$ ratio in glassy submarine basalts. This plot is used to demonstrate the effects of mixing atmospheric argon ($^{40}\text{Ar}/^{36}\text{Ar} = 295$) with mantle argon of high $^{40}\text{Ar}/^{36}\text{Ar}$. The dashed line probably represents the mixing line of steepest slope and may correspond to the maximum ^{40}Ar of the oceanic crust. The data are from refs 8, 15–17 and unpublished.

from which more sophisticated solutions can evolve. Several possible perturbations on the model are envisaged.

First, loss of ^{40}Ar to the atmosphere from upper mantle without simultaneous transfer of K to the sialic crust would increase the K content of the depleted upper mantle while decreasing the K content of the sialic crust, but would not change the K content of the non-depleted mantle.

Second, modification of the thickness of the zone of depletion can decrease or increase possible solutions for the K and Ar contents of the mantle, but would not change the solutions for the K content of the sialic crust.

Third, if the K concentration of the sialic crust is less than 2.5%, a higher percentage of ^{40}Ar must have degassed directly from the mantle without coherent formation of the sialic crust. This would result in higher K concentrations for the depleted mantle. Furthermore, the ^{40}Ar of the sialic crust would be lower than the value used in this model to calculate $\Sigma^{40}\text{Ar}$ and would result in lower solutions for the K content of the non-depleted mantle. For example, if the K concentration of the sialic crust is 1% instead of 2.5% as used in the model calculation, $\Sigma^{40}\text{Ar}$ and K concentration of the non-depleted mantle would be 13% lower.

Several implications of the idea of preferential degassing can be tested by comparing data on samples thought to represent the

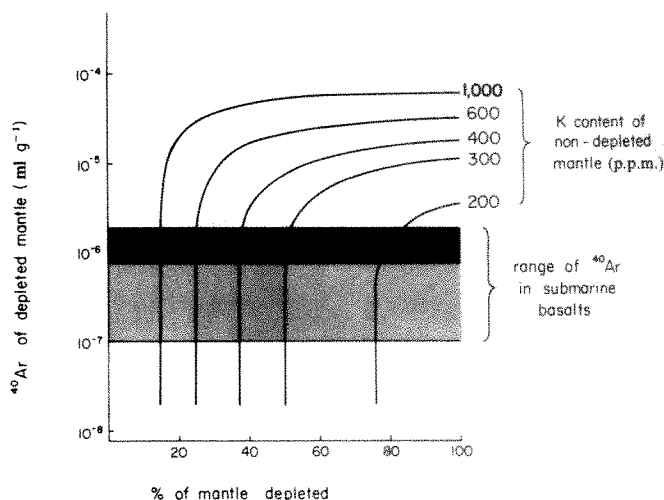


Fig. 3 The ^{40}Ar content of the present day upper mantle compared with the per cent of the mantle from which the atmosphere-sialic crust formed. The iso-K curves represent solutions of the initial K content of the mantle derived by recombining the atmospheric-sialic component of ^{40}Ar with the ^{40}Ar presently in the depleted portion of the mantle.

depleted and non-depleted mantles. Ocean ridge basalt probably originated in the depleted zone, while basalts associated with 'plumes' and many continental ultramafics may originate in the deep mantle. The most reliable samples of deep mantle may be continental ultramafic rocks containing anomalous ^3He , ^{21}Ne , or ^{129}Xe or exhibiting a 'planetary' type rare gas abundance pattern. The planetary-type rare gas abundance pattern is characterised by a high $^{84}\text{Kr}/^{130}\text{Xe}$ ratio compared with the atmosphere ratio and a low $^{20}\text{Ne}/^{36}\text{Ar}$ ratio compared with the ratio in oceanic ridge basalts¹⁰. Deep mantle-derived samples include: a kaersutite inclusion in nephelinitic basalt from New Zealand containing excess ^3He and ^{21}Ne and exhibiting a planetary rare gas abundance pattern³; josephinite, a rare terrestrial nickel-iron alloy containing elemental silicon and thought by some to have originated from the core-mantle boundary and containing excess ^3He , ^{21}Ne , and ^{129}Xe ^{6,7}; a kearsutite inclusion in alkali basalt from Dish Hill, California, containing excess ^3He and exhibiting a planetary type rare gas abundance pattern⁵; two olivine megacrysts from South African

kimberlite pipes containing excess ^3He and ^{129}Xe and a planetary rare gas abundance pattern and samples of kimberlite, carbonatite and diamond from South Africa^{5,11}.

The model calculations imply that the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of the deep mantle is similar to that of atmosphere (see Table 1). This stems from the assumptions that the deep mantle has remained a closed system and the atmosphere now contains more than 80% of all the ^{40}Ar and more than 99% of the ^{36}Ar that would have currently been in the upper mantle if it had remained a closed system. It is also important that according to the model calculations the upper mantle has been depleted in ^{36}Ar by a factor of >100 whereas it has been depleted in K by a factor not greater than 6; the net result being, that $^{40}\text{Ar}/^{36}\text{Ar}$ ratios as high as 16,000 can be expected in the depleted upper mantle. A comparison of the available data with the model is summarised in Fig. 4 which is a histogram comparing the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in basalts dredged from plume areas and continental ultramafics uncontaminated by crustal material, with submarine ridge basalts. The $^{40}\text{Ar}/^{36}\text{Ar}$ of the plume basalts and ultramafics range from 295 to 1,200 with the majority falling below 500, while ridge basalts range up to 16,000 with the majority falling above 1,000. It is interesting that, all continental ultramafics containing ^3He , ^{21}Ne , or ^{129}Xe , have a $^{40}\text{Ar}/^{36}\text{Ar}$ ratio below 850. This observation has led several workers to suggest that the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of the mantle source is similar to that of atmosphere³⁻⁵. The possibility of a mantle with a low $^{40}\text{Ar}/^{36}\text{Ar}$ ratio was first proposed on the basis of the initial ratio derived from K-Ar isochron plots of basalts and dolerites¹², although these samples may have been contaminated by atmospheric or crustal argon. For deep mantle ultramafics which have excess ^3He and relatively low $^{40}\text{Ar}/^{36}\text{Ar}$ ratios, crustal contamination can be ruled out because of the high concentrations of ^4He in crustal rock. On the other hand, the high concentration of ^{36}Ar in the atmosphere makes it conceivable that atmospheric contamination after extrusion has produced the low $^{40}\text{Ar}/^{36}\text{Ar}$ ratios without completely eradicating the other rare gas isotopic anomalies. However, stepwise heating experiments on low $^{40}\text{Ar}/^{36}\text{Ar}$ ultramafics have demonstrated the low ratio exists even in the high temperature fractions which are least likely to be contaminated by atmospheric argon⁴.

The model presented above also requires that the deep-mantle samples have higher rare gas contents than upper mantle samples. The evaluation of this implication from existing data is difficult because crustal contamination and degassing may have altered the concentrations. The ^{36}Ar concentration in the glassy margins of oceanic basalts is variable, but after accounting for the effects of atmospheric contamination and degassing, the ^{36}Ar concentration of the upper mantle under oceanic ridges has been estimated to be $<2 \times 10^{-10} \text{ ml g}^{-1}$ (ref. 1). With the exception of diamonds, the available ^{36}Ar data on the continental ultramafic samples used in Fig. 4 fall in the range $0.38\text{--}3.6 \times 10^{-8} \text{ ml g}^{-1}$, distinctly higher than estimates of the ^{36}Ar in the upper mantle under the oceanic ridges. The high abundance of ^{36}Ar in these samples may reflect the ability of these minerals to preferentially accommodate rare gases in their structure relative to other phases in the mantle and does not prove that the deep

mantle has a higher concentration of rare gases than the upper mantle. Nonetheless, the data are consistent with and support the model of preferential degassing of the upper mantle. The only ultramafic samples known at present with anomalous ^3He or ^{129}Xe , which have $^{40}\text{Ar}/^{36}\text{Ar}$ ratios $>1,000$, are ultramafic xenoliths from Hawaii^{12,13}. This observation may reflect the possibility that the mantle is more extensively depleted in oceanic regions than under the continents, or that these xenoliths originated in the depleted upper mantle.

The concept of preferential formation of the atmosphere and sialic crust from the upper mantle provides a means of depleting K and other incompatible elements in the mantle beneath oceanic ridges and is consistent with rare gas data on mantle derived rocks. It is especially useful in accounting for possible similarities in the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of the atmosphere and rocks of deep mantle origin.

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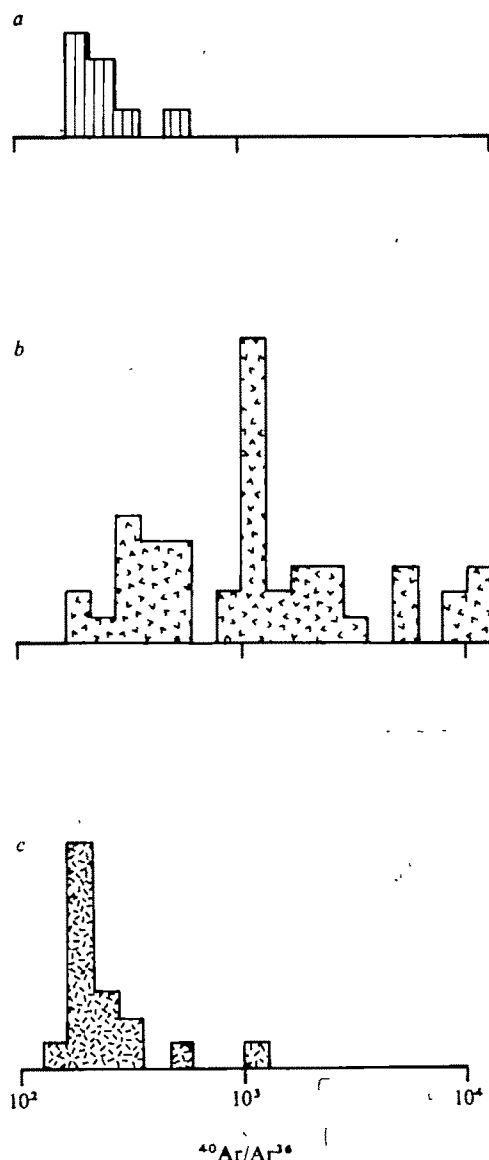


Fig. 4 A histogram comparing the $^{40}\text{Ar}/^{36}\text{Ar}$ ratios of: a, continental ultramafics exhibiting ^3He , ^{21}Ne , and ^{129}Xe anomalies; b, glassy submarine basalts from oceanic ridges; and c, glassy submarine basalts from plume areas. Data are from refs 4-8, 11, 15-17 and unpublished.

Table 1 Model calculations of the K and Ar concentrations in major units of the Earth*

	K (p.p.m.)	^{40}Ar ($10^{-6} \text{ ml g}^{-1}$)	^{36}Ar ($10^{-8} \text{ ml g}^{-1}$)	$^{40}\text{Ar}/^{36}\text{Ar}$
Atmosphere	—	9.08†	3.37†	295.5
Sialic crust	12,500-25,000	175-350	0.50-2.00‡	8,750-70,000
Upper mantle	125-422	3.20§	0.02-0.04§	8,000-16,000
Lower mantle	660	45	10.00	450

*See model constraints in text.

†Total argon of atmosphere is divided by the mass of the mantle.

‡The ^{36}Ar content of the sialic crust is estimated from the range of concentrations in crustal rocks¹.

§Estimated from glassy submarine basalts¹.

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Sapphirine-bearing rocks with sedimentary and volcanogenic protoliths from the Arunta Block

At least 15 separate localities for sapphirine-bearing rocks have been found in the Arunta Block, a Precambrian metamorphic complex in central Australia (Fig. 1), although only two have been documented in any detail¹⁻³. Such rocks are the most spectacular examples of a suite of rocks with a distinctive, characteristic chemistry. This suite includes, *inter alia*, the better known cordierite-anthophyllite rocks, and is widely distributed both within the Arunta Block and world-wide. From field observations it is proposed here that two different geological processes, one sedimentary or diagenetic and the other volcanic, generated rocks that are now indistinguishable in their whole-rock chemistry and mineral assemblages. It had formerly been proposed that sapphirine-bearing rocks of volcanogenic and sedimentary origins were mineralogically and chemically distinct⁴.

Rocks in this chemical suite have compositions in which >90% of the oxide total is contributed by SiO₂, Al₂O₃, MgO, and FeO; representatives are either silica-undersaturated or contain only small quantities of modal quartz. Metamorphic grade affects the proportion of modal quartz for a given SiO₂ content, as well as the other minerals which may include talc, chlorite, phlogopite-biotite, anthophyllite, gedrite, cummingtonite, cordierite, sillimanite, kyanite, corundum, staurolite, orthopyroxene, kornerupine (boron-bearing), garnet, sapphirine and spinel. These minerals and whole-rock compositions may be shown diagrammatically on a quaternary SiO₂, Al₂O₃, and MgO+FeO plot (Fig. 2). Such a plot cannot take account of the effect of MgO/FeO on mineral assemblages: staurolite and garnet may occur if MgO/FeO (whole-rock composition) is low, whereas sapphirine requires high MgO/FeO. The effects of metamorphic facies and variations within the chemical constraints are shown in Table 1, using examples from various localities within the Arunta Block.

Whole-rock analyses from the chemical suite define a trend slightly more aluminous than the tie quartz-chlorite in Fig. 2, as do estimates of composition derived from mineral assemblages. This agrees substantially with previously published statements^{5-7,14} that some cordierite-anthophyllite rocks originated as chlorite-rich rocks, but implies an additional aluminosilicate component, such as kaolinite, illite, phengite, muscovite or feldspar. Sapphirine-bearing rocks (and hypersthene-garnet-bearing rocks), being higher metamorphic facies equivalents of cordierite-anthophyllite rocks, may have similar origins. Figure 2 also shows that even apparently 'sedimentary-type' sapphirine-bearing rocks could not have originated as mixtures of clay minerals, although for such rocks magnesite-rich protoliths should be considered.

On the basis of geological setting two major groups of rocks belonging to the chemical suite are recognised in the Arunta Block.

Group 1: sedimentary type. In the Reynolds Range sapphirine-bearing and chemically akin rocks crop out intermittently over a kilometre along the (regional) unconformity between an eroded sequence of pelites and an overlying quartzite⁸. Similar rocks, also sapphirine-bearing, crop out in the same stratigraphic position, some 10 km to the east, and similar, though slightly more siliceous, rocks crop out on the same unconformity several kilometres to the south. A hundred kilometres to the east, near Delmore Downs, specimens of quartz-chlorite-anthophyllite-cordierite granofels have been collected at the same regional unconformity. In the Reynolds Range a pod of sapphirine-anthophyllite-cordierite-enstatite granofels with cordierite-rich granofels also occurs several hundred metres above the unconformity within a pelitic sequence.

The geological processes that generated these magnesian minerals must have been compatible with the deposition and diagenesis of the enclosing non-volcanic sedimentary sequences. A possible protolith would be a mixture of magnesite, a clay (illite or kaolinite), haematite and quartz (see ref. 9) formed by surface waters with high pH and CO₂ content. An alternative origin might have been by way of meerschaum or sepiolite deposits generated from alkaline groundwaters¹⁰. As these rocks are low in CaO, they are not metamorphosed marls, unlike the sapphirine-bearing rocks of southern Madagascar¹¹. These rocks contain appreciable FeO, and include examples lower in SiO₂ when compared to the chemically similar white schists of sedimentary origin in Zambia and Afghanistan¹².

Group 2: volcanic type. Rocks belonging to the chemical suite are also widely distributed in the granulite terrain of the Harts and Strangways Ranges within metamorphosed volcanic and volcanoclastic sequences of acid and basic composition¹³. Occurring as pods and layers associated with distinctive cordierite quartz-rich granofels, they are subdivided on field setting into two subclasses. Those with discordant relationships originated as chlorite-rich pods within zones of extensive chloritic alteration cross-cutting the original volcanic stratigraphy. Others occur as a concordant basal layer in a three-tier

Table 1 Assemblages in examples of the chemical suite selected from the Arunta Block

Chemical control	Prograde	Retrograde
High SiO ₂	High MgO Qz-Cd-Opx High FeO Qz-Cd-Hy ⁺ Si ± Ga Qz-Ch-Anth-Cd ± Ga	RR Qz-Ky-Cd-Ged EC EC, MM Qz-Anth-Cd-Ch EC DD
Low SiO ₂	High MgO Cor-Sp-Sap-Si-Hy-Cd En-Cd-Sap-Ph High FeO Sp-Ga-Hy-Cd	MM RR MM, EC Cor-St-Ch St-Ga-Ch EC

Minerals: Anth, Anthophyllite; Cd, Cordierite; Ch, Chlorite; Cor, Corundum; En, Enstatite; Ga, Garnet; Ged, Gedrite; Hy, Hypersthene; Ky, Kyanite; Opx, Orthopyroxene; Qz, Quartz; Si, Sillimanite; St, Staurolite; Ph, Phlogopite; Sp, Spinel.

Localities (see Fig. 1): DD, Delmore Downs; EC, Edwards Creek; MM, Mount Milton; RR, Reynolds Range.

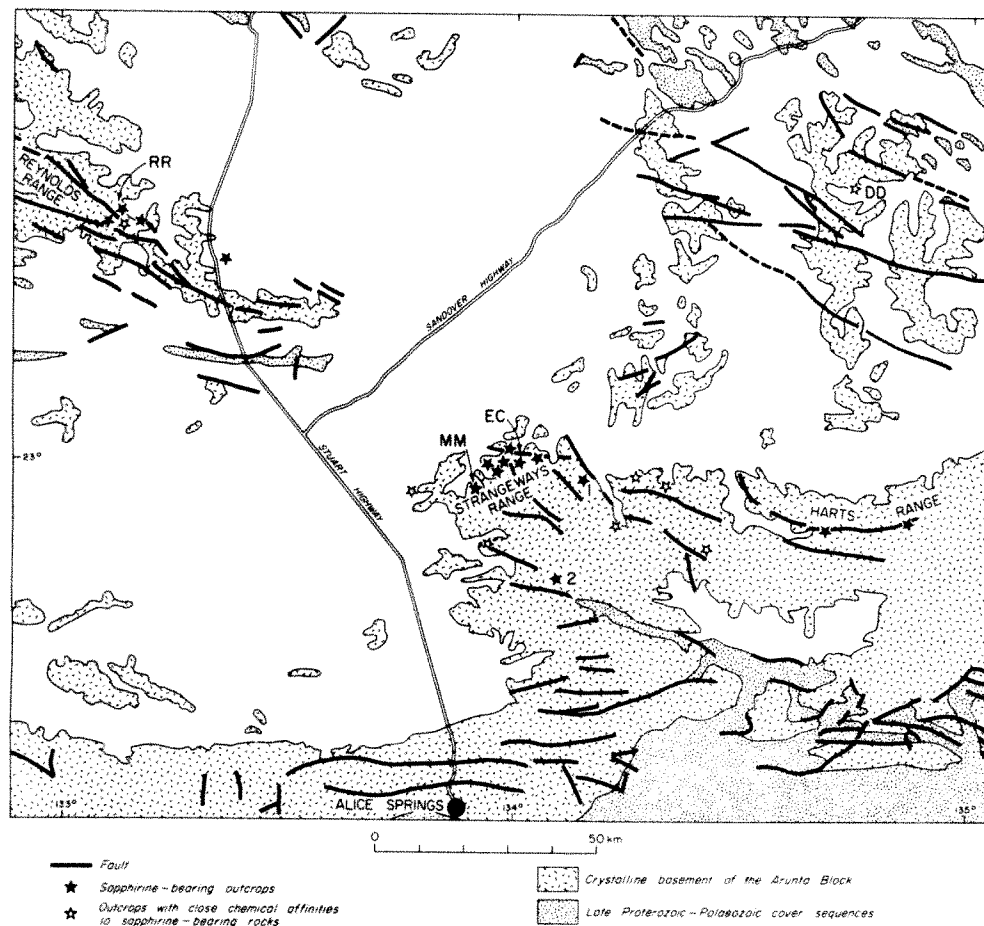


Fig. 1 Distribution of sapphirine-bearing outcrops, and outcrops of chemically allied rocks in the Arunta Block, central Australia. Localities referred to in text: RR, Reynolds Range; DD, Delmore Downs; MM, Mount Milton; EC, Edwards Creek. 1, Woodford and Wilson (1976); 2, Hudson and Wilson (1966).

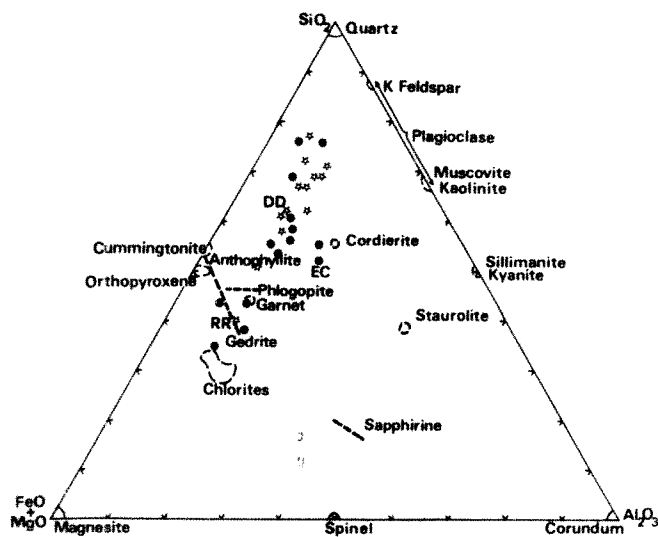


Fig. 2 Ternary plot into plane molar SiO_2 , Al_2O_3 , $\text{MgO} + \text{FeO}$, showing both minerals characteristic of the 'chemical suite' and also whole rock compositions, from the literature and from the Arunta Block as indicated. Stars indicate sedimentary origin, dots volcanogenic origins. Note overlap of fields.

association with impure marbles and/or calc-silicate granofels and banded gneisses consisting of quartz and iron minerals (magnetite, grunerite, and so on). These are interpreted as metamorphosed distal deposits from exhalative activity (Fig. 3). At some localities both subclasses crop out in close proximity; elsewhere only one type is present. This interpretation is similar to that proposed for some cordierite-anthophyllite rocks in the Canadian Shield^{6,14}, an interpretation enhanced by the presence of pyrite, galena, sphalerite, and chalcopyrite in many of the layered outcrops¹⁵. Sapphirine-bearing outcrops in the Yilgarn Shield have been interpreted as altered mafic volcanic rocks¹⁶. Relatively unmetamorphosed per-magnesian chloritic rocks of volcanic origin are rare but are known, for example, from the Woodlawn orebody in southeastern Australia¹⁷. Previously sedimentary origins have been proposed for some sapphirine-bearing (and chemically related) rocks in the Strangways Range^{3,18}.

Only field relationships are presently useful in distinguishing the two groups. Chemical analyses (from the Arunta Block and elsewhere) of the two types show such an overlap (see Fig. 2) that they provide no obvious discriminants.

Several outcrops shown in Fig. 1 were discovered by other members of a project team from the Bureau of Mineral Resources studying the Arunta Block. Constructive criticism and discussion proffered by colleagues at BMR and the Northern Territory Geological Survey, and by B. J. Hensen, N. Herriman, D. F. Sangster, W. Schreyer, and R. H. Vernon is

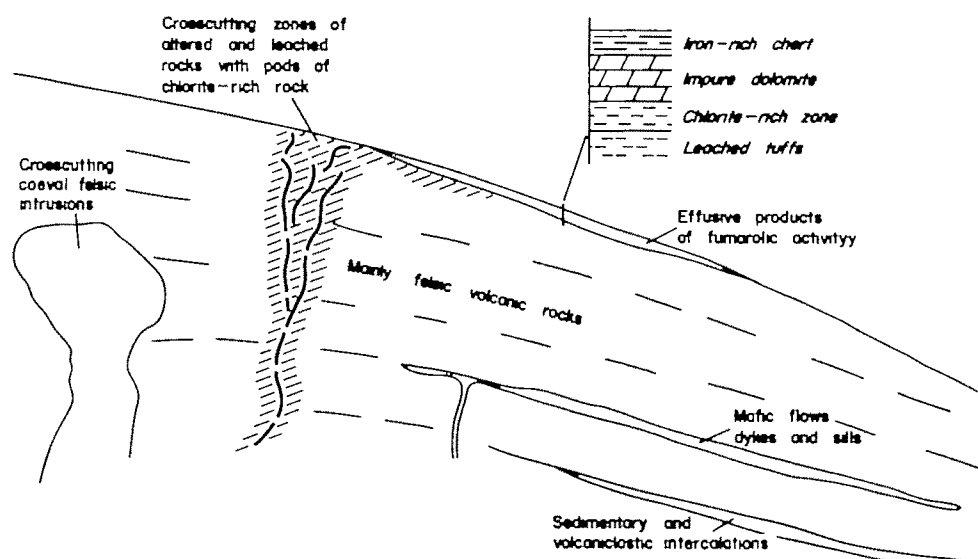


Fig. 3 Inferred settings for the formation of protoliths to sapphire-bearing rocks (and the chemically allied rocks) in the Strangways Range.

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Sediment loads in the Amazon River

ESTIMATES published in the past 20 years of the sediment load delivered to the sea by the Amazon River range from 4 to 10×10^8 tonnes yr^{-1} . Estimates published around 1960, when few data were available on either the sediment concentration or the river discharge, were in the range $9\text{--}10 \times 10^8$ tonnes yr^{-1} (refs 1, 2). Estimates published in 1967 and 1968, after significant new data had been collected, were in the lower range of $4\text{--}5 \times 10^8$ tonnes yr^{-1} (refs 3, 4). Our more comprehensive data collected mostly since 1970 (and especially in 1977) suggest that the earlier higher estimates may have been more nearly correct. We show here that the mean annual load of suspended sediment at Óbidos, Brazil is between 8 and 9×10^8 tonnes yr^{-1} . Most of this sediment is discharged onto the continental shelf.

During the high-water seasons of 1976 and 1977, we measured sediment loads in the Amazon River basin. Our most

complete set of measurements were made during May and June of 1977, when we followed the annual high-water stage down the Amazon and measured the suspended-sediment loads at six places on the mainstem and in the two largest tributaries (Fig. 1). Because our ship moved downriver at about the same speed as the flood wave (considering the sampling stops we made en route), we came close to following the same relative flood stage for about 3,000 km from Peru to near the mouth of the Amazon. This gave us added insight into how the sediment load changed downriver.

The amounts of sediment being carried in suspension in 1977 were measured in samples of river water collected from RV Alpha Helix with a large-volume, depth-integrating sampler. The depth-integrating sampler is lowered to the river bed and raised back to the surface at a uniform vertical rate; its intake nozzle is designed to admit the sediment-water mixture at stream velocity. The resulting sample is thereby weighted for differences in velocity in different parts of the water column. One only need multiply the concentration of sediment measured in such a sample by the corresponding water discharge to obtain the suspended-sediment load^{5,6}. At each of the locations listed in Table 1, we took depth-integrated samples at three to seven stations spaced more or less evenly across the width of the river. Further information on our sampling and laboratory procedures, as well as a complete tabulation of the suspended-sediment data, are published elsewhere⁷.

Water discharges reported in Table 1 for the five mainstem sections in Brazil were estimated from the stage-discharge relationships (rating curves) that have been developed for these stations by Hidrologia S.A. and Companhia de Pesquisa de Recursos Minerais (CPRM). River stage is measured daily and river discharge is measured monthly or bimonthly at these stations. At Iquitos, where no independent measurements were available, we made a rough calculation of water discharge from our velocity data and the area of the cross-section measured by sonar (depth) and radar (width). The discharge of the Rio Madeira is estimated from measurements that Hidrologia S.A. makes monthly at its furthest downstream gaging station on the Madeira, about 200 km upriver of where we collected samples of suspended sediment. Discharge of Rio Negro, whose flow is not measured, was estimated from the difference between discharge at Óbidos and at other measured and unmeasured tributaries as detailed in the footnote to Table 1.

Suspended-sediment loads remain fairly constant in the mainstem between Iquitos and Manacapuru, indicating that the sediment in this part of the river is derived from the Andes while the jungle-draining tributaries that enter the river in Brazil bring in large amounts of water but little additional sediment. This

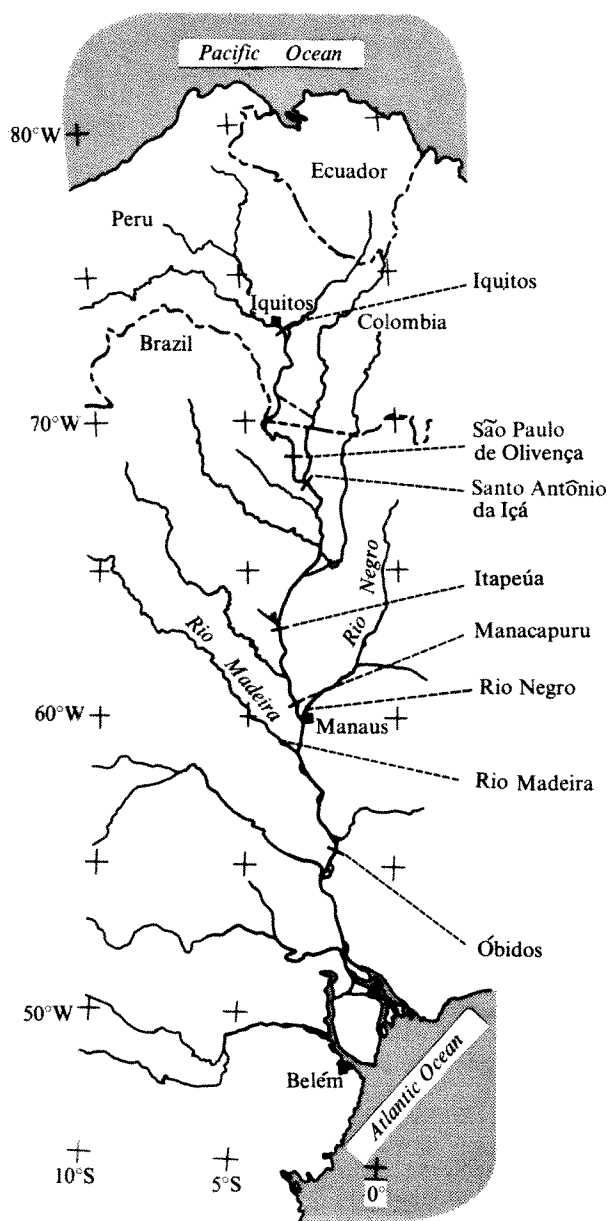


Fig. 1 Map of Amazon River, showing locations on mainstem and tributaries where sediment loads were measured in 1977.

feature was noted earlier by Sioli⁸ and was elucidated by Gibbs³, who estimated that 82% of the suspended solids discharged by the river were derived from the 12% of the total area of the Amazon basin that lies in the mountainous terrain of the Andes. The epitome of the jungle tributaries is Rio Negro which contributes ~20% of the water flow of the total Amazon system but only a trivial proportion of the sediment. Rio Madeira, on the other hand, has its headwaters in the Andes of Bolivia, and it carries a significant load of sediment.

The downstream decrease in the discharge-weighted concentrations shown in Table 1 was noted earlier by Gibbs³, but our data disagree with his in several places (Fig. 2). We were unable, in either 1976 or 1977, to detect the concentrations $>400 \text{ mg l}^{-1}$ that Gibbs reported during high-water seasons in the upper river. At Óbidos in the lower river, we measured higher concentrations than he did. After discussions with Gibbs we agree that the differences between our data and his are probably related to differences in sampling procedure. However, this does not rule out the possibility that the differences are real differences between one year and another.

Because the data in Table 1 represent only a single day's sediment load near the annual peak of river flow, we cannot extrapolate the figures by themselves to obtain long-term loads. However, by combining our sediment data with other unpublished data on the annual cycle of river discharge and the concentration of suspended sediment at low discharge, we can make a new estimate of the average annual load of suspended sediment at Óbidos. The section at Óbidos is the furthest downstream point on the mainstem at which river discharge is measured regularly.

The mean monthly discharges at Óbidos for a 4-yr period are shown in Fig. 3a. These averages were computed from the daily measurements of river stage and the less frequent (mostly bimonthly; some monthly) measurements of river discharge that were made in this period by CPRM and Hidrologia S.A. for the Departamento Nacional de Águas e Energia Elétrica (DNAEE).

The concentration of suspended sediment at low water was measured at Óbidos on 21 November 1963, by Ames of the

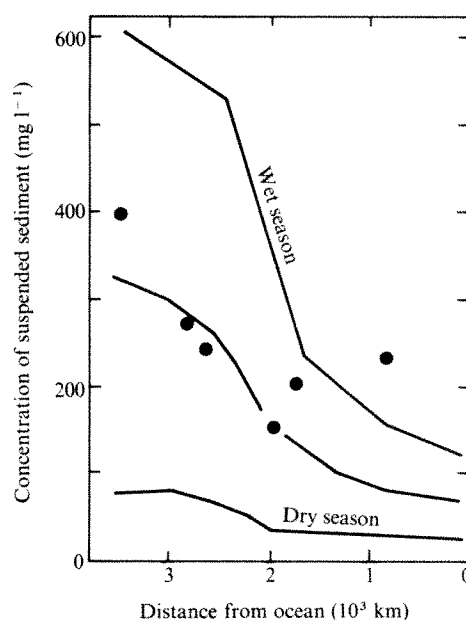


Fig. 2 Concentrations of suspended sediment in Amazon River mainstem. Solid lines represent concentrations reported by Gibbs³ for wet, intermediate, and dry seasons. Dark circles are the discharge-weighted concentrations listed in Table 1.

USGS during the first concerted efforts to measure the flow of the Amazon⁹. River discharge on that day was measured at $72,500 \text{ m}^3 \text{ s}^{-1}$. Ames collected 13 point samples of suspended sediment from different depths and at different locations across the river, and he calculated a discharge-weighted concentration of about 50 mg l^{-1} .

If we assume that our measurement of 235 mg l^{-1} represents the discharge-weighted concentration of suspended sediment at high discharge at Óbidos and that Ames' measurement of 50 mg l^{-1} represents the concentration at low discharge, and if we assume that the relationship between mean monthly discharge and mean monthly concentration follows the power relation plotted in Fig. 3b, we can compute monthly sediment loads for the average monthly discharges shown in Fig. 3a, and we can sum the monthly loads to obtain an estimate of the mean annual load of suspended sediment at Óbidos. This procedure gives an estimate of $9.3 \times 10^8 \text{ tonnes yr}^{-1}$. A slightly more conservative estimate can be computed if we assume that the mean concentration in the month of highest discharge is only 200 mg l^{-1} and that the concentrations in the other months range correspondingly between this value and the minimum

Table 1 Suspended-sediment loads measured in Amazon River and its tributaries, 1977

Location	Date	Water discharge ($\text{m}^3 \text{s}^{-1}$)	Suspended sediment	
			Concentration (mg l^{-1})	Load ($10^6 \text{ tonnes d}^{-1}$)
Iquitos	20 May	48,000	400	1.7
São Paulo de Olivença	22 May	70,000	275	1.7
Santo Antônio do Iça	23 May	80,000	245	1.7
Itapeúa (Coari)	26 May	110,000	150	1.4
Manacapuru	27 May	130,000	200	2.2
Rio Negro	28 May	50,000*	5	0.02
Rio Madeira	1 June	40,000	330	1.1
Óbidos	2 June	230,000	235	4.7

* Estimated by subtracting from discharge at Óbidos ($230,000 \text{ m}^3 \text{s}^{-1}$) the following: discharge of mainstem at Manacapuru (130,000), estimated discharge of Rio Madeira at Novo Aripuanã (40,000), and estimated discharge of Rio Trombetas (5,000) and other tributaries (5,000) between Manacapuru and Óbidos.

concentration of 50 mg l^{-1} ; in this case, the estimated annual suspended-sediment load at Óbidos is $8.2 \times 10^8 \text{ tonnes yr}^{-1}$.

How much of this sediment is discharged onto the continental shelf at the mouth of the river? Óbidos is about 850 km (river distance) above the mouth of the Amazon. The tributaries that enter the river below Óbidos add another 10% to the water discharge but do not add any significant amount of sediment. The question centres on how much of the sediment is deposited on the wide alluvial plain and in the extensive delta that lie between Óbidos and the sea. Even if this amount is substantial—as much as 20% of the sediment that passes Óbidos, for example—the remaining sediment load would place the Amazon behind only the Yellow River of China and the Ganges-Brahmaputra system of India and Pakistan as a supplier of sediment to the oceans⁴.

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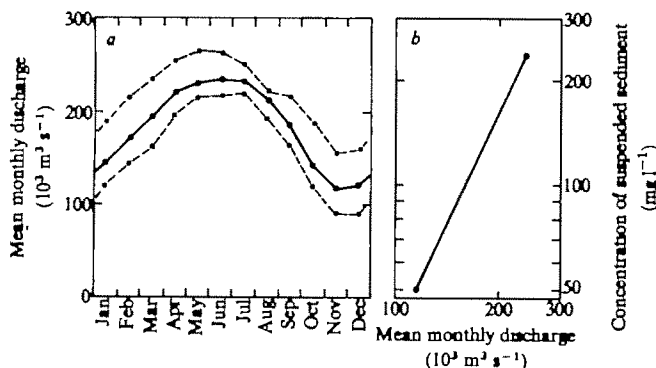


Fig. 3 a, Mean monthly discharges of the Amazon River at Óbidos, 1971-75. Maximum and minimum monthly mean discharges are shown by dashed lines; averages are shown by the solid line. Note that the greatest monthly mean flow for the 4-yr period is only 3 times the smallest. b, Assumed relation between mean monthly concentration of suspended sediment and mean monthly river discharge at Óbidos.

Photolysis of highly chlorinated dibenzo-*p*-dioxins by sunlight

CHLORINATED dibenzo-*p*-dioxins (CDDs) are now widely recognised as contaminants of commercial chlorinated phenols and related products. These compounds possess a remarkable range of toxicity; the LD_{50} for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) in female rats is $45 \mu\text{g per kg}$, that of the octachloro compound (OCDD) is $> 1 \text{ g per kg}$ (ref. 1). Although the degree of toxicity is related to the extent of chlorination, positional isomerisation also plays a critical and perhaps dominant part, 1,2,3,4-TCDD is at least 800 times less toxic than 2,3,7,8-TCDD (ref. 2). The extreme toxicity of some of these compounds, coupled with the large variations with extent of chlorination and positional isomerisation, make accurate

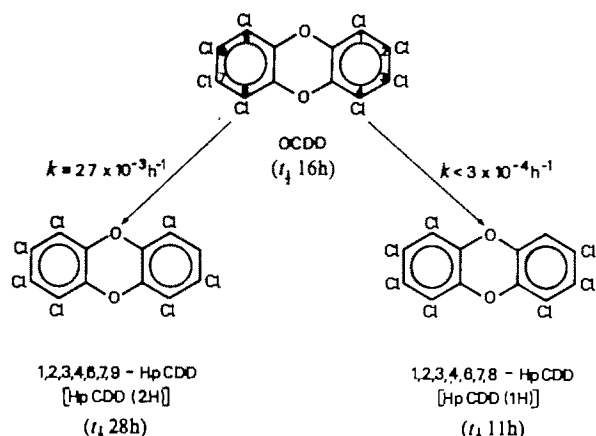


Fig. 1 Photolysis of OCDD in hexane. The rate constants and half lives quoted here derive from an experiment involving consecutive daily exposures of samples in hexane solution contained in matched quartz photolysis cells. The samples were exposed from 09.00 to 15.00 in late September/early October 1977 at Princes Risborough. The kinetics were all found to be first-order when the total time exposed was used (minimum correlation coefficient 0.986 for 10 data points). Hours of direct sunlight did not produce such a good correlation. The experiments have been repeated outside at other times of the year and in the laboratory and once allowance is made for the different incident light intensities the results are in agreement. For example, the half lives of OCDD were found to be 7.0 h at the beginning of August and 9.0 h in mid September. The computed ratios of the rate of OCDD destruction to the rate of conversion of OCDD to HpCDD(2H) are, however, independent of the solar intensity and the same results have been found in laboratory experiments using a fluorescent blacklight for the photolyses. In all cases the concentrations of the solutions used were such that less than 10% of the incident light was absorbed.

identification of CDDs essential in any assessment of environmental or safety implications. The study reported here is concerned with the photolysis of octa-, hepta (Hp)- and some hexa (Hx)-chlorodibenzo-*p*-dioxins and shows that the structural isomers which are expected to be the most toxic are most rapidly photodecomposed. We also report that despite photolytic reductive dechlorination which gives rise to less highly chlorinated dioxins, the reaction mechanism favours the formation of the less toxic isomers.

OCDD is a common contaminant of the widely used biocide pentachlorophenol occurring at a concentration of $\sim 1,000 \mu\text{g}$ per g in the commercial product³. Previous work has shown that photolytic reductive dechlorination can occur^{4,5} and the possible safety implications of this have been considered⁵ but without any detailed consideration of the kinetics or the mechanism of reaction.

Figures 1 and 2 summarise the principal results of our work which, although designed to study the photolysis of OCDD, has also provided data of relevance to other CDDs. The first photolysis experiments, conducted with a solution of OCDD in hexane (approximately $1 \mu\text{g}$ per ml) containing traces of HpCDD isomers, indicated that the OCDD and one of the HpCDD isomers were photolytically decomposed but that the other HpCDD increased in concentration during photolysis. Figure 3 shows gas chromatography (GC) traces illustrating this behaviour. There was also evidence from the GC traces of an increase in concentration of another compound which has a relative retention time on GC in the region expected for a HxCDD⁶. As photolysis continued, and the concentration of OCDD decreased, the concentration of the HpCDD isomer that had previously increased reached a steady state and then decreased. The same behaviour was found when the initial ratios of concentrations of HpCDD and OCDD were changed. The HpCDD is therefore generated by photolysis of OCDD. Photolytically-induced reductive dechlorinations have been reported before for chlorinated dioxins^{4,5} but the identification of the formation of a specific HpCDD facilitates an investigation of the mechanism of reaction.

Highly chlorinated dibenzo-*p*-dioxins can be formed by controlled heating of sodium chlorophenoxides⁶. We prepared OCDD from purified sodium pentachlorophenoxide and confirmed the structure by mass spectroscopy. Other CDD compounds cannot be prepared in the pure state in this way. HpCDD and HxCDD were prepared by the controlled heating of mixtures of sodium pentachlorophenoxide and specific sodium tetrachlorophenoxides^{6,7} and we confirmed their identity by GC analyses on QF-1 and OV-101 columns. The relative retention times with respect to OCDD on OV-101 were in agreement with published values⁸.

Comparison of the GC traces from the photolysed solution on two GC columns with samples of authentic compounds prepared as above showed that the HpCDD which increased in concentration was the 1,2,3,4,6,7,9-HpCDD (or HpCDD(2H)). Similarly, the peak in the HxCDD region could be assigned to the 1,2,4,6,7,9-HxCDD (or HxCDD(2,7H)).

To study the kinetics of photolytic degradation of both the HpCDDs, we minimised the formation of HpCDD from OCDD by varying the ratios of sodium penta- and tetrachlorophenoxides used in the synthesis of CDDs. The ratio of concentrations of HpCDD and OCDD chosen was at least 20 times the concentration ratio at which the photolytic production and destruction of HpCDD(2H) were found to be equal. These solutions also contained HxCDD isomers and the half lives for these compounds in sunlight are shown in Fig. 2.

All the solutions were exposed in matched quartz photolysis cells placed in a rack so that each solution was exposed to the same intensity and spectrum of sunlight. The rate constant for conversion of OCDD to HpCDD(2H) in these conditions (Fig. 1) can be calculated from the ratio of concentrations of these two compounds at the photostationary state for HpCDD(2H). As no ratio of concentrations was found for which the concentration of HpCDD(1H) increased during photolysis, only an upper limit to the rate can be calculated.

It is clear from Fig. 1 that OCDD is short lived when exposed to sunlight in hexane solution. From the rate constants we deduce that reductive dechlorination to produce less chlorinated dioxins is a minor photolytic pathway accounting for $< 10\%$ of the OCDD destroyed. The GC traces showed the photolytic formation of compounds with retention times longer than that for OCDD: these compounds are not simple CDDs and have not been identified.

There are two groups of chlorine atoms in OCDD: those bonded to carbon atoms in the 1-position, which are adjacent to an oxygen substituent (1,4,6 and 9 in Fig. 1) and those bonded to carbon atoms in the 2-position (2,3,7 and 8 in Fig. 1). The half lives (Figs. 1 and 2) indicate that CDDs with chlorine atoms in the 2-position photolyse more rapidly than those with chlorine atoms in the 1-position. This seems to be due partly to the

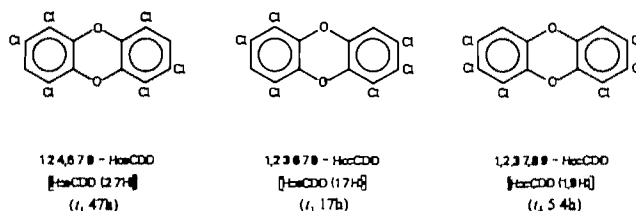


Fig. 2 Photolytic half lives for various hexachlorodibenzo-*p*-dioxin isomers exposed to sunlight in hexane solution in the same conditions as the solutions used for Fig. 1. The solution containing HxCDD(1,9H) also contained some HxCDD(1,6H) which is produced on heating sodium 2,3,4,6-tetrachlorophenoxide^{6,7}. The GC procedure used for the kinetics did not resolve these isomers, initially present in a ratio of $\sim 1:3$, HxCDD(1,6H):HxCDD(1,9H). The decay of the composite peak followed first-order kinetics for a period of over five half lives; as the major component of the peak was the HxCDD(1,9H) the half life found is ascribed to this compound. The half life of the HxCDD(1,6H) must be very similar to the HxCDD(1,9H), otherwise the decay of the composite peak would have been more complex.

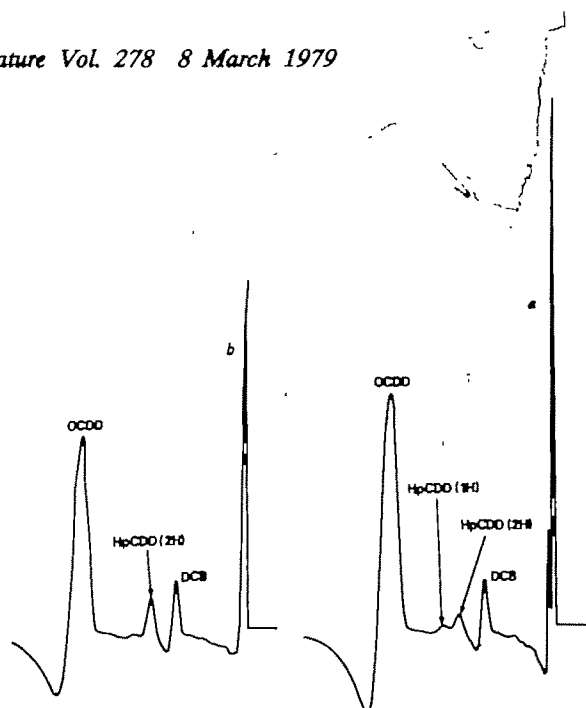


Fig. 3 GC traces from a hexane solution of approximately $4 \mu\text{g ml}^{-1}$ OCDD containing traces of HpCDD impurities before (a) and after (b) exposure of the solution in a quartz photolysis cell to sunlight. The peak labelled DCB is from decachlorobiphenyl ($0.05 \mu\text{g ml}^{-1}$) used as an internal standard for the GC analyses. These chromatograms were run on a Pye 104 gas chromatograph using EC detection. A 1-m column packed with 5% QF-1 on Diatomite C-AW-DCMS 80–100 mesh was used with a column temperature 210°C , detector temperature 300°C and nitrogen gas flow rate 120 ml min^{-1} . Analyses were also carried out with a 1-m column containing 3% OV-101 on Chromosorb W-HP 100–120 mesh, operated with the same temperatures as for the QF-1 column and a nitrogen gas flow rate of $\sim 50 \text{ ml min}^{-1}$. The concentration of OCDD in solutions was found by comparison with standard solutions and approximate HpCDD concentrations found by comparison of peak areas of HpCDD peaks with those for OCDD with allowance for the relative ECD responses of the compounds⁸. Decay constants were found by comparison of photolysed samples with diluted solutions of the unphotolysed solution. The relative retention times for the compounds of interest are:

Compound	Relative retention times		Literature values OV-101 (ref. 8)
	QF-1	OV-101	
OCDD	1.00	1.00	1.00
HpCDD(1H)	0.62	0.59	0.58
HpCDD(2H)	0.57	0.53	0.52
HxCDD(1,6H)	0.39	0.34	0.34
HxCDD(1,7H)	0.36	0.31	0.31
HxCDD(1,9H)	0.44	0.36	0.36
HxCDD(2,7H)	0.32	0.28	0.27
Decachlorobiphenyl	0.42	0.51	—

greater reactivity of the 2-position chlorines, shown by the higher rate of formation of HpCDD(2H) than HpCDD(1H). However, other factors are also involved because compounds possessing the same number of chlorine atoms in the 2-positions photolyse more rapidly as chlorine atoms are removed from the 1-positions ($t_1\{\text{HxCDD}(1,9\text{H})\} < t_1\{\text{HpCDD}(1\text{H})\}$) and $t_1\{\text{HxCDD}(1,7\text{H})\} < t_1\{\text{HpCDD}(2\text{H})\}$). Extrapolation of these results leads to the prediction that 2,3,7,8-TCDD should be the most photolabile of the chlorinated dibenzo-*p*-dioxins.

Available evidence on CDDs^{1,2}, and the similarities between the behaviour of the CDDs and other compounds with similar molecular shapes⁹, suggest that the presence of chlorine atoms in the 2,3,7 and 8 positions is critical for toxic effects (enzyme inhibition studies suggest at least three chlorine atoms¹⁰). The observations we report are therefore reassuring from the environmental point of view as the molecules that are likely to be the most toxic are the most susceptible to photodegradation, and the photodegradation of highly chlorinated dibenzo-*p*-

dioxins is not a facile route for the generation of the highly toxic, less chlorinated compounds.

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The evolution of the cell-cell recognition system

THE invention of sexuality was one of the milestones in the evolution of organisms. Indeed, in both unicellular and multicellular organisms, sexuality, by allowing the exchange of genetic material between cells, has been the major driving force of evolution. On the other hand, sexuality requires surface structures for the specific recognition and mating of the cells of the two gametic types. One of us (A.M.) has recently suggested that the cell-cell recognition system is phylogenetically linked to the appearance of sexuality¹. A phylogenetic connection between sexuality and cell recognition has also been suggested by Bennett *et al.*². Here, we will elaborate on this idea.

We can only speculate on the organisation of the cell membrane in the primitive prokaryotes; it may have been just a lipid bilayer. The major advance in this organisation must have been the insertion of proteins and/or glycoproteins in the bilayer. This step provided the cell membrane with an immense potential for modulating its molecular organisation; and this may have been the basis for the species-specific organisation of the cell membrane and hence of cell recognition. We do not know this happened concurrently with the appearance of the sexuality factors; in any case, it may be assumed that the sexuality factors were responsible for encoding certain proteins which became inserted into the membrane of the cells carrying such factor(s).

Jacob *et al.*³ have suggested that in bacteria, conjugation depends on an antigen, the F-antigen, at the bacterial surface of the F⁺ of *Hfr* males, which 'would allow the host to recognise cells which do not carry the episome and to conjugate with such cells'. Hence, originally the mating reaction would have resulted from the interaction between surface components specific for the (+) cells (which in *Escherichia coli* seem to be located at the tip of the F-pilus⁴) with some surface component of the (–) cells. Indeed, one might visualise a situation in which the molecules encoded by the sex factor(s) at the surface of the (+) cells were similar to lectins or multivalent antibodies and interacted with some of the glycoprotein components of the surface of the (–) cells in a process similar to 'anchorage modulation' or cap

formation⁵. The suggestion of Jacob *et al.* is supported by recent work of Kennedy *et al.*⁶ which shows that the proteins coded for by the *tra* operon (the transcriptional unit containing the genes required for the synthesis of the F-pili and the stabilisation of the mating aggregates, for DNA transfer and for surface exclusion which prevents the interaction of F cells with other donors) are associated with the cell envelope. The fact that the *tra* operon also codes for a membrane protein (PTraT, which is responsible for surface exclusion)⁷ is particularly important in connection with the origin of the specificity of cell-cell recognition and interaction. It is worth mentioning at this point that the incompatibility system in higher plants also depends on proteins at the surface of the pollen tube whose synthesis is controlled by the S locus (see ref. 8 for review).

Apparently, very early in the course of evolution not only did the molecular organisation of the cell surface fall under genetic control, but also the surface structures involved in conjugation were, in turn, able to control the activity of the genetic apparatus. The observations of Skurray and Reeves⁹ are an example of this. These authors found that in the F⁻ cells certain functions, notably DNA synthesis, are inhibited during conjugation. On the other hand, the location on the bacterial membrane of the initiator site of DNA replication³ is well known. The next step in the evolution of recognition in the mating reaction in lower eukaryotes must have been the establishment of specificity of the 'molecular fit' of the receptor sites. For example, in the yeast *Hansenula wingei* it has been shown that mating results from the interaction of two glycoproteins at the surface of the cells of the two mating types¹⁰⁻¹². In *Clamydomonas*¹³, the putative area of fusion between mt⁺ and mt⁻ cells seems to be a structurally specialised area of the cell surface which differentiates during gamete maturation. However, precise knowledge of the molecular composition and organisation of the receptor sites is still lacking.

We suggest that the cell-cell communication system has evolved from the membrane structures originally meant for cell recognition in the mating process. In this connection, we can cite the experiments of Miyake (see his review, ref. 14) on the ciliate *Blepharisma*. In this organism, the preconjunction events of cell interaction between mating types I and II are mediated by two 'gamones' (gamones 1 and 2) secreted by the cells. Both gamones are able to induce cell union events between cells of the same mating type. In this case, however, the union remains sterile, that is, none of the nuclear events—meiosis, exchange and fusion of gametic nuclei—that occur when cells of the two different mating types unite, will follow. By taking advantage of a mutant, *doublet*, which is provided with two sets of oral openings (cell union occurs in the oral region), chains of cells of the same mating type could be constructed by submitting them to the action of gamone 1. If at this point one singlet (that is, of the wild type with one oral opening) cell of the complementary mating type (pretreated with gamone 2) is attached to one end of such a chain, the conjugation events are immediately activated in a chain-like sequences, that is, they start on the cell closest to the 'inducer' cell and propagate from cell to cell.

Let us now leap from the unicellular to the multicellular organisms. We suggest that this event in evolution was concurrent with the segregation of the somatic from the germ cell line. The dichotomy between the two cell lines involves (1) in the somatic cell line, the silencing of the genetic apparatus coding for the surface structures responsible for the recognition between male and female individuals; (2) in the germ line, the retention of a fully (or at least largely) derepressed genome.

According to this hypothesis the cells of the germ cell line have retained their ancestral surface organisation whereby only cells belonging to the opposite sex can interact with one another. In this connection it may be significant that in vertebrates the primordial germ cells originate far away from the gonadal ridge and migrate as single cells.

On the other hand, although the matter has not been investigated with this question in mind, the formation of mouse chimaeras¹⁵⁻¹⁷ shows that genetically male and female embry-

onic cells do not discriminate between one another. Also, hybrid histotypic aggregates can be formed in culture from such species as far apart as chick and mouse¹⁸⁻²⁰. (However, we must remember that *in vitro* conditions may alter the organisation of the cell surface in such a way that some properties such as the species specificity are lost, while the tissue specificity is retained.) These experiments suggest that the structures discriminating between male and female are not expressed at the surface of these cells.

The retention of a largely derepressed genome by the cells of the germ line is inferred from the fact that in the oocyte, the complexity of the transcripts is several times greater than in the somatic cells (see, for example, ref. 21). Although we know of no such direct evidence in the case of the male germ cells, it has been shown that at least in *Drosophila*, spermatocytes exhibit lampbrush chromosomes comparable to those of the oocyte²².

It is therefore not surprising to find at the surface of the germ cells—both eggs and spermatozoa—antigens and other molecules which are also present at the surface of highly specialised somatic cells. One such example is the presence of acetylcholine receptors at the surface of the *Xenopus*²³ and of the mouse oocyte²⁴. The role of these receptors is obscure; in the mouse oocyte they are no longer detectable in the two-cell embryo²⁴.

Finally, we should mention the relevance here of some of the information now available on the T locus in the mouse. Indeed, it has been shown that mutations at this locus result in both segregation distortions in males and fertility alterations²⁵ and that they also affect cell interactions during the early stages of embryonic development. The antigens encoded by the T locus are expressed at the surface of the spermatozoon but not of any somatic cell, thus suggesting that they may have an important role in gamete differentiation and during early embryonic development²⁶. The location on the same chromosome (and in very close proximity to each other) of the T locus and the H-2 locus which specifies the major histocompatibility antigens may also suggest an evolutionary relationship between the genetic system controlling gamete differentiation, cell recognition and the immune system of the higher vertebrates.

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Microtubules, cell wall deposition and the determination of plant cell shape

THE shape of plant cells depends largely on the orientation of cellulose microfibrils in their cell walls¹. In almost all cells where the microfibrils are regularly arranged, cortical microtubules lie parallel to the wall microfibrils. Experimental evidence indicates that these microtubules influence the arrangement of the deposition of wall microfibrils². How microtubules determine the orientation of microfibrillar deposition is not yet known^{3,4}, but it has been speculated that they may influence the alignment of cellulose synthetase enzymes on the plasma membrane⁵. Microtubules act as a cytoskeleton in the formation and maintenance of cell shape in many types of cells⁶ including plant cells which lack a cell wall⁷, or before a wall is deposited⁸, and it may be that microtubules, in those plant cells where they are arranged congruently with recently deposited wall microfibrils, directly determine cell shape. However, using coumarin to inhibit cell wall regeneration by protoplasts of *Mougeotia* I demonstrate here the inability of microtubules to directly determine cell shape.

Spherical protoplasts of this freshwater alga, isolated following enzymatic digestion of its cell wall, readily regenerate a new wall and revert to their original cylindrical cell shape⁹. The ratio of length to diameter (axial ratio) of these cells is very large, making them ideal for investigations on the mechanisms controlling the development of cell asymmetry. Following attachment to a substratum, these protoplasts can be burst by osmotic shock to reveal the cytoplasmic face of the plasma membrane and microtubules associated with it¹⁰. Cortical microtubules become linked by cross bridges to the membrane during the first 3 h of protoplast culture (unpublished data). Intact protoplasts treated with 0.2% colchicine or 10^{-4} M isopropyl *n*-phenylcarbamate (IPC) lose their cortical microtubules¹⁰ and regenerate a cell wall in which the microfibrils are randomly orientated. These cells remain spherical (unpublished data).

Coumarin (1,2-benzopyrone) inhibits the biosynthesis of cell wall components, especially cellulose¹¹, and prevents the deposition of microfibrils on the surface of regenerating tobacco

leaf protoplasts¹². This drug does not affect the cortical microtubules of plant cells¹³. Freshly isolated protoplasts of *Mougeotia* were washed in protoplast maintenance medium (PMM), which contains 0.2 M mannitol, 0.2 M sorbitol, 2 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 2 mM NaH_2PO_4 at pH 5.5. A stock solution of 50 mg ml⁻¹ coumarin (Sigma) in ethanol was diluted with PMM to 250 $\mu\text{g ml}^{-1}$. Protoplasts were incubated in this solution at 20 °C on the same 16/8 h light/dark regime they had experienced before removal of their cell wall. Control protoplasts were maintained in PMM containing 0.5% ethanol. Wall regeneration by the protoplasts was monitored by staining with 0.1% Calcofluor M2R New (American Cyanamid), a fluorescent stain for β -polysaccharides¹⁴, as well as by transmission electron microscopy.

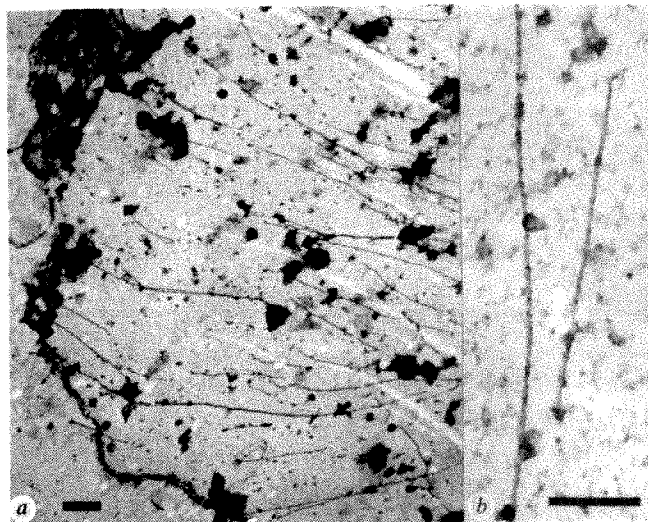


Fig. 2 *a*, Transmission electron micrograph of part of a *Mougeotia* protoplast plasma membrane. Protoplasts were allowed to settle on formvar/carbon-coated grids treated with poly-L-lysine. They were then burst by osmotic shock in a microtubule stabilising buffer containing 1 mM MgSO_4 , 2 mM EGTA and 100 mM PIPES at pH 6.9 and negatively stained with uranyl acetate. This protoplast had been incubated in 250 $\mu\text{g ml}^{-1}$ coumarin for 24 h before bursting. Microtubules are conspicuous on the membrane. Scale marker = 1 μm . *b*, High magnification micrograph of the plasma membrane of a burst coumarin-treated protoplast showing microtubules. Scale marker = 0.5 μm .

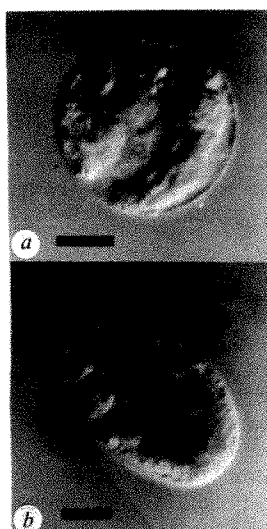


Fig. 1 *a*, Photomicrograph of a protoplast of *Mougeotia* that has been cultured in 250 $\mu\text{g ml}^{-1}$ coumarin in PMM for 24 h; it lacks a cell wall and remains spherical. Scale marker = 10 μm . *b*, Normal regeneration of a *Mougeotia* protoplast after 24 h in PMM without coumarin. The cell has a wall and is ovoid in shape. Scale marker = 10 μm .

Over 50% of untreated protoplasts regenerate a cell wall after 5 h in culture, and by 24 h over 90% have a wall. Such regenerating cells become ovoid after about 12 h, cylindrical within 2 d, and by the third day of culture many have divided⁹. Protoplasts treated with coumarin for up to 3 d do not develop a cell wall that is detectable by electron microscopy or by Calcofluor fluorescence, and they remain spherical (Fig. 1). These protoplasts could be ruptured by osmotic shock (further evidence for their lack of a cell wall) and contain microtubules attached to the plasma membrane (Fig. 2). The cortical microtubular arrays are indistinguishable from those in untreated cells. If coumarin-treated protoplasts were transferred to PMM lacking coumarin they develop a cell wall and become cylindrical; the time course of this belated development is similar to that of the controls.

If coumarin does not affect microtubules, as suggested by this work and that of Itoh¹³, they apparently have no direct cytoskeletal function in developing the shape of these cells as they do in *Ochromonas*⁷, *Pediastrum*¹⁵ and some higher plants¹⁶. The finding that coumarin-treated protoplasts do not deposit a cell wall and that they remain spherical clearly implies that the wall is necessary for establishing cell shape as well as for its maintenance. Experiments with colchicine and IPC suggest that, as in

higher plants, cortical microtubules control cell shape by influencing the pattern of microfibril deposition.

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Non-selective isolation of human somatic cell hybrids by unit-gravity sedimentation

DATA from somatic cell hybridisation have made important contributions to the understanding of genetic control of phenotypic expression, the nature of malignancy in tumours, gene mapping and the basic genetic lesions causing metabolic diseases^{1–5}. Broader application has been limited by the absence of biochemical markers in most cell strains and lines, making it difficult to eliminate parental cells and isolate hybrids. Recent non-selective methods of isolating hybrid cells require either expensive and elaborate equipment⁶ or examination of large numbers of clones with a low yield of hybrids⁷. We now present a method for isolating tetraploid clones from fused human fibroblasts non-selectively and in high yield. Our technique is applicable to tetraploid cells formed from any pair of diploid cells as long as the synkaryons so formed can proliferate. The method depends on the separation of fused cells from the unfused parental cells because of their greater size. A mixture of cells settles out according to size in a shallow bovine serum albumin (BSA) gradient at unit gravity, yielding fractions enriched in double-nucleated cells from which tetraploid clones can be isolated in high yield.

Our goal was to fuse human fibroblasts with polyethylene glycol and then fractionate them by means of a 'staput' chamber⁸. A thin layer of fused cell suspension was pipetted on top of a shallow gradient solution in the chamber and left to settle at unit gravity. In these conditions, sedimentation velocity was almost directly proportional to the square of the cell radius⁸, so that the larger fused cells settled out faster than the smaller unfused cells. After the specific time for differential sedimentation had elapsed, cell fractions were collected from the bottom of the gradient.

To keep the fibroblasts in a monodispersed state for effective separation and to maintain their maximal viability for cloning, we found that BSA dissolved in otherwise serum-free culture medium (Ham's F-10) was superior to Ficoll or fetal calf serum as the gradient material. Because the purpose of the gradient was to prevent adjacent layers from mixing during collection, the percentage of BSA in the gradient could be varied, for

example, from 1 to 3%, without affecting the separation significantly. Addition of anti-clumping agents such as 12 mM xylocaine or 0.01% EDTA⁹, although reducing clumping to a minimum, also reduced cell viability by up to 40%. Substitution of phosphate-buffered saline (PBS) for culture medium led to slightly increased clumping, and the presence of fetal calf serum in either the culture medium or PBS caused marked clumping of the fibroblasts.

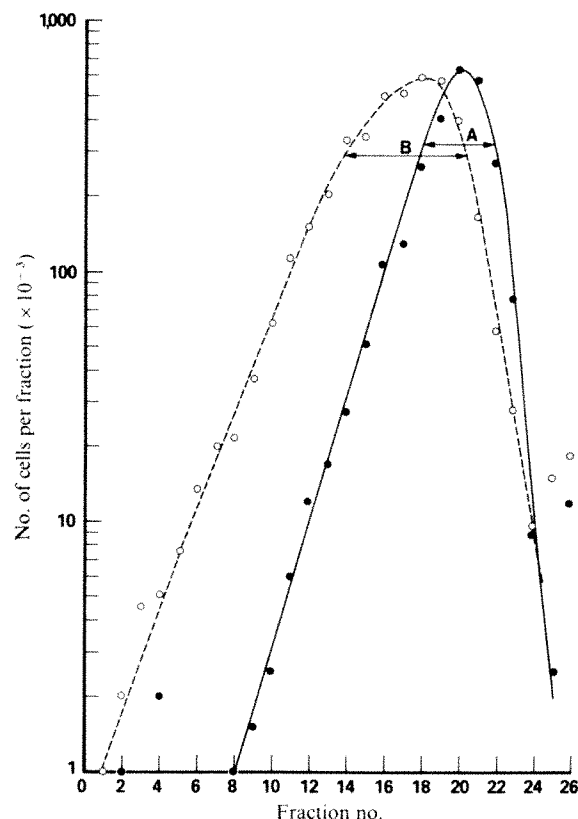
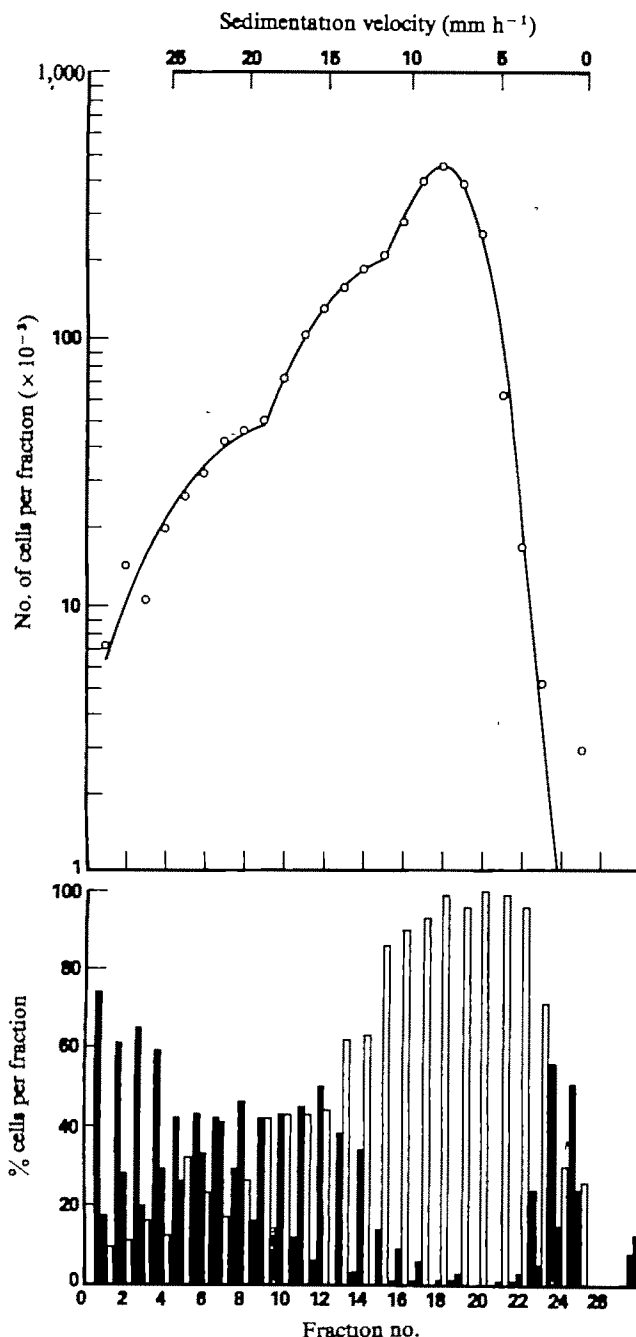


Fig. 1 Sedimentation profiles of unsynchronised fibroblasts and fibroblasts synchronised by serum starvation. Approximately 5×10^6 human skin fibroblasts cultured in Ham's F-10 medium supplemented with 20% fetal calf serum, glutamine and antibiotics, or a similar number of fibroblasts synchronised by serum starvation (0.2% of fetal calf serum in Ham's F-10 medium for 3 d) were collected with trypsin (0.125%) and EDTA (0.02%). Cells were suspended in 20 ml of Ham's F-10 medium and layered as a thin band on top of a gradient of 2–3% bovine serum albumin in Ham's F-10 medium in the staput apparatus with a 10-ml pipette. After sedimentation at 4 °C for 3 h, 50-ml fractions were collected from the bottom of the gradient. Each fraction was centrifuged at 200g for 10 min, and the pellets were suspended in 0.5 ml of complete medium for cell counts. ○, Unsynchronised fibroblasts; ●, fibroblasts synchronised by serum starvation.

A further complication with cultured fibroblasts was the variability in size among the diploid parental cells, in part due to their being in different phases of the cell cycle. To bring them to a homogeneous size range before fusion and thus maximise the size difference between the fused and unfused cells after fusion, techniques known to induce cell synchrony were tried. We found that serum starvation reduced considerably the variation in cell size (Fig. 1), so that the width of distribution at half peak height was 2.4 units (B) for the serum-starved population compared to 4.1 units (A) for the untreated cells. Thus by this treatment fewer larger cells and a predominance of smaller cells were obtained. Synchronisation by leaving cells to grow to confluence, or by single or double thymidine block was less successful than serum starvation in reducing the variation of cell size, and furthermore, thymidine treatment tended to increase clumping.

After synchronising human skin fibroblast strains by serum starvation, we fused two strains with polyethylene glycol 1000 (ref. 10) and fractionated the cells according to size in the staput chamber containing a gradient of 2–3% BSA dissolved in serum-free Ham's F-10 culture medium (Fig. 2). Because cells with more than two nuclei do not divide¹¹, they could be ignored for subsequent cloning purposes. However, fractions that contained the highest ratio of double-nucleated cells to single-nucleated cells should have the best chance of yielding tetraploid clones. Therefore, the earliest fractions (1–8) collected from the bottom of the sedimentation chamber with an average ratio of double-nucleated to single-nucleated cells of 2:1 were used for cloning. In contrast, the original unsorted sample had an average ratio of 0.17:1.

Of the 72 clones successfully propagated for identification by karyotyping¹², 18 (25%) had a modal number of 46 (diploid), nine (12.5%) had both diploid and tetraploid cells and 45 (62.5%) were tetraploid. Several tetraploid clones were carried through multiple passages, each maintaining their complement of 92 chromosomes.



Vigorous growth capability in both parent cell strains was essential for successful propagation of tetraploid clones. For example, when we fused a female skin fibroblast strain with a male skin fibroblast strain that was much more vigorous in growth, we found that of the 45 tetraploid clones, at least 24 were apparently from male-male fusions with two Y chromosomes. Only nine clones were judged to be from female-female fusions, as shown by the absence of a Y chromosome, and 10 were judged to be from male-female fusions, as shown by the presence of only one Y chromosome. The identification of the last two categories was tentative, as the complete tetraploid chromosome number of 92 was seldom obtained. The number of chromosomes counted was most often approximately 80, mainly because of the technical difficulty in spreading so many chromosomes properly for counting and identification.

To overcome this difficulty of identifying true hybrids cytogenetically, we used the isozymes of glucose-6-phosphate dehydrogenase (G6PD) as markers^{13,14}. A fibroblast strain with the G6PD_A isozyme was fused with another strain with the G6PD_B form. After separation in the staput chamber, clones propagated from fractions 1–8 were identified cytogenetically as diploid or tetraploid. All the tetraploid clones were then subjected to electrophoresis to identify their G6PD profile. Tetraploids formed from homologous fusion between the same parent cell types would show either the A or the B form. Hybrid tetraploids formed from heterologous fusion between the two parent cell types would show the hybrid heteropolymer between the A and B form in the ratio of 1:2:1. Out of 202 clones isolated from fractions 1–8, 114 were growing sufficiently vigorously for karyotyping. Of these, 31 were tetraploid, seven were mixed tetraploid and diploid and 76 were diploid. Electrophoretic separation of G6PD isozymes in 30 of the tetraploid clones showed that six had the typical hybrid pattern, 21 had only the A form, one had the appearance of the hybrid form mixed with the A form, 1 had the B form and one had both A and B. Thus, of the 30 clones judged to be tetraploids, 20% were found to be pure hybrid clones.

We have thus shown that by exploiting the different sizes of hybrid cells and their unfused parents, hybrid tetraploid clones can be obtained in high yield. The staput apparatus provides a relatively simple technique for separating somatic cell hybrids and is applicable to a wide variety of cell types which have not been studied previously because of their lack of biochemical selection markers. Furthermore, because hybrid clones can be isolated without perturbing the genetic composition of the parental cell strains by chemical or physical selection, the exact biochemical and genetic mechanisms that account for complementation between some types of inborn error of metabolism may be more easily revealed and questions of gene dosage and gene inactivation can be examined in the hybrid clones without constraint.

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Fig. 2 Separation of human skin fibroblasts by unit-gravity sedimentation in the staput apparatus after fusion with polyethylene glycol. Fibroblasts synchronised by serum starvation were seeded in T-75 culture flasks (Corning) at a density of 4×10^6 cells per flask (2×10^6 cells from each of two fibroblast cell strains) and incubated for 24 h in complete medium (Ham's F-10 supplemented with 20% fetal calf serum). They were fused with polyethylene glycol 1000 according to the procedure of Davidson *et al.*¹⁰ and incubated for about 16 h before collection for separation in the staput apparatus, as described in the legend to Fig. 1. After sedimentation at 4 °C for 4 h, 50-ml fractions were collected from the bottom and centrifuged at 200g for 10 min. The pellet was suspended in 0.5 ml of complete medium for cell count, Giemsa staining and cloning. The entire operation was performed in sterile conditions. —, No. of cells per fraction; S, sample cell load before sedimentation. Open columns, one nucleus per cell; solid columns, two nuclei per cell; hatched columns, more than two nuclei per cell.

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Infection of the Mongolian gerbil with the cattle piroplasm *Babesia divergens*

DISEASES of cattle caused by piroplasms are one of the major constraints on the growth of the livestock industry in the tropics. *Babesia divergens*, which is the common cause of redwater in European cattle, can be transmitted to splenectomised goats, sheep and deer by the inoculation of blood from infected cattle¹. Furthermore, it has been shown experimentally that *B. divergens* is transmissible in this way to chimpanzees². Evidence is now accumulating to suggest that it is this species of *Babesia* which is capable of causing fatalities in splenectomised humans in Europe^{3,4}. Our inability to adapt cattle piroplasms to small laboratory animals has restricted efforts to develop new drugs

Table 1 *B. divergens* parasitaemias of infected gerbils up to 96 h post-inoculation

Time post-inoculation (h)	No. of infected erythrocytes per 1,000			
	G1	G2	G3	G4
24	<1	<1	<1	<1
48	<1	<1	<1	<1
72	58	17	7	7
96	372	50	236	67

G1 and G2 were splenectomised gerbils; G3 and G4 were intact gerbils.

Table 2 Summarised reactions of splenectomised Friesian calves to the inoculation of approximately 10^8 *B. divergens*-infected erythrocytes

Calf no.	No. of infected erythrocytes inoculated	Origin of parasites inoculated	Parasitaemia		Febrile response		Haematology	
			Prepatent period to parasitaemia of 1 per 1,000 RBC (d)	Maximum parasitaemia per 1,000 RBC	Prepatent period to febrile response of $\geq 39.5^\circ\text{C}$ (d)	Maximum febrile response ($^\circ\text{C}$)	Lowest PCV (%)	Highest MCV (μm^3)
M1010	1.8×10^8	Gerbil	4	143	6	40.7	15.1	46
M1011	5.5×10^8	Gerbil	4	180	4	41.2	10.2	47
J488	9.7×10^7	Calf	3	186	3	40.5	8.9	44

PCV, packed corpuscular volume; MCV, mean corpuscular volume.

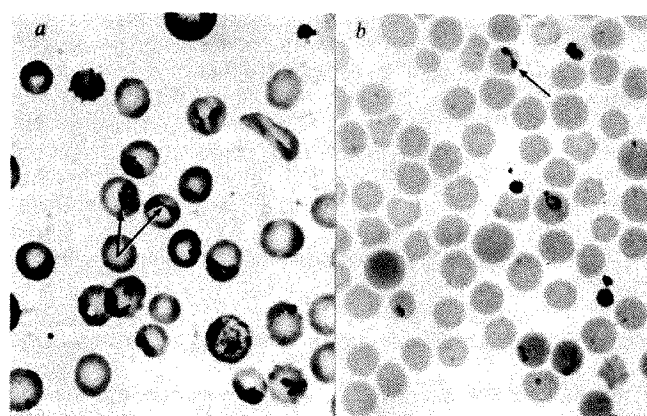


Fig. 1 *B. divergens* piroplasms infecting *a*, gerbil, and *b*, bovine erythrocytes. Giemsa-stain, $\times 2,200$. Arrows indicate characteristic divergent forms of the parasite.

and vaccines against them. However, during a recent investigation of a human fatality⁵, we were able to transmit the parasite to gerbils by inoculation of infected blood from the patient. Although final evidence to implicate *B. divergens* in this instance is still being assessed, we decided to investigate the possibility of infecting gerbils with *B. divergens*-infected blood from cattle. We now report the successful adaptation of *B. divergens* to the Mongolian gerbil (*Meriones unguiculatus*).

A splenectomised Friesian calf reacting to inoculation with a strain of *B. divergens* originally isolated from Eire had a *B. divergens* parasitaemia of 61 infected erythrocytes per 1,000 cells. A sample of 75 ml of blood was taken from the calf's jugular vein into Anticlot (Clinton Laboratories) and centrifuged at 3,400g for 20 min. The resultant pellet was resuspended and washed three times in phosphate-buffered saline (PBS) (pH 7.2). The pellet was finally reconstituted so that 0.7 ml contained $\sim 5 \times 10^8$ *B. divergens*-infected erythrocytes.

Four gerbils, two intact and two splenectomised, were each inoculated intraperitoneally with 0.7 ml of the reconstituted blood. Thin blood smears were made daily from the gerbils' tail-tips and stained with Giemsa. Parasites were seen in blood smears from all animals from day 1 post-inoculation and parasitaemias are shown in Table 1. Parasites present in the blood of infected gerbils exhibited considerable pleomorphism (Fig. 1*a*); however, several of these forms were characteristic of *B. divergens* piroplasms in infected bovine erythrocytes (see Fig. 1*b*).

On day 4 post-infection all the gerbils had significant *B. divergens* parasitaemias. One intact animal with a 23% infection of *B. divergens* piroplasms was killed and 0.1 ml of blood containing 2×10^7 infected erythrocytes was inoculated intraperitoneally into two further gerbils (G5 and G6). On the fourth day after inoculation gerbil G5 had a parasitaemia of 14 infected erythrocytes per 1,000 and a red blood cell (RBC) count of 8.9×10^9 per ml; gerbil G6 had a parasitaemia of 59 per 1,000 erythrocytes and an RBC of 6.2×10^9 per ml. They were killed and ~ 1.5 ml of blood was recovered from each animal, representing 1.8×10^8 and 5.5×10^8 *B. divergens*-infected erythrocytes, respectively. The erythrocytes were washed and

resuspended in PBS as before. Splenectomised calf M1010 was then inoculated intravenously with parasites from gerbil G5 and splenectomised calf M1011 with parasites from gerbil G6. A further 0.2 ml of blood from each infected gerbil was inoculated into a splenectomised control gerbil. Both control gerbils subsequently experienced similar reactions to those of the animals used in the first passage of bovine blood.

The reactions of the two splenectomised calves and that of a splenectomised control calf inoculated with 9.7×10^7 *B. divergens* parasites (Eire strain) are summarised in Table 2.

We have thus demonstrated (1) that gerbils can be infected with *B. divergens* by the intraperitoneal inoculation of infected bovine blood; (2) that the parasite is then transmissible to further gerbils; (3) that the parasite can be used to infect splenectomised calves once again after two gerbil passages.

The epizootiological significance of an alternative rodent host for piroplasms affecting cattle merits further investigation. Furthermore, these preliminary findings seem to indicate that the Mongolian gerbil could provide a small animal model for *B. divergens* and other species of cattle piroplasms. This system may prove suitable for immunological investigations and the screening of anti-babesial drugs.

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T-cell lines which cooperate in generation of specific cytolytic activity

THE unidirectional mixed leukocyte culture (MLC) is characterised by cell proliferation and by the development of cytolytic T lymphocytes (CTL)¹⁻³. In murine MLC, the generation of CTL seems to involve proliferation of at least two subpopulations of responding T cells—T cells of the Ly-2⁺3⁺ phenotype, which differentiate into CTL, and cells of the Ly-1⁺ phenotype, which seem to 'help' or 'amplify' the CTL response^{4,5}. The mechanism by which 'amplifier cells' augment the CTL response is unclear; recent reports have shown, however, that CTL precursors present in populations of long-term MLC or of immune spleen cells^{7,8} can respond to supernatant fluid obtained from allogeneic MLC or mitogen-stimulated spleen cell cultures in the absence of specific antigen. Several groups using such conditioned medium have reported the successful long-term culture of normal and antigen-specific immune T cells in the absence of alloantigen stimuli⁹⁻¹¹. Whereas amplifier supernatant fluids seem to substitute functionally for a T-cell subpopulation (Ly-1⁺?) in the absence of alloantigen, there is only indirect evidence that amplifier cells can be activated by alloantigen to release one or several soluble factors that influence the differentiation or proliferation of

Table 1 Peak levels of ³H-thymidine incorporation

Responding cell	Factor	Stimulating cell		
		Medium	C57BL/6	DBA/2
L2	Medium	197 ± 52	1,104 ± 58	7,501 ± 1,011
	LCA SF	558 ± 384	10,182 ± 5,354	17,228 ± 4,380
	2 ⁰ MLC SF	1,097 ± 444	1,646*	8,705 ± 1,883
L3	Medium	107 ± 48	1,030 ± 368	1,296 ± 176
	LCA SF	702 ± 490	30,865 ± 12,043	53,313 ± 13,744
	2 ⁰ MLC SF	925 ± 585	3,242*	13,035 ± 1,522

The incorporation of ³H-thymidine by L2 or L3 cells cultured with irradiated C57BL/6 (syngeneic) or DBA/2 (allogeneic) spleen cells, or medium alone, was measured in the presence of either LCA or 2⁰ MLC supernatant fluid (SF), or medium alone. Values are mean d.p.m. ± s.e.m. Results from 12 different experiments are included. For details, see Fig. 1.

*Mean d.p.m. calculated from results obtained in two different experiments

CTL¹²⁻¹⁴. We have used modifications of techniques previously described for the long-term culture of immune T cells to isolate several cloned lines of MLC-reactive T cells, including a non-cytolytic T-cell line which, when co-cultured with alloantigen, permits another cell line to proliferate and express specific cytolytic activity.

Primary MLC were prepared by culturing, for 14 d, C57BL/6 (H-2^b) responding spleen cells with irradiated DBA/2 (H-2^d) spleen cells¹⁵. Cells were then collected and restimulated at low density with irradiated DBA/2 spleen cells¹⁶. After 48 h of secondary MLC, cells were collected and cultured at limiting dilution with irradiated DBA/2 spleen cells using medium consisting of equal volumes of 48-h secondary MLC supernatant fluid and Dulbecco's modified Eagle's medium (DMEM)¹⁷ containing 20% fetal calf serum (FCS). Clones of cells appeared in 30% of the wells 8 to 10 d after culturing. Screening of all clones by a micro-⁵¹Cr release assay¹⁸ revealed cytolytic activity by some, but not all, of the cells. Several lines of such cells, some of which are cytolytic, have been maintained in culture for 7 months by weekly transfer with irradiated DBA/2 spleen cells and supernatant fluid obtained from either 48-h secondary MLC (2⁰ MLC) or 48-h cultures of Lewis rat spleen cells stimulated with concanavalin A (LCA). Two of these cell lines have been studied intensively. Both are T cells, as determined by treatment with anti-Thy 1.2 sera: one, designated L2, is non-cytolytic; the other, designated L3, is cytolytic.

The proliferative response of L2, L3, or a mixture of L2 and L3 (L2+L3), indicated by incorporation of ³H-thymidine (³H-TdR), was measured on days 3 and 5 in nine different experiments (Fig. 1a, b). The L2 cells cultured with the original stimulating alloantigen (DBA/2) exhibited peak proliferative responses on day 3, with an average stimulation index of 6.8, when compared with the response of L2 cells cultured with irradiated syngeneic C57BL/6 cells. The proliferative response of L2 cells cultured alone or with irradiated C57BL/6 cells did not change significantly between days 3 and 5, and in terms of viable cells recovered, there was only a 1-to-3-fold increase over L2 cell input compared to a 21- to 44-fold increase in viable cells recovered from cultures of L2 cells and irradiated DBA/2 spleen cells.

In contrast, the proliferative response of L3 cells cultured with irradiated alloantigen was not greater than that observed with L3 cells cultured with syngeneic spleen cells (stimulation index of 1.3 for peak response), and was 5.4 times lower than the response of L2 cells cultured with alloantigen. There was no change in the proliferative response of L3 cells between days 3 and 5; viable cells recovered from cultures of L3 cells with irradiated DBA/2 or C57BL/6 cells increased only 1- to 4-fold over L3 cell input compared to a 0- to 1-fold increase for L3 cells cultured alone.

When L2 and L3 cells were cultured together with irradiated DBA/2 spleen cells, the extent of maximal ³H-TdR incorporation on day 3 appeared to be approximately the sum of the values observed with separate populations of L2 and L3 cells (average stimulation index of 14.2 compared to L2+L3 cells

cultured with irradiated syngeneic C57BL/6 spleen cells). However, the total cell recovery from cultures of L2 + L3 cells with irradiated DBA/2 cells was, on average, twice the sum of the cell recoveries from separate cultures of L2 and L3 cells.

Cytolytic activity was measured with a ^{51}Cr release assay¹⁵ after 3 and 5 days of culture. Although L2 cells proliferated when stimulated with alloantigen in culture (Fig. 1a), these cells never expressed any detectable cytolytic activity (Fig. 1c). Likewise, L3 cells expressed only barely detectable levels of cytolytic activity (mean of <2 lytic units per culture). However, L2 and L3 cells cultured together with irradiated DBA/2 alloantigen developed high levels of cytolytic activity against P-815 target tumour cells (DBA/2 origin) (mean of 71 lytic units per culture). On the other hand, L2 and L3 cells cultured together with irradiated syngeneic spleen cells developed only little lytic activity (mean <4 lytic units per culture). Thus, the inclusion of L2 cells with irradiated DBA/2 alloantigen resulted in an average 22.4-fold increase in cytolytic activity.

Like other investigators⁹⁻¹¹, we have found that 2^0 MLC or LCA supernatant fluid makes possible the proliferation of T cells and the generation of cytolytic activity. The peak proliferative responses for L2 and L3 cells cultured with various supernatant fluids are shown in Table 1. The incorporation of ^3H -TdR by L2 cells cultured with irradiated allogeneic (DBA/2) or syngeneic C57BL/6 spleen cells was significantly higher, on the average, if LCA supernatant fluid was added, as was the total cell recovery. However, the addition of 2^0 MLC supernatant fluid to L2 cells cultured with irradiated DBA/2 cells did not result in any significant increase in either ^3H -TdR incorporation or total cell recovery, except that, in a single experiment, the addition of 2^0 MLC supernatant fluid to L2 cells cultured with irradiated C57BL/6 cells increased total cell recovery 10-fold without any significant increase in ^3H -TdR incorporation. Thus, in the absence of alloantigen, L2 cells cultured with irradiated syngeneic cells could be stimulated to proliferate by the addition of either 2^0 MLC or LCA supernatant fluid. Cytolytic activity, however, was not detectable under any conditions in which L2 cells proliferated.

The effect of 2^0 MLC or LCA supernatant fluid on the peak proliferative response of L3 cells was much greater than the effect on L2 cells (Table 1). The addition of LCA supernatant

fluid to L3 cells cultured with irradiated syngeneic or allogeneic spleen cells increased both the average ^3H -TdR incorporation and cell recoveries 30- to 41-fold, compared to the effect of culture medium only. The effect of 2^0 MLC supernatant fluid was much less than that of LCA supernatant fluid. As seen in Table 1, neither L2 nor L3 cells gave an appreciable proliferative response when they were cultured with 2^0 MLC or LCA supernatant fluid in the absence of additional irradiated cells.

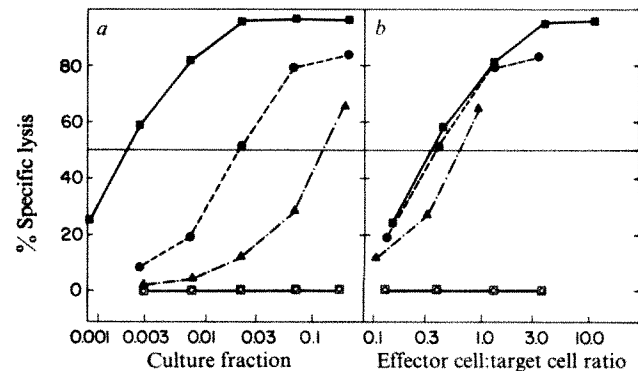


Fig. 2 Cytolytic activity generated by L3 cells cultured with irradiated syngeneic C57BL/6 spleen cells in the presence of LCA supernatant fluid (□), 2^0 MLC supernatant fluid (○), or medium alone (△) after 5 d of culture. Closed symbols, P-815 target cells. Open symbols, AKR-A target cells. a, Cytolytic activity expressed in terms of per cent specific lysis per culture fraction. b, Cytolytic activity expressed in terms of per cent specific lysis at various ratios of effector cells to target cells. For details, see Fig. 1.

These results differ from those reported by Gillis and Smith¹⁰, who found that such supernatant fluids alone were capable of stimulating the proliferation of T cells without addition of cells.

In marked contrast to the results observed with L2 cells, cytolytic activity was detectable whenever L3 cells were stimulated to proliferate. There was a good correlation between the magnitude of the proliferative response and the extent of CTL generation. Figure 2 shows the results obtained in a single representative experiment in which cytolytic activity was

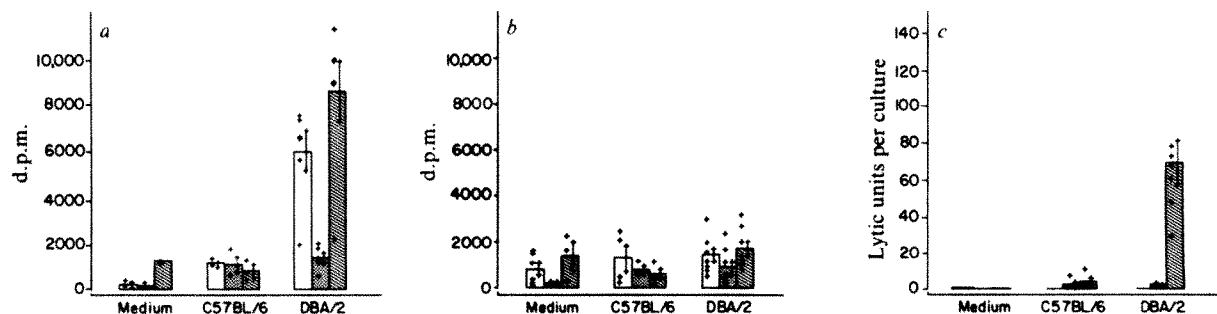


Fig. 1 a, Tritiated-thymidine (^3H -TdR) incorporation by L2 cells (□), L3 cells (▨), and a mixture of L2 and L3 cells (▩) when the cells were cultured alone or with irradiated syngeneic (C57BL/6) or allogeneic (DBA/2) spleen cells in culture medium for 3 d. b, ^3H -TdR incorporation measured after 5 d of culture. c, Cytolytic activity measured against P-815 target cells after 5 d of culture. Results from nine different experiments are presented in which, typically, 1.6×10^4 L2 and/or L3 cells (7 to 8 d after previous transfer) in 0.1 ml were transferred to 3 ml culture wells (Linbro 24-well plate, type 76-033-05) containing 6×10^6 irradiated (1,500 rad) syngeneic (C57BL/6) or allogeneic (DBA/2) spleen cells, or culture medium (DMEM supplemented with 2% heat-inactivated fetal calf serum, 10 mM MOPS buffer, $5 \times 10^{-5}\text{M}$ 2-mercaptoethanol, plus penicillin/streptomycin¹⁷), and either 48-h 2^0 MLC or LCA supernatant fluid (final concentration, 33%), or culture medium, in a final volume of 1.5 ml. Culture plates were then incubated in a humidified atmosphere of 5% CO_2 at 37°C . After 3 and 5 d of culture, individual wells were collected and assayed for both ^3H -TdR incorporation and cytolytic activity. A 0.1-ml culture fraction of each well was placed in triplicate wells of a flat-bottomed microwell tissue culture plate and pulsed with $1 \mu\text{Ci}$ ^3H -TdR ($0.36 \text{ Ci mmol}^{-1}$) for 4 h at 37°C . Cells were then collected on to filter paper strips with an Otto Hiller cell harvester and counted for 2 min in a Searle liquid scintillation counter. Results are expressed as d.p.m. $\pm$ s.e.m. Cytolytic activity was determined with a ^{51}Cr release assay¹⁵. Tumour target cells were labelled with $\text{Na}_2^{51}\text{CrO}_4$ and mixed with serial threefold dilutions of effector cells (replicate wells) in round-bottomed microtitre plates. The plates were incubated for 3.5 h at 37°C and then centrifuged, after which one-half of the supernatant volume in each well was counted in a Packard well-type scintillation counter. The per cent of specific cytotoxicity at various effector-to-target cell ratios was calculated by the formula

$$\% \text{ specific lysis} = \frac{\text{c.p.m. exp.} - \text{c.p.m. spontaneous release}}{\text{c.p.m. max. release} - \text{c.p.m. spontaneous release}} \times 100$$

Maximum release was determined by freeze-thaw lysis of target cells in water; spontaneous release was determined from the amount of isotope released during the assay by target cells incubated with medium only. One lytic unit is defined as the number of effector cells required to lyse 50% of 10,000 target cells within 3.5 h.

compared in terms of culture fraction as well as effector-to-target cell ratio for L3 cells cultured with irradiated C57BL/6 spleen cells in the presence of medium, 2^0 MLC, or LCA supernatant fluid. Measured in lytic units (LU) per culture (Fig. 2a), the addition of LCA supernatant resulted in the expression of the greatest cytolytic activity observed (526 LU per culture) which was approximately 11 times greater than that observed with 2^0 MLC supernatant fluid (46 LU per culture) and 67 times greater than that observed with medium alone. Cytolytic activity was directed specifically towards the original stimulating alloantigen, since there was no detectable lysis when syngeneic or third-party allogeneic tumour cells were used (EL-4, AKR-A, respectively). These differences in CTL activity correlated very well with ^3H -TdR incorporation as well as cell recovery.

When evaluated on the basis of effector-to-target cell ratio, the cytolytic activity generated by L3 cells in response to the addition of 2^0 MLC or LCA supernatant fluid, or of medium alone in the presence of irradiated syngeneic cells, was approximately equal (Fig. 2b). Thus, 50% lysis of 10,000 target cells was observed at effector-to-target cell ratios of 0.36, 0.31 and 0.58, respectively. When L3 cells are cultured with irradiated cells and either medium, 2^0 MLC, or LCA supernatant fluids, they appear to have similar killing potential. Thus, the cytolytic activity of L3 cells is related primarily to the number of cells developing during culture; the 2^0 MLC or LCA supernatant fluids seem to stimulate the proliferation of T cells.

These experiments can be summarised as follows: two cloned lines of T cells, L2 and L3, have been obtained from MLC prepared by co-culturing responding C57BL/6 spleen cells with irradiated DBA/2 alloantigen. L2 cells proliferate in response to alloantigen, but do not express cytolytic activity, whereas L3 cells, which do not proliferate in response to alloantigen unless exposed to 2^0 MLC or LCA supernatant fluid, express cytolytic activity specific for the original stimulating alloantigen. The magnitude of the cytolytic activity seems to be related primarily to the extent of L3 proliferation. The results observed in cultures prepared with L2 and L3 cells together with alloantigen are consistent with the interpretation that alloantigen stimulation of L2 cells results in proliferation and in the release of one or more soluble amplifier factors which bring about the proliferation of L3 cells with a concomitant increase in cytolytic activity. In preliminary experiments, supernatants have been obtained after 2 d from cultures of L2 cells stimulated with alloantigen. L3 cells grown in the presence of 'L2-cell supernatant fluid' together with irradiated allogeneic cells generated cytolytic activity that was 12 times greater than that observed in the absence of 'L2 cell supernatant fluid'. The availability of separate T-cell lines with amplifier or cytolytic activity will simplify the study of interactions involved in the development of cell-mediated immune responses.

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Association of a B-cell alloantigen with susceptibility to rheumatic fever

THE possibility that genetic factors determine susceptibility to rheumatic fever following infection with Group A streptococci was suggested by epidemiological and family studies¹. However, with the exception of a slightly increased frequency of Lewis group secretors², the identification of a genetic marker in the patient group has been unsuccessful. In particular, the distribution of HLA-A and B allotypes did not reveal any consistent difference from controls³⁻⁵. We report here that a novel B-cell alloantigen has been found at a significantly increased frequency among patients with rheumatic fever, providing further evidence for the value of B-cell alloantisera as reagents for the demonstration of disease associations with genetic determinants relevant to the immune system. This alloantigen was of particular interest because it was not found to be associated with any recognised HLA-D locus determinant.

The observation that B lymphocytes preferentially express differentiation alloantigens has led to a new approach to the use of genetic markers that might be of special significance to the immune system⁶. These are recognised primarily with alloantibodies that develop as a result of immunisation with paternal antigens during pregnancy. The majority of such alloantisera recognise alloantigens that have extensive biological and chemical homologies with the I-region antigens of the murine histocompatibility system and have been termed 'Ia-like' or simply 'Ia' (ref. 7). Some of these Ia alloantigens are closely related to HLA-D alleles defined in the mixed lymphocyte culture (MLC) reaction, and have been designated HLA-DR. Other B-cell alloantigens are not as readily related and seem to be primarily significant in demonstrating the immunogenetic associations of particular diseases^{8,9}.

Mononuclear cells were isolated from 61 patients with documented rheumatic fever (Jones criteria) attending clinics either in Bogota, Colombia, or New York City, and from age, sex and race matched control individuals. Reagent B-cell alloantisera were selected for their very low T-cell reactivity and further absorbed with pooled platelets. Two assays for B-cell alloantigens were used: mononuclear cells were stimulated with pokeweed mitogen for 4-6 d before staining using an indirect immunofluorescent technique¹⁰, and 'B' cells isolated by depletion of T lymphocytes were used in a modified Amos two-stage microcytotoxicity test⁹.

Twenty-eight lymphoblastoid lines were derived from individuals known to be homozygous for defined HLA-D alleles; 17 were derived from unselected individuals who were defined for all HLA determinants and known to be heterozygous for HLA-D. DR specificities were assigned according to the pattern of reagent serum reactions with the reference cell lines⁹. In the case of sera which detected an alloantigen jointly expressed on cell lines derived from individuals with different HLA-D alleles such as Dw4, Dw7 or Dw10, the specificity of the sera was designated 4 × 7 × 10 (ref. 9). The χ^2 statistic included

Table 1 B-cell alloantigen typing in patients with rheumatic fever

Designation	Reagent serum DR Specificity	New York series (%)		Bogota series (%)		Combined series	
		Normal (52)	Rheumatic fever (21)	Normal (60)	Rheumatic fever (41)	χ^2	Relative risk
883	Undefined	17	71	16	75	50.144	12.94
1,818	1	31	20	24	20	0.906	0.686
1,558	2	28	33	20	30	0.878	1.463
1,033	3	24	25	20	15	0.745	0.68
924	4 × 10	26	25	20	30	0.463	1.26
1,038	4 × 7 × 10	28	35	32	40	1.397	1.54
1,995	5	12	0	10	15	0.125	1.21

the Yates correction for continuity. The relative risk was calculated according to Woolf¹¹ as equal to (patients positive/patients negative) × (controls negative/controls positive). Values greater than one indicate the degree of positive association of a marker with disease.

Reagent serum 883 was identified from among over 200 unselected B-cell alloantisera through its reaction in preliminary studies with a high proportion of patients with rheumatic fever. This and other sera with established DR specificities were used to determine their alloantigen frequencies in two populations of patients with rheumatic fever (Table 1). Serum 883 reacted with 75% of the patients in the Bogota series and 71% of the patients in New York, whereas the frequency of reaction in the two control populations was 16 and 17%, respectively. The association of alloantigen 883 defined by serum 883 with rheumatic fever for the combined series was significant at $P < 0.001$ and the relative risk was 12.9. We have not shown the unremarkable frequency of reaction with serum 883 among patients with a variety of other diseases, including tuberculosis, leprosy, post-streptococcal glomerulonephritis, multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus.

The expression of a number of Ia-like alloantigens, including some which were related to HLA-D specificities, was studied with 19 B-cell alloantisera. The frequency of alloantigens detected by these reagents among patients was not significantly different from their respective control groups. The results for six sera with representative specificities are included in Table 1.

Attempts were made to define the specificity of alloantiserum 883 using 28 B-cell lines derived from individuals homozygous for the various MLC D-locus alleles (Dw1, Dw2, Dw3, Dw4, Dw5, Dw6, Dw7, Dw10 and Dw11) and selected for their suitability as typing cells. Serum 883 did not react with any of these defined homozygous cell lines. In contrast, both peripheral blood B cells and B-cell lines derived from 17 individuals known to be heterozygous at the D locus gave strong reactions with serum 883 in three instances. However, the reactivity could not be correlated with a defined MLC specificity. Similarly, using B lymphocytes from 52 normal subjects, no significant association of the presence of alloantigen 883 could be established with any HLA-A, B or D alleles.

The recognition of alloantigen 883 through the disease association with rheumatic fever emphasises the usefulness of directly screening disease populations with multiple antisera as a means of gaining insight into genetic factors that otherwise might not be as readily apparent. The interpretation that alloantigen 883 is an allele of a segregant series that is either a second Ia locus unrelated to HLA-D or a totally distinct B-cell locus is favoured by three observations: the absence of any relation of alloantigen 883 to a defined HLA-D allele; the lack of a reciprocal decrease of any DR alloantigens among the patient group that would have been expected if the DR alloantigens were alleles of alloantigen 883; the occurrence in certain individuals of alloantigen 883 as a third specificity in addition to two conventional and non-cross-reacting DR alloantigens and their associated HLA-D determinants.

The association of this B-cell alloantigen with rheumatic fever provides direct support for the concept that an immunogenetic factor is relevant to the development of rheumatic fever after streptococcal infection.

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NK cell-sensitive T-cell subpopulation in thymus: inverse correlation to host NK activity

NATURAL KILLER (NK) cells have been shown to have an important role in resistance to some tumours (see ref. 1 for review) and it has been suggested that these NK cells may represent a T cell and macrophage-independent surveillance mechanism² similar in principle to the theory proposed by Burnet³ and Thomas⁴. Recent observations have led to the speculation that NK cells may also have a physiological role *in vivo* as NK cells were closely associated with the rejection of bone marrow grafts^{5,6}. Although the target 'antigens' of NK cells have not yet been fully defined⁷, a wide range of malignant and non-transformed cells, including normal thymocytes¹⁷ and bone marrow cells⁸ are also sensitive to low levels of NK-mediated cytotoxicity. We provide evidence here to support the view that the NK cells may be involved in the control of normal T-cell subpopulations within the thymus.

Thymocytes from 2-week-old A/Sn mice were lysed in a 3 h ⁵¹Cr release assay with nylon wool passed CBA spleen cells

as NK effectors (Fig. 1). As shown below in Fig. 4, this lytic effect was dose-dependent at effector to target cell ratios below 25:1, whereas higher numbers of effector cells resulted in a marked decrease in lysis for unknown reasons. The active killer cell shared the cell surface characteristics of mouse NK cells (data not shown), and showed the same strain distribution as previously reported for mouse NK cells¹ (Fig. 3). Moreover, cold targets inhibition assays have shown that NK cells recognise the same target structure on A/Sn thymocytes as on the NK-sensitive tumour targets YAC-1 (data not shown). Low but significant levels of sensitivity to NK-mediated lysis were also exhibited by bone marrow, peripheral blood and spleen cells from 1-2-week-old A/Sn mice (Fig. 1). Conversely the organ distribution of NK reactivity in adult mice invariably showed that the thymus is the organ of least NK cytolytic potential (shown for strain CBA in Fig. 1) as previously reported⁹.

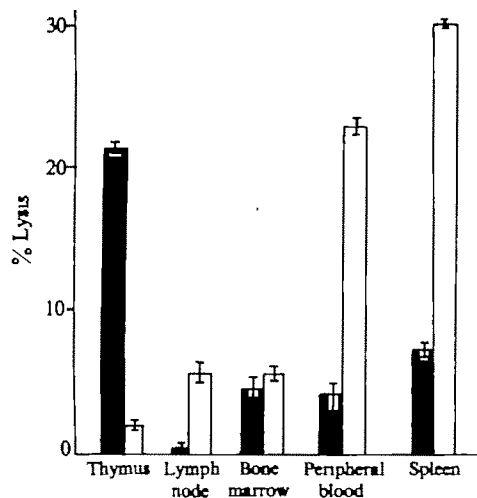


Fig. 1 Organ distribution of NK-sensitive cells in various lymphoid organs of 2-week-old A/Sn mice. Lymphocytes were enriched from peripheral blood by Ficoll-isopaque gradients followed by iron-magnet treatment to remove monocytes-macrophages. Spleen cells were treated with hypotonic shock followed by passage through nylon-wool adherence column to enrich for T lymphocytes. Cells from each organ were used as targets (solid bars) in a conventional 3 h ⁵¹Cr release assay using nylon wool-passed CBA spleen cells as NK effector cells at an effector to target cell ratio of 10:1 as described previously⁵. Open bars indicate organ distribution of NK reactivity in a standard NK assay using the YAC-1 tumour as target.

The NK sensitivity of thymocytes varied markedly with the age of the thymus donor. The highest sensitivity was observed with thymocytes from very young donors (<1-2 weeks) whereafter a gradual decrease of NK sensitivity occurred (Fig. 2). The mice that were used as thymocyte donors were also tested for their spleen NK activity. As shown in Fig. 2, the onset of NK activity around the third week after birth occurred simultaneously with a fall in the NK sensitivity of the thymocytes from the same mice. In old mice, NK activity as well as thymocyte sensitivity was low or absent.

The genetic control of thymocyte sensitivity was also investigated in several other strains of mice previously classified as high or low NK responders in extensive genetic studies (see ref. 1 for review). Again the inverse correlation between thymocyte sensitivity and NK activity was striking (Fig. 3). Thus, thymocytes from mice of low NK reactive strains (A, A.CA and AKR) displayed higher sensitivity to lysis than thymocytes from NK high reactive (CBA or DBA/2 × C57BL/6 F₁ hybrids) or mid reactive strains (C57BL/6 and DBA/2). Ten separate experiments yielded a mean lysis of 13.4% for A/Sn and 4.8% for CBA thymocytes ($P < 0.001$) from age-matched mice. The same relative differences in thymocyte sensitivity appeared when NK effector cells were taken from CBA, C57Bl or A strains (Fig. 3). As high NK levels were invariably associated

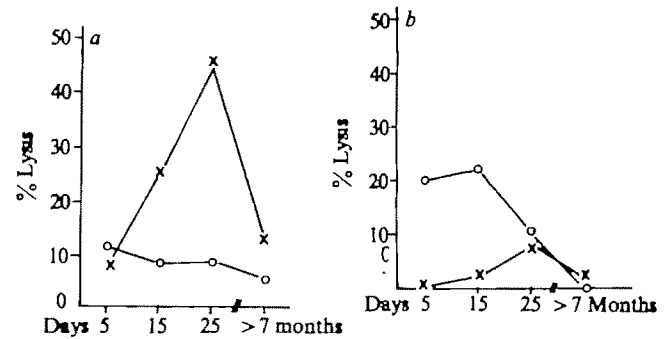


Fig. 2 Inverse correlation between the age dependence of splenic NK activity and the sensitivity of thymocytes to NK-mediated lysis. Thymocytes from three mice of each age were pooled and used as target cells in a 3 h cytolytic assay with nylon wool-passed CBA spleen cells as effector cells at an effector to target cell ratio of 50:1 (○). Spleen cells from the same mice were used as effector cells in a 3 h NK assay at an effector to target cell ratio of 50:1 with the YAC-1 tumour as target (×). *a*, Experiment with the CBA strain, *b*, with the A/Sn strain as thymocyte target and spleen cell effector donor.

with low levels of sensitive thymocyte targets it was important to determine the effect of NK boosting agents on thymocyte sensitivity in the same animal.

It is known that NK activity can be markedly augmented by interferon as well as several interferon inducing agents^{10,11}; all known to exert anti-tumour effects *in vivo*^{12,13}. When two such agents, tilorone (Fig. 4) and polyI:C (unpublished observation) were administered to young strain A mice a substantial increase in NK activity was apparent. However, thymocytes from these same animals were much less sensitive to NK-mediated lysis than thymocytes from controls, which again demonstrates the inverse relationship between levels of NK reactivity and thymocyte sensitivity to lysis.

There are various possibilities to consider when trying to explain this inverse relationship. The NK target distribution may be a direct consequence of the NK activity. Thus, in organs with high NK activity (spleen, peripheral blood, lymph node) or in individual mice of high reactive age or genotype, the natural target could simply be eliminated by the cytolytic effect of the NK cells, a process which would be enhanced by activation of the NK cells with tilorone. It is equally possible, however, that the NK-sensitive thymocytes and NK cells lie on different branches of a single differentiation pathway and thereby compete for a common progenitor. A third alternative is that

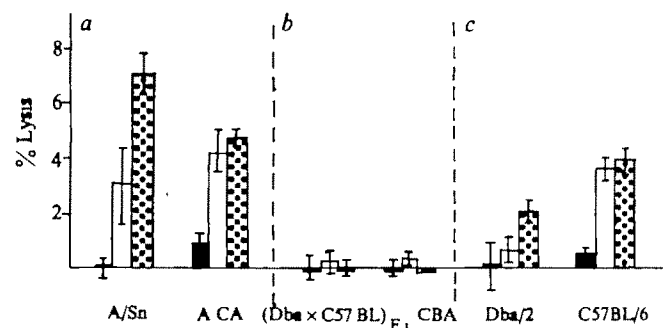


Fig. 3 Strain distribution of thymocyte sensitivity for NK lysis and its inverse correlation with genetically controlled NK reactivity. *a*, NK-low reactive strains; *b*, NK-high reactive strains; *c*, NK-intermediately reactive strains. Thymocytes were pooled from three mice at 3 weeks of age and used as target cells in a 3 h NK assay. Bar values represent the mean % lysis of triplicate samples $\pm$ s.e.m. Nylon wool-passed spleen cells from A/Sn (■), C57BL/6 (□), and CBA (▨) mice were used as a source of NK cells at an effector to target cell ratio of 10:1. These mouse strains were previously classified as to their genetically controlled NK reactivity¹.

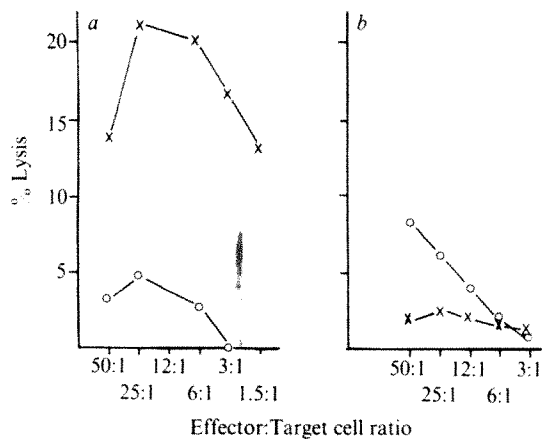


Fig. 4 Increased NK reactivity (b) and decreased NK sensitivity of thymocyte targets (a) following administration of the interferon inducer tilorone. Two-week-old A/Sn mice were each given 0.8 mg tilorone orally and 14 h later thymocytes from treated (○) or sham treated (×) littermates were tested as target cells in a 3 h cytolytic assay with nylon wool-passed CBA spleen cells as effectors (a). The total number of thymocytes recovered from tilorone-treated or control mice was 65 and 60×10^6 , respectively, indicating that the NK-sensitive population had not been eliminated by a cortisone-mediated effect¹⁶, which would have caused a marked cellular depletion of the thymus. b, increased NK reactivity in spleen cells from the same tilorone-treated (○) and control (×) mice as tested in a standard 3 h NK assay against the YAC-1 tumour target. The % lysis values are low as these mice are below optimum NK age (Fig. 2).

NK activity and the frequency of natural targets may both be regulated by a common extrinsic agent such as interferon. Trinchieri *et al.*¹⁴ have demonstrated that interferon can have opposing effects of increasing NK activity and decreasing target sensitivity for NK lysis. However, as the role of interferon in the age and genotype regulation of NK cells is not yet established, the two former explanations may be more likely. Preliminary results indicate that the NK-sensitive targets belong to the immature, cortisone-sensitive subpopulation of thymocytes. As NK cells are present in the bone marrow but not in the thymus, we will test the hypothesis that these 'precursor' T cells are destroyed in the marrow before, or during, their migration to the thymus.

In summary we have found an NK-sensitive subpopulation of T cells in the thymus. The frequency of these natural targets was inversely correlated to the level of NK reactivity in the thymocyte donor as judged by four separate criteria of organ distribution, age dependence, genotype variation and the effect of interferon inducers. Note that T-cell lymphomas in particular seem to be the most sensitive tumours for NK-mediated effects^{2,15}. It is an exciting possibility that the autologous NK target may in fact correspond to the target cell for leukaemic transformation and NK cells therefore may have a central role as a homeostatic control mechanism for this potentially neoplastic population.

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Activation of complement by cytoskeletal intermediate filaments

INTEREST in cytoplasmic intermediate (10 nm) filaments has grown since the successful immunohistochemical differentiation of these filaments from microtubules and microfilaments^{1,2}. Despite the widespread occurrence of intermediate filaments in various cell types, little is known about their function^{1,3,4}. A cytoskeletal nucleus-anchoring role has been suggested based on their intracellular distribution, their connections to the plasma membrane and nucleus, and their marked insolubility in detergents and salt solutions^{5,6}. The presence of autoantibodies against intermediate filaments in the sera of patients^{7,8} suggests that these filaments can be involved in pathogenetic processes. We report here that in studies on possible pathogenetic mechanisms involving intermediate filaments, antibody-independent binding of complement components to intermediate filaments has been observed. Initial observations were made using cultured human embryonal fibroblasts, as cytoplasmic filaments present in these cells have been characterised by using both immunological markers and drugs capable of selectively altering the morphology of microfilaments, intermediate filaments and microtubules^{1,7}. Subsequent studies showed complement binding to similar cytoplasmic structures of other cultured cells and also cells in tissue sections.

Cultured cells were made permeable to serum macromolecules by treatment with the non-ionic detergent NP40 (ref. 9) before incubation with serum. Complement components bound to cellular constituents were detected by incubation with fluorochrome-conjugated antibodies against human C1q, C4 and C3. Antibody conjugates detecting fibrin and immunoglobulins G, M and A served as controls. Direct immunofluorescent staining of fibroblasts incubated with fresh normal human serum revealed strong binding of the C1q subcomponent of C1 and of C4 and C3. A fine fibrillar cytoplasmic distribution of all three complement components was seen (Fig. 1a) using cultured fibroblasts originating from three different human fetuses. No binding of immunoglobulins or fibrin could be demonstrated. There was no staining if the detergent treatment was omitted, nor did staining occur with anti-complement conjugates alone. The complement binding phenomenon thus seemed to be independent of serum antibodies and could be demonstrated using 50 normal human sera without detectable antibodies against fibroblasts. Furthermore, purified C1q¹⁰ bound to cultured fibroblasts in a way similar to C1q of serum. C4 and C3 binding was inhibited in the presence of 10 mM EDTA and EGTA, which inhibit complement activation by chelating Ca^{2+} and Mg^{2+} (ref. 11). Binding of C1q occurred despite inhibition of C4 and C3 binding. The results indicate that C1q binding initiates Ca^{2+} - and Mg^{2+} -dependent complement activation by the classical pathway.

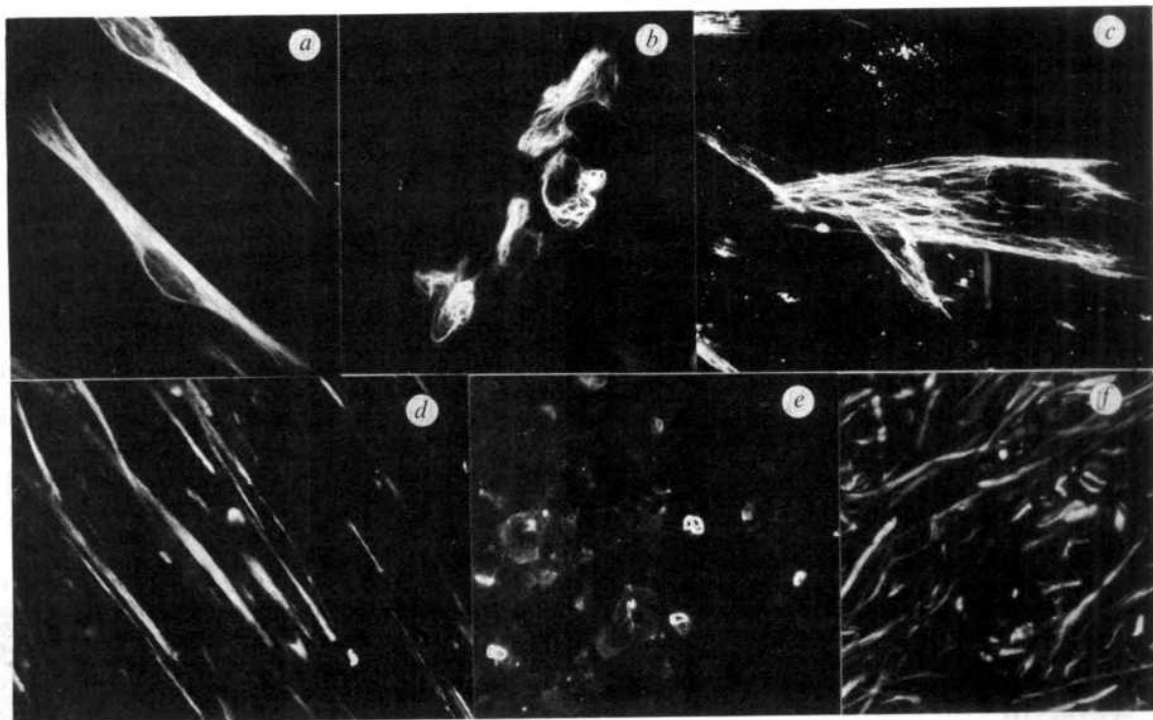


Fig. 1 Direct immunofluorescent staining of cultured cells (a–e) and human fetal connective tissue (f) demonstrating specific binding of complement components to cytoskeletal intermediate filaments. A fine fibrillar cytoplasmic network corresponding to the distribution of intermediate filaments is visualised in cultured human fetal fibroblasts. *a*, The cells were cultivated on coverslips in RPMI 1640 (Grand Island Biological) supplemented with 10% heated fetal calf serum (56 °C, 60 min), fixed in 3.5% phosphate-buffered paraformaldehyde to conserve cellular morphology, and treated with a 0.05% solution of the non-ionic detergent Nonidet P40 (NP40, BDH) to make cells permeable to serum macromolecules⁹. The cells were incubated with normal human serum (diluted 1:10 in saline) for 30 min at room temperature, washed in saline and treated with fluorescein isothiocyanate-conjugated rabbit anti-Clq conjugate (Behringwerke). *b*, Fibroblasts treated with vinblastine sulphate (Lilly, 10 $\mu\text{g ml}^{-1}$) for 4 h were similarly treated with serum and stained for bound Clq. Note typical perinuclear bundles of intermediate filaments decorated by bound Clq. *c*, Fibroblasts treated with 0.1% detergent Triton X-100 (BDH) in saline for 15 min and then extracted with low and high ionic strength extraction buffers LAMES and HAMES⁶ containing 1 mM enzyme inhibitor phenylmethylsulphonylfluoride (Sigma) still exhibited an insoluble network of intermediate filaments attached to the substratum. This network retained the ability to bind complement. Binding of complement component C3 demonstrated after staining with TRITC-anti-human C3c (Behringwerke). *d*, Fibroblasts depleted of culture medium become permeable to serum constituents and exhibit irregular binding of Clq to intermediate filaments without pretreatment with detergent. Treatment otherwise as described in *a*. *e*, After incubation with serum, lymphoma cells K562 exhibit binding of complement component Clq to perinuclear fibrillar structures very similar to intermediate filaments in fibroblasts treated with microtubule disrupting drugs (*b*). *f*, Binding of complement components to cytoplasmic fibrils of fetal human mesenchymal cells was similar to the binding observed with cultured fibroblasts. Cryostat section of dermal connective tissue was treated as described in *a*. Magnifications $\times 500$.

The cytoplasmic structures binding Clq were readily identified. The localisation of bound Clq was indistinguishable from that produced by antibodies against intermediate filaments but was clearly distinct from that seen with antibodies against actin containing microfilaments or against microtubules^{3,7}. A typical change in the complement binding pattern, consistent with reorganisation of intermediate filaments³, was achieved by treating fibroblast cultures with the microtubule disrupting drugs, demecolchicine and vinblastine⁷ (Fig. 1*b*). Disruption of microfilaments³ with cytochalasin B did not alter the pattern of complement binding.

Intermediate filaments bound complement even after the cells had been subjected to treatment with non-ionic detergent Triton X-100 and subsequent extraction with high and low ionic salt solutions^{5,6} (Fig. 1*c*). Earlier studies have shown that these treatments leave only intermediate filaments in the cell residues, microfilaments and microtubules having been disrupted⁵. This further confirmed that Clq is bound to intermediate filaments and not to other cytoskeletal structures. Intermediate filaments able to bind complement were also demonstrated in nonviable fibroblasts permeable to serum proteins without detergent treatment, in cultures depleted of nutrient medium for 24 h (Fig. 1*d*). Preliminary studies have shown that cultured cells such as glia and glioma MG-251 cells bind complement in a similar way to fibroblasts, whereas perinuclear coiling bundles were observed in lymphoma cells K562 (ref. 12) (Fig. 1*e*). Further studies indicated that complement binding is not a property unique to cultured cells; this was shown most clearly in cryostat sections of fetal connective tissue (Fig. 1*f*).

The results suggest that Clq binding and complement activation through the classical pathway by intermediate filaments of different cell types is an antibody-independent recognition mechanism. Antibody-independent activation of Clq seems to be physiologically important and has been shown to operate against bacterial lipopolysaccharides¹³, virus-infected cells¹⁴, parasites¹⁵ and autologous cellular constituents such as heart subcellular membranes¹⁶, erythrocyte membranes¹⁷ and DNA¹⁸. Clq binding to intermediate filaments followed by classical complement pathway activation and accumulation of inflammatory cells¹¹ may be an elimination mechanism for poorly soluble cytoskeletal residues of damaged cells. It is tempting to speculate that failure of this mechanism results in chronic inflammatory reactions or extra-cellular deposition of intermediate filament residues in the form of morphologically and immunologically identical hyaline¹⁹ or amyloid^{20,21}.

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Diminished microtubules in fibroblast cells derived from inherited dystrophic muscle explants

MUSCULAR DYSTROPHY of the Duchenne type is inherited in a recessive, sex-linked fashion. The aetiology of the disease is still unclear but biochemical and morphological studies strongly suggest a cell surface membrane abnormality in Duchenne muscular dystrophy¹⁻⁷. Even though there are artefacts due to surface condensation of water, freeze-fracture studies of skeletal muscle from patients with Duchenne dystrophy show a depletion of intramembrane particles in the protoplasmic and environmental faces of the muscle plasma membrane^{8,9}. In contrast, Ketelsen's freeze-fracture studies indicated that the number of particles in the inner membrane fracture face in muscular dystrophy increased two- or threefold beyond normal¹⁰. In other studies, it was demonstrated that patients and carriers of Duchenne muscular dystrophy exhibited diminished cap formation in their lymphocytes^{11,12}. Although these experiments clearly support a membrane defect in muscular dystrophy, whether this is related to the aetiology of the disease is not yet clear. Several of these studies indicate that this membrane defect is an early and perhaps primary one in the aetiology of Duchenne's muscular dystrophy⁷. Even though the primary lesion in muscular dystrophy is usually thought to be

myogenic or neurogenic the reported abnormalities in the lymphocyte^{11,12} and erythrocyte membrane¹³ indicate that the expression of muscular dystrophy may not necessarily be restricted to these tissues. There have been many studies on the dynamic interrelationships between cytoplasmic structures and the cell surface^{14,15} and models have been proposed which suggest that changes in the state of the cytoplasmic microfilaments and microtubules can alter the mobility and distribution of membrane surface receptors¹⁶. In an attempt to determine if the membrane defect in muscular dystrophy might be correlated with a cytoskeletal alteration, we have studied the distribution of cytoplasmic microfilaments (actin) and microtubules (tubulin) in fibroblasts derived from muscle explants of both normal and dystrophic inbred chickens using indirect immunofluorescence methods with monospecific antibodies directed against actin and tubulin. We report here that fibroblasts from explants of cardiac and skeletal muscle of dystrophic chickens show a dramatic reduction in the appearance of intact interphase microtubules compared with those from normal chickens.

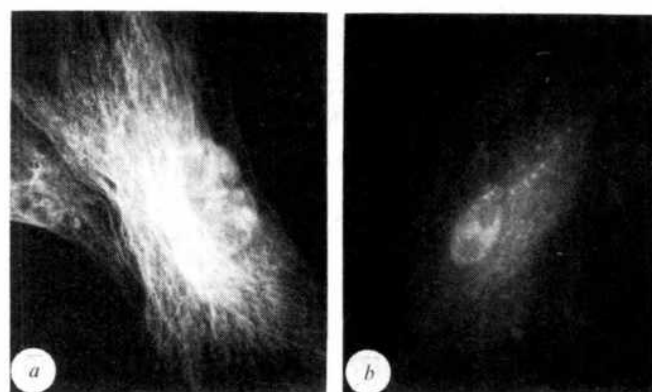


Fig. 2 Cells derived from normal cardiac explants (a) of a 7-d-old chicken possess full cytoplasmic microtubule complexes, whereas similar cells obtained from dystrophic cardiac muscle (b) possess diminished cytoplasmic microtubule complexes. Tubulin antibody stain. Scale bar, 10 μ m.

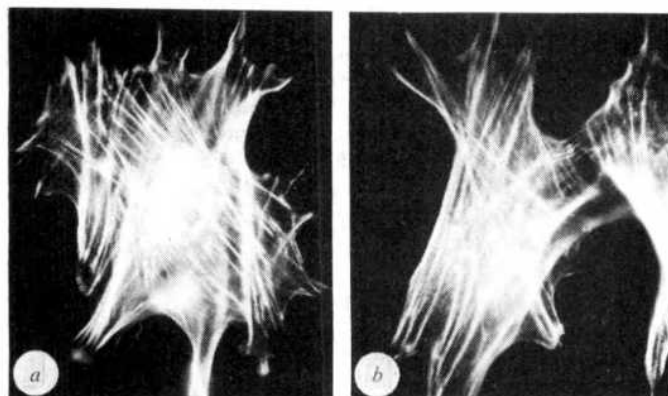


Fig. 1 Cells derived from cardiac explants obtained from 7-d-old normal (a) and dystrophic (b) chickens exhibit no apparent difference in morphology or distribution of cytoplasmic actin stress fibres. Actin antibody stain. Scale bar, 10 μ m.

In our initial studies, we chose muscle explants from fertilised eggs and newborn chickens. The birds of line 413 are characterised by the onset of inability to right themselves about 3 weeks after hatching. The dystrophic chicken lines are considered to be a good model for human dystrophy because the progressive developmental onset of the disease resembles that seen in Duchenne's dystrophy. Line 413 birds are similar to the line 304 birds from which they were derived with regard to muscle fibre diameter, cytochemistry, acetylcholinesterase (AChE) levels, electromyography and serum enzymes¹⁷.

Small pieces (<1 mm³) of cardiac and skeletal muscle were placed on glass coverslips in 35-mm Petri dishes and allowed to attach firmly for 10 min before addition of 2 ml CMRL-1066 culture medium (Gibco) containing 10% horse serum and 5% fetal bovine serum. After 7 days incubation, a monolayer of cells grew out from these muscle explants which seemed morphologically to be primarily fibroblasts. In similar studies it has been reported that the cells which grow out from these muscle explants may in fact be aberrant precursors of myoblasts or an abnormal form of fibroblastic connective tissue¹⁸. The coverslips with attached fibroblast cells were rinsed in phosphate-buffered saline (PBS), fixed in 3% formaldehyde-PBS for 20 min, and then extracted in acetone at -10 °C for 7 min. The cells were rehydrated from acetone in PBS and incubated with the antibody (0.1 mg ml⁻¹ for anti-actin; 0.05 mg ml⁻¹ for anti-tubulin) for 45-60 min at 37 °C. Following four 15-min rinses in PBS, the cells were incubated in fluorescein-conjugated goat anti-rabbit IgA (Mely) diluted 1:6 with PBS. Finally, they were rinsed

Table 1 Presence of cytoplasmic microtubule complex in different muscle types

Muscle tissue type	No. of animals used	Age of animal	No. of cultures counted	No. of cells +CMTC	No. of cells -CMTC	Total no. of cells	% +CMTC
Normal cardiac	6	12-d embryo	8	577	203	800	72%
Dystrophic cardiac	5	12-d embryo	7	471	231	702	67%
Normal skeletal	6	12-d embryo	8	545	245	800	68%
Dystrophic skeletal	5	12-d embryo	7	507	193	700	72%
Normal cardiac	6	1-d chick	8	636	164	800	80%
Dystrophic cardiac	5	1-d chick	7	247	453	700	35%
Normal skeletal	6	1-d chick	8	583	235	818	71%
Dystrophic skeletal	5	1-d chick	7	238	462	700	34%
Normal cardiac	6	14-d chick	8	650	150	800	81%
Dystrophic cardiac	5	14-d chick	9	256	644	990	28%
Normal skeletal	5	14-d chick	8	650	150	800	81%
Dystrophic skeletal	5	14-d chick	9	229	671	900	25%

+CMTC, full cytoplasmic microtubule complex; -CMTC, diminished cytoplasmic microtubule complex. Normal control cardiac and skeletal fibroblast cells possess 60–80% +CMTC regardless of the age of the animal. The remaining control cells with a -CMTC are accounted for by those that are in mitosis, or other phases of the cell cycle in which the +CMTC is not apparent. Also, the cell population is not homogeneous and other cell types not containing full CMTC may be present. Fibroblast cells derived from dystrophic cardiac and skeletal muscle explants exhibit a loss in organisation of the cytoplasmic microtubules after hatching of the chick. This reduction of organisation and complexity of the CMTC becomes strikingly evident in the 14-d-old dystrophic chick.

four times for a total of 60 min and mounted on glass slides in a drop of glycerol: PBS (9:1 by volume at pH 9.5–10.0)¹⁹.

Figure 1 shows fibroblasts derived from normal and dystrophic cardiac explants obtained from 7-d-old chickens. These cells were stained with anti-actin to show the distribution of actin microfilaments and, as can be seen in Fig. 1, no differences are apparent. Figure 2 also shows fibroblasts derived from normal and dystrophic cardiac explants obtained from 7-d-old chickens which were studied for their microtubule distribution. The normal cells (Fig. 2a) contain a full complex of microtubules while the dystrophic cells (Fig. 2b) contain a diminished complex of microtubules. Figure 3 illustrates fibroblasts derived from normal and dystrophic skeletal muscle treated identically to those in Fig. 2. These observations indicate that there is a dramatic alteration in the distribution of intact interphase microtubules in cells derived from dystrophic tissue.

These differences in the distribution of microtubules cannot be accounted for on the basis of membrane impermeability to the tubulin antibody in the dystrophic cells for the following reasons: first, in the experiments with antibodies against actin, both the normal and dystrophic cells stained identically; second, the microtubules in the dystrophic cells are only diminished during interphase. The dystrophic cells grow at approximately the same rate as the normal cells and mitotic spindles composed primarily of microtubules seem to be identical by immuno-

fluorescence. Also, recent observations have indicated that rounded and flattened cell morphologies are variable in the expression of microtubules²⁰, but in this case, both the normal and dystrophic cells maintain a flattened morphology and thus, the diminished complex of microtubules in dystrophic fibroblasts does not seem to be related to overall cell shape. These results are consistent with a defect in the control of expression of interphase microtubule organising centres.

Other possible interpretations of these findings can be extrapolated from the recent work of others^{18,19}. Dise *et al.*²¹ reported results which indicated that an alteration occurs in the regulation of erythrocyte membrane function by calcium in human muscular dystrophy and Irish *et al.*²² have reported an alteration in the calcium binding component of the troponin complex (troponin-C) in avian muscular dystrophy. As microtubules are sensitive to intracellular calcium levels²³, our results could be interpreted to support a calcium alteration in muscular dystrophy.

In an attempt to analyse these findings further, a study of various stages of development was undertaken. As can be seen in Table 1, the diminished complex of microtubules is not present in fibroblasts derived from explanted cardiac and skeletal muscle obtained from embryonic material. Explants from 1-d-old dystrophic chickens show the first indication of loss of microtubules and this reduction is maximised approximately 2 weeks after hatching. These studies indicate that diminished microtubules in fibroblasts derived from inherited dystrophic muscle explants may be a progressive developmental defect and studies are now in progress to analyse the microtubules in lymphocytes and platelets from carriers and individuals with Duchenne's muscular dystrophy.

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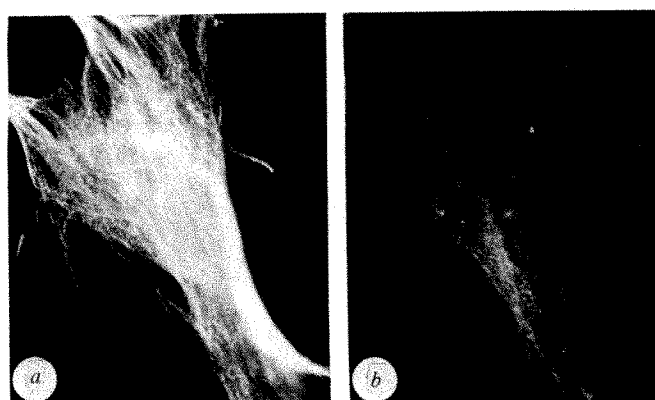


Fig. 3 Cells derived from normal skeletal muscles (a) of a 7-d-old chicken contain a full complex of cytoplasmic microtubules, whereas the cells obtained from the dystrophic skeletal muscle (b) possess diminished cytoplasmic microtubule complexes. These cells were prepared for immunofluorescence identically to those in Fig. 2. Scale bar, 10 μ m.

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Possible role of retinoic acid binding protein in retinoid stimulation of embryonal carcinoma cell differentiation

RETINOIDS, naturally occurring and synthetic analogues of vitamin A, are involved in the maintenance and differentiation of epithelial tissue^{1–3}. Furthermore, it has been reported that these compounds inhibit the growth of certain nontransformed and virally and chemically transformed cell lines^{4,5}. Recently, it has been shown that retinoic acid stimulates the differentiation of embryonal carcinoma cells^{6,7}, undifferentiated early embryonic-like cells present in teratocarcinomas. Embryonal carcinoma cell lines differ widely in their capacity to differentiate: a distinction has been made between 'pluripotent' and 'nullipotent' cell lines^{8,9}. In contrast to the latter, pluripotent embryonal carcinoma cells differentiate when injected into mice⁸ or when cell aggregates are formed *in vitro*¹⁰; some cell lines differentiate quite readily even when grown in monolayer culture. We have shown⁷ that retinoic acid not only stimulates the differentiation of pluripotent embryonal carcinoma cell lines, but also induces differentiation of the so-called nullipotent embryonal carcinoma cell lines nulli SCC1 and 6050 AJ. We show here that this effect is not limited to retinoic acid but that several other retinoids have the ability to stimulate differentiation of at least one embryonal carcinoma cell line, PCC4.aza1R. Furthermore, we present evidence to suggest that the action of retinoids may be mediated by a retinoic acid binding protein.

PCC4.aza1R embryonal carcinoma cells do not differentiate when maintained in the exponential phase or when grown to confluency; however, when cell aggregates are formed in a bacteriological dish and transferred to a tissue culture dish, outgrowth of differentiated cells is observed after a 2-d incubation period^{7,10}. The differentiated cells can be distinguished from PCC4.aza1R cells by their morphology and the production of plasminogen activator: outgrowths of PCC4.aza1R aggregates containing differentiated cells secrete plasminogen activator as indicated by the fibrin-agar overlay technique¹¹, whereas those containing no differentiated cells do not. The proportion of PCC4.aza1R aggregates giving rise to differentiated cells depends on the duration of incubation of the cell aggregates in bacteriological dishes. In standard conditions, at least 7 d of incubation are required before any PCC4.aza1R aggregates will produce differentiated cells; however, in the presence of 10^{-5} M retinoic acid, 100% of 1-day-old aggregates give rise to

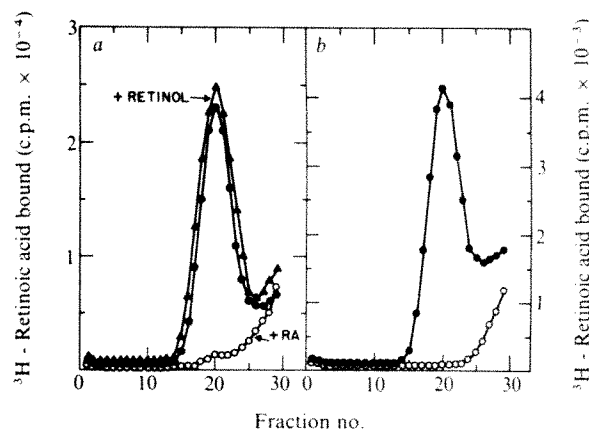


Fig. 1 Sucrose gradient analyses of the binding of ^3H -retinoic acid to cytosol and nuclear supernatants of PCC4.aza1R cell homogenates. Cells grown in monolayer culture in Dulbecco's modified Eagle's medium were collected and washed three times in phosphate-buffered saline (PBS). After resuspension in 10 mM Tris-HCl buffer (pH 7.4) containing 2 mM MgCl_2 and 7 mM β -mercaptoethanol, cells were homogenised with a Dounce homogeniser. Lysed cells were centrifuged for 5 min at 1,000 g. The supernatant was collected and centrifuged at 110,000g for 1 h. The resulting supernatant is referred to as the cytosol fraction. The nuclear pellet was resuspended in 0.32 M sucrose plus 3 mM MgCl_2 and layered at the top of 1.7 M sucrose and centrifuged at 22,000 r.p.m. for 1 h in a SW27 rotor. The resulting pellet contains purified nuclei. The nuclei were homogenised by sonication and centrifuged at 110,000g for 1 h. The resulting supernatant is referred to as the nuclear supernatant. *a*, The cytosol supernatant was incubated with 5×10^{-8} M ^3H -retinoic acid ($30.9 \text{ Ci mmol}^{-1}$) alone (●), ^3H -retinoic acid plus 5×10^{-6} M unlabelled retinoic acid (○) or unlabelled retinol (▲). Incubation mixtures were subjected to sucrose gradient centrifugation on linear 5–20% (w/v) sucrose gradients in PBS for 18 h at 220,000g. Fractions were collected and analysed for tritium content with a liquid scintillation spectrometer. *b*, Sucrose gradient analyses of nuclear supernatant of PCC4.aza1R cells incubated with ^3H -retinoic acid (○) and nuclear supernatant prepared after intact cells were incubated with 10^{-6} M ^3H -retinoic acid for 2 hr at 37°C (●). Bovine serum albumin (68,000), ovalbumin (43,000) and myoglobin (17,000) sedimented in separate gradients at fraction numbers 3, 5, and 20, respectively.

differentiated cells^{7,10}. Using the aggregation procedure, we tested several analogues for their ability to stimulate differentiation of PCC4.aza1R cells (Table 1). In the control, (in the absence of retinoids), none of the PCC4.aza1R aggregates produce differentiated cells whereas in the presence of retinoic acid (no. 4) 100% of the aggregates differentiate. In general, the acid analogues are the most potent stimulators, although some (nos 12 and 16) are inactive.

A retinoic acid binding protein has recently been detected in the cytosol and nuclei of several tissues^{12–15}, and we have tested for the presence of such a protein in embryonal carcinoma cells. The 110,000g supernatant fraction of PCC4.aza1R cells readily binds ^3H -retinoic acid, demonstrating on sucrose gradient analysis a single, symmetrical receptor peak with a sedimentation coefficient of approximately 2S (Fig. 1a). A similar binding protein is observed in the cytosol of embryonal carcinoma cell lines OC 15 and nulli SCC1 (unpublished observations). Unlabelled retinoic acid competes with ^3H -retinoic acid for the binding site but retinol does not (Fig. 1a). Retinoic acid has a high affinity for the binding protein; an apparent dissociation constant of 2.3×10^{-8} can be calculated from the data in Fig. 2. No retinoic acid was detectable when isolated nuclei were homogenised and the supernatant fraction incubated with ^3H -retinoic acid. However, binding of retinoic acid was observed in nuclear fractions prepared from cells preincubated with ^3H -retinoic acid (Fig. 1b). This situation is very similar to that of steroid hormone receptors¹⁶, where nuclear binding is only detectable after hormone-receptor interaction in the cytoplasm.

Table 1 Comparison of the capacity of retinoids to stimulate the differentiation of embryonal carcinoma cells with their ability to compete for the binding site of the retinoic acid binding protein

Basic chemical structure	R	No.	% of aggregates differentiating			Competition for binding site
			10^{-5} M	10^{-6} M	10^{-7} M	
	CH ₂ OH	1	T	0	0	—
	CH ₂ OCH ₃	2	0	0	0	—
	CHO	3	50	0	0	—
	COOH	4	100	100	100	+
	COOCH ₃	5	0	0	0	—
	CH ₂ OCOCH ₃	6	100	10	0	—
	CH ₂ OH	7	0	0	0	—
	CH ₂ OCH ₃	8	0	0	0	—
	COOH	9	100	100	100	+
	COOC ₂ H ₅	10	0	0	0	—
	COOH	11	100	100	100	+
	COOH	12	0	0	0	—
	COOH	13	100	80	0	+
	COOC ₂ H ₅	14	0	0	0	—
	COOH	15	100	100	100	+
	COOH	16	0	0	0	—

Embryonal carcinoma cells (10^5 cells per dish) were added to Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 U ml^{-1} each of penicillin and kanamycin and $100 \mu\text{g ml}^{-1}$ streptomycin in a bacteriological dish (6 cm diameter). In these conditions cells do not adhere to the dish but proliferate and form aggregates. After 48 h incubation, aggregates were transferred to a tissue culture dish containing complete medium supplemented with the indicated retinoid dissolved in ethanol or with ethanol alone in the control (0.15% v/v final concentration). The aggregates attach to the dish and grow out. After 2 d of incubation the percentage of aggregates containing differentiated outgrowths was determined either on the basis of morphology or plasminogen activator production by the fibrin-agar overlay method¹¹. These criteria correlated very well with each other. Differentiation in control conditions (in the absence of retinoids) was zero. Analysis of competition for the binding site was carried out by incubating the $110,000g$ supernatant fraction, obtained as described in the legend to Fig. 1, with $5 \times 10^{-8} \text{ M } ^3\text{H-retinoic acid}$ ($30.9 \text{ Ci mmol}^{-1}$) plus 100-fold excess of the indicated retinoid for 1 h at 4°C . Competition was determined by sucrose gradient analysis as described in the legend to Fig. 1. Analogues 1 and 9 are retinol and retinoic acid, respectively; + and — indicate, respectively, the analogue's ability and inability to compete for the binding site. T, toxicity.

To investigate the possibility that the observed binding protein might be involved in the action of retinoids on embryonal carcinoma cells, the ability of the various retinoids to stimulate

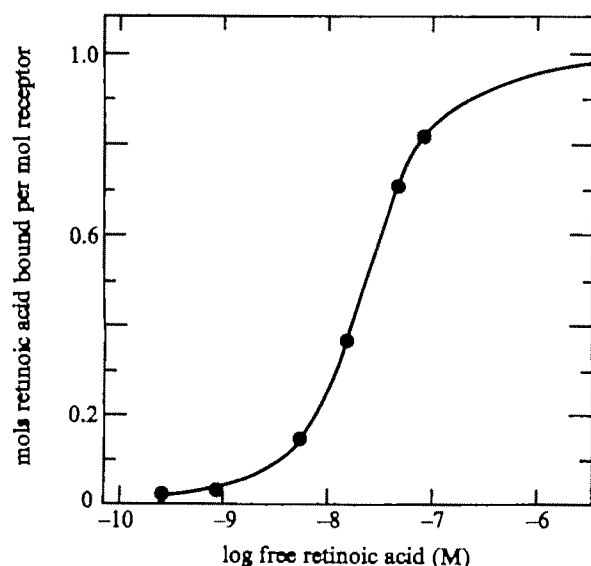


Fig. 2 Plot of $^3\text{H-retinoic acid}$ binding by PCC4.aza1R cytosol as a function of retinoic concentration. Binding assays were carried out as described in Fig. 1. The dissociation constant was determined according to the procedure described by Wiggert *et al.*¹⁴ and represents an average of the dissociation constants of all binding sites in the 2S fraction.

differentiation of PCC4.aza1R cells was compared with their capacity to compete with $^3\text{H-retinoic acid}$ for binding sites on the retinoic acid binding protein. Table 1 shows that these two activities correlate well with each other. Retinyl acetate seems to be an exception because it does not compete for the binding site but is moderately active; however, this might be due to metabolism of the retinyl acetate into an active analogue. Modification of the cyclohexene ring of retinoic acid often does not interfere with the ability of the analogue to stimulate differentiation or to compete for the binding site, whereas substitution of the carboxylic acid group affects these activities severely. This suggests that the carboxylic acid group plays an important part in the interaction of these compounds with the binding protein.

The results presented here show that the induction of differentiation of embryonal carcinoma cells is not limited to retinoic acid but that several other analogues are also active. This activity correlates well with the ability of these compounds to compete for the binding site of the retinoic acid binding protein and suggests that their action on differentiation may be mediated by this binding protein. The results on the binding of retinoic acid to nuclear fractions indicate a similarity with the binding of steroid hormones to their receptors¹⁶ and suggest that retinoids may act as a mechanism similar to that of steroid hormones, that is, that complexes of retinoic acid and binding protein are transported into the nucleus, whence they modify gene transcription. However, on the basis of their observations that retinoids are involved in glycosylation reactions, De Luca *et al.*¹⁷ have proposed that retinoids may act through an altered biosynthesis of specific glycosylated intermediates. This hypothesis cannot as yet be ruled out.

Several of the retinoids which are potent stimulators of differentiation of embryonal carcinoma cells are also very effective in reducing the occurrence and retarding the growth of certain tumours^{3,18-20}. The test described above might be useful as an *in vitro* screening procedure to identify those compounds with potential anti-tumour activity.

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Folate deficiency and decreased brain 5-hydroxytryptamine synthesis in man and rat

FOLATE deficiency was once considered to belong exclusively to the field of haematology. However, recently there have been reports of neuropsychiatric symptoms in folate-deficient patients¹⁻⁴. Among the most frequent symptoms are mild polyneuropathies, fatigue, mild depression and abnormal intellectual functioning. These symptoms, which can occur in the absence of any severe haematological changes at routine haematological testing, are responsive to folate therapy. Excess folate can also produce undesirable side effects. One study on the effect of pharmacological doses of folate (15 mg daily) had to be abandoned after 1 month because of side effects such as altered sleep patterns, malaise, irritability and overactivity⁵; the

same clinical complaints were found in some of our patients who received folic acid therapy and in whom folate levels were very high (M. I. B., unpublished data). In studies on rats, behavioural deficits were found in animals fed diets that were either deficient⁶ or over-supplemented⁷ with folate. Both active and passive avoidance behaviour was affected. In this study we have looked for biochemical changes associated with folate deficiency and over-supplementation. Because the regional distribution of 5-methyltetrahydrofolate in the brain is similar to that of 5-hydroxytryptamine (5-HT)⁸ we looked at the latter. We found that the concentration of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) is low in the lumbar cerebrospinal fluid (CSF) of folate-deficient patients who have folate-responsive neuropsychiatric symptoms. We also found low brain 5-HT levels in the brains of rats fed folate-deficient or over-supplemented diets. Thus, there is a close association between folate-dependent behavioural effects and low brain 5-HT.

Table 1 gives CSF data for controls and for patients with folate deficiency. All the folate-deficient patients had neuropsychiatric symptoms such as mild polyneuropathies, fatigue and mild depression and were selected according to diagnostic criteria described previously^{2,3}. The folate deficiency was caused by a long-lasting folate-deficient diet with or without decreased absorption of folate due to gastrointestinal disorders. The low levels of 5-HIAA in the CSF of the folate-deficient patients were not due to deficiency of precursor for 5-HT synthesis, as CSF tryptophan, which to a certain extent reflects the brain level⁹, was normal.

In two previous studies, one on psychiatric patients¹⁰ and one on patients with senile dementia¹¹, folate deficiency was not found to be associated with low CSF 5-HIAA. In a third study, anticonvulsant treatment was found to be associated with low CSF folate and high CSF 5-HIAA¹². The high CSF 5-HIAA was probably due to increased precursor availability, as CSF tryptophan was also high. The elevated tryptophan and 5-HIAA levels were clearly associated with blood levels of anticonvulsants which were within or above the therapeutic range. In the first two of these studies, CSF folate was measured in groups of patients, who were then divided into normal and low-folate groups for comparison of CSF amine metabolite levels. In the third study, the patients were grouped according to whether or not they were on anticonvulsant medication. Low folate levels are not usually associated with neuropsychiatric symptoms which are responsive to folate therapy^{1,2} so it is unlikely that there were many patients in these studies with folate-responsive signs. On the other hand, in our study, where folate deficiency is associated with low CSF 5-HIAA, the low-folate patients were selected on the basis of behavioural and neurological symptoms which respond to folate administration. This suggests that low CSF 5-HIAA is associated not so much with folate deficiency itself as with neuropsychiatric effects of folate deficiency, which are responsive to folate therapy.

Table 1 Folate, tryptophan and 5-HIAA in controls and folate-deficient patients

		Controls		Folate-deficient patients		P
		Mean $\pm$ s.e.	n	Mean $\pm$ s.e.	n	
Serum folate (ng ml ⁻¹)	L. Casei method	7.48 $\pm$ 1.61	5	3.26 $\pm$ 0.73	6	<0.025
	Radioactive method	6.11 $\pm$ 0.51	9	2.41 $\pm$ 0.35	7	<0.005
CSF folate (ng ml ⁻¹)	L. Casei method	22.3 $\pm$ 1.9	9	11.3 $\pm$ 1.6	7	<0.005
	Radioactive method	19.7 $\pm$ 1.3	9	8.8 $\pm$ 1.3	7	<0.005
CSF tryptophan (ng ml ⁻¹)		362 $\pm$ 57	9	280 $\pm$ 61	7	NS
CSF 5-HIAA (ng ml ⁻¹)		20.9 $\pm$ 2.4	8	10.3 $\pm$ 2.3	7	<0.005

CSF was taken by lumbar puncture in a sitting position at 9.00 a.m. Controls were patients suffering from neuropsychiatric disorders not known to affect brain amine metabolism and who had CSF taken for diagnostic purposes. The characteristics of the folate-deficient patients are described in the text. There was no significant difference between the ages of the control patients (mean $\pm$ s.d. 38.6 $\pm$ 16.4 yr) and the folate-deficient patients (50.0 $\pm$ 10.1 yr). The methods for determination of folate have been described previously². Tryptophan and 5-HIAA in CSF were measured by the method of Anderson and Purdy¹⁹.

Brain amine levels in rats given folate-deficient or over-supplemented diets are given in Table 2. Experimental conditions were exactly the same as those in earlier experiments in which folate deficiency⁶ and over-supplementation⁷ were found to cause deficits in active and passive avoidance behaviour. In both groups, noradrenaline and dopamine were unchanged, whereas 5-HT was low. These data strengthen the association between alterations of behaviour and brain 5-HT in folate deficiency and suggest that excess folate may have biochemical effects that are in some respects similar.

The mechanism of the effect of folate on brain 5-HT metabolism is unknown. The fact that the distribution of 5-HT in brain is similar to that of 5-methyltetrahydrofolate⁸ suggests that a methylation reaction plays an important part, but in spite of the varied metabolic actions of folate in brain¹³ there are no methylation reactions known to alter the brain 5-HT content. However, there is one other possible mechanism that could be tested. In rats folate deficiency can induce thiamine deficiency¹⁴ and thiamine deficiency is frequently found in folate-depleted patients¹⁵. Recently, it has been shown that rats on a thiamine-deficient diet develop an impairment of the cerebellar 5-HT system¹⁶. Thus, the influence of folate deficiency on 5-HT may be mediated by thiamine deficiency. However, another mechanism must be invoked to explain the effects of excess folate. Here, one possibility is an effect on the functional activity of tryptophan hydroxylase, the rate-limiting enzyme in 5-HT synthesis; this enzyme requires tetrahydrobiopterin (BH₄) as cofactor. The last step in *de novo* synthesis of BH₄ is the reduction of BH₂ and this step is carried out by tetrahydrofolate reductase¹⁷. Excess folate might competitively inhibit this enzyme, thus preventing formation of new BH₄ and decreasing the activity of tryptophan hydroxylase.

Table 2 The effect of folate on rat brain biogenic amine content

Diet	5-HT	Dopamine	Noradrenaline
Purina	342 ± 11	897 ± 32	634 ± 35
Control (folate deficient + 2 µg folate per g diet)	346 ± 26	954 ± 36	623 ± 20
Folate deficient	242 ± 36*	945 ± 42	702 ± 37
Folate excess (folate deficient + 100 µg folate per g diet)	238 ± 36*	990 ± 37	672 ± 71

Charles River CD rats (60–70 g) were randomly divided into 4 groups and placed on the diets shown above for 3 weeks. To avoid diurnal changes in brain amine content the animals were all killed in the morning. Brain amine content was measured by liquid chromatography with electrochemical detection (S.N.Y., unpublished method). A brain extract was prepared according to the method of Ahtee *et al.*²⁰ except that the brains were homogenised in the presence of epinine, which was used as an internal standard. The sample preparation involves partial purification of the amines on a column of Amberlite CG 50. The amines are eluted from the column with 3 ml of 1.2 M HCl and 0.05 ml of the eluate was injected directly into the chromatograph (model LC-40; Bioanalytical Systems). Chromatography was carried out on a 2.1 mm × 50 cm column of VYDAC SCX pellicular resin with a buffer 50 mM in both acetate and citrate at pH 5.1. The amines were detected using a carbon paste electrode set at 0.8 V against the Ag/AgCl reference electrode. The amines were eluted in the order noradrenaline, dopamine, epinine, 5-HT. At a flow rate of 0.6 ml min⁻¹ each run took 20–25 min. All values are given as the mean of 10 determinations ± s.e. in ng ml⁻¹.

*Animals treated with diets containing no or excess folate have significantly less 5-HT than control animals ($P < 0.05$).

Although the mechanisms of these effects must remain speculative at the moment, we have shown that a vitamin deficiency can decrease synthesis of a neurotransmitter and influence behaviour in man. Folate deficiency and excess cause similar behavioural and biochemical changes in rats. In humans, the behavioural effects of these two conditions have some symptoms in common such as irritability and altered sleep patterns. Whether folate excess decreases brain 5-HT synthesis in man is unknown, but our data suggest that excess of even a water-

soluble vitamin such as folate can have detrimental effects. The data on folate deficiency is of interest in view of the relatively high incidence of folate deficiency in psychiatric patients¹⁸. In some patients decreased 5-HT levels due to folate deficiency may be an important cause of psychiatric symptoms.

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Fibrin ϵ -(γ -glutamyl)lysine crosslink acceleration by red blood cells

FIBRIN, the skeletal protein of thrombi, is derived from its soluble plasma precursor, fibrinogen. Fibrinogen has a molecular weight of ~340,000. It is composed of three pairs of subunit polypeptide chains, A α , B β , and γ , of molecular weights 68,000, 56,000 and 49,000, respectively, joined by disulphide bonds. The serine protease thrombin releases fibrinopeptides A and B from the amino terminal end of the A α - and B β -chains of fibrinogen to leave the α - and β -chains of fibrin monomer. Fibrin monomers then align by noncovalent interactions to give a fibrin polymer gel. Under the influence of thrombin-activated factor XIII (factor XIIIa, activated fibrin stabilising factor, plasma transglutaminase) and calcium ion, covalent ϵ -(γ -glutamyl)lysine crosslinks are rapidly formed between γ -chains of adjacent fibrin monomers to give the γ -dimer subunit of partially crosslinked fibrin¹. The γ -crosslinking transforms the clot into a covalently bonded polymer, rendering it insoluble in 3 M urea and other agents that disrupt noncovalent interactions². Extensive crosslinking of the α -chain to give the α -polymer subunit of fully crosslinked fibrin requires several hours in plasma and confers relative plasmin resistance to the clot³. We report here an increased rate and extent of α -crosslinking when red blood cells are present in the fibrin clot.

Fresh whole blood was collected in a 9:1 dilution of 3.8% citrate and 1 M ϵ -aminocaproic acid (EACA). Red blood cells

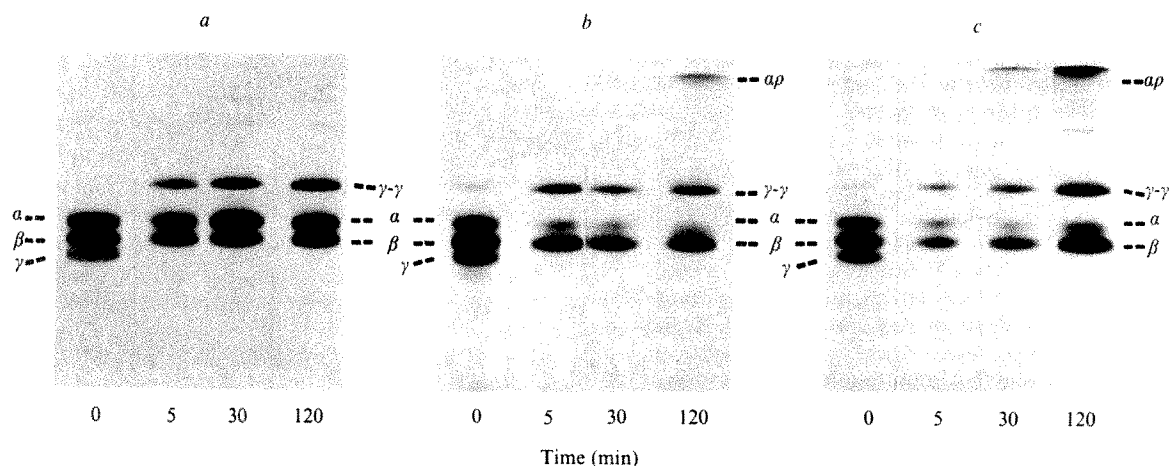


Fig. 1 5% SDS-polyacrylamide gel electrophoresis of reduced samples of a time course fibrin crosslinking study of clots from plasma (a) whole blood (b) and plasma plus RBCs (c). Crosslinking of α -chain to form α -polymer (α p) is greater when RBCs are present in clot.

(RBCs) and plasma were separated by centrifugation and red cells were filtered through a 1:1 mixture of microcrystalline cellulose and α -cellulose to remove white cells and platelets⁴. The red cells were then washed 10 times in 5 volumes of 0.9% NaCl, pH 6.5. The washed red cells were added to platelet-poor plasma from which they were separated to give a final haematocrit of 40–50%. Aliquots of 200 μ l whole blood, 100 μ l platelet-poor plasma and 200 μ l RBCs plus platelet-poor plasma were added to separate sets of four 3 $\times$ 50 mm glass test tubes. EDTA was added to a final concentration of 1% in the first tube of each set to prevent fibrin crosslinking. The mixture was clotted with human thrombin (Fibrindex, Ortho Diagnostics, or ultrapure thrombin) at a final concentration of 5 NIH units per ml. The resulting fibrin clot from the plasma sample was wound on a glass micropipette to express serum from the clot. Serum and red cells were expressed from the red cell containing clots between layers of filter paper. Clots were then washed three times in distilled water, dropped into 200 μ l of a solution of 2% mercaptoethanol–2% SDS–8M urea–0.065M Tris-HCl, pH 7.4, and placed in a boiling water bath for 5 min to stop the reaction.

Additional samples in each set of tubes were clotted with thrombin in the presence of calcium (final concentration of thrombin and calcium, 5 NIH units per ml and 80 mM, respectively). Samples were incubated at room temperature for 5, 30 and 120 min to allow fibrin crosslinking to occur before the fibrin clots were separated and processed as described above. As earlier studies had shown complete γ -crosslinking by 5 min, samples clotted for 5 min or longer were washed in 3 M urea to enhance removal of plasma proteins and red cells from the fibrin mesh.

Analysis of reduced samples by SDS-polyacrylamide gel electrophoresis⁵ revealed complete γ -crosslinking in clots of blood, plasma and plasma plus red cells by 5 min. The α -chain of fibrin crosslinked much more rapidly and extensively in whole blood and in plasma plus red cells than in plasma alone (Fig. 1). Disappearance of the α -chain was associated with appearance of the α -polymer. The presence of 0.1 M EACA inhibited plasminogen activation to plasmin and there was no build up of α -chain degradation products with time.

Gels stained with Coomassie brilliant blue were scanned using a Gelman ACD-15 densitometer. Areas under scans of the α -chain were compared with the sums of the areas under scans of the γ -chain, β -chain and γ -dimer to give a ratio (termed the α -ratio) of free α -chain to the sum of other fibrin chains. The α -ratio was plotted against time to quantitate the rate of disappearance of α -crosslinking. Such plots demonstrated that even extensively washed RBCs accelerated fibrin α -crosslinking in platelet-poor plasma to a rate and extent similar to that in whole blood.

To determine whether platelet factor XIII activity accelerated fibrin crosslinking in plasma, crosslinking studies were done as described above using platelet-rich and platelet-poor plasma. The rate and extent of fibrin α -crosslinking were not significantly different in platelet-rich and platelet-poor plasma.

In one of a series of studies designed to determine whether the release of intrinsic transglutaminase⁶ or other intracellular substance from red cells during clotting might be responsible for accelerated fibrin crosslinking, washed red cells were treated with *p*-hydroxymercuribenzoate (PMB) to block the sulphhydryl groups on their cell surface. Red cells were incubated in 5 volumes of 10 mM PMB in 0.9% NaCl, pH 6.5, for 20 min, and then washed three times to remove excess PMB. When these red cells were resuspended in plasma, they no longer accelerated the α -crosslinking of fibrin (Fig. 2). As PMB does not cross intact red cell membranes⁷, the crosslink-accelerating activity of RBCs must reside in or on the external surface of the cell membrane and be independent of intrinsic transglutaminase activity.

To further define the activity of red cell membranes in fibrin crosslinking, erythrocyte ghosts were prepared and their ability to accelerate fibrin crosslinking in plasma was determined. Erythrocyte ghosts prepared by hypotonic lysis⁸ did not accelerate fibrin crosslinking. As the fibrin crosslink accelerating activity of red cells was not diminished significantly by extensive washing in 0.9% NaCl, but was lost from hypotonically prepared red cell membranes, the activity must be due to a substance in or on the membranes that is inactivated or released by hypotonic media.

Additional experiments failed to relate intracellular red cell constituents to the acceleration of fibrin crosslinking by red cells. Glutathione in final concentrations of 1 mM, 3 mM and 10 mM had negligible effects on the rate of fibrin crosslinking in plasma

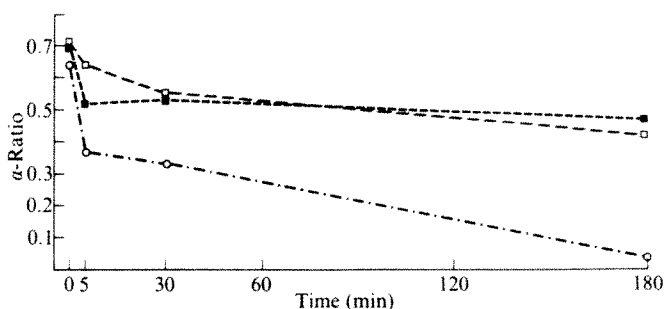


Fig. 2 Rate and extent of disappearance of α -chain in clots of plasma (□) plasma plus RBCs (○) and plasma plus *p*-hydroxymercuribenzoate-treated RBCs (■). The α -ratio reflects disappearance of free α -chain as this is crosslinked to form the α -polymer.

when compared with the effects of intact RBCs. The supernatant from red cell membrane ghost preparations also failed to accelerate fibrin crosslinking in plasma to the same extent as intact red cells. Generally, no difference in the rate and extent of crosslinking was observed, although some samples showed minimal (<10%) acceleration of crosslinking.

Because of the rapid rate of formation of γ -crosslinks, the effect of red cells on the rate of that reaction could not be adequately assessed. When the rate of crosslinking of the γ -chain of fibrin was evaluated at 4 °C in plasma and in whole blood the reaction was still virtually complete at 5 min and no significant differences in the rate could be determined.

Our data are most consistent with the presence of a sulphhydryl-containing membrane protein located in or on the external surface of red cells that accelerates fibrin crosslinking. RBCs may thus have a more important physiological role in the acceleration of fibrin crosslinking and stabilisation of thrombi than previously recognised. Conclusions about *in vivo* fibrin crosslinking derived from studies of clotted plasma or fibrinogen may be misleading because of the absence of RBCs in such coagulum.

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Viroid replication is inhibited by α -amanitin

VIROIDS are infectious single-stranded circular RNA molecules¹ which cause disease in higher plants. Although the complete molecular structure of one viroid has recently been established², the mechanism of viroid replication and pathogenesis is still unknown. The viroid molecule carries insufficient genetic information to code for a complete viroid-specified replicase. Replication must therefore depend on host cell enzymes. We report here the inhibition by α -amanitin, of ³H-uridine incorporation into viroid RNA in infected tomato protoplasts. The inhibitory intracellular concentrations of α -amanitin which we determined suggest the involvement of the nuclear DNA-dependent RNA polymerase II in viroid replication.

Healthy plant cells contain two types of known RNA-synthesising enzymes, namely the DNA-dependent RNA polymerase system³ and the recently described RNA-dependent RNA replicase system^{4,5}. Viroids could therefore either be replicated through complementary RNA, as are most RNA viruses, or through a DNA template. This DNA template could be already present in the host cell in a repressed state or could be

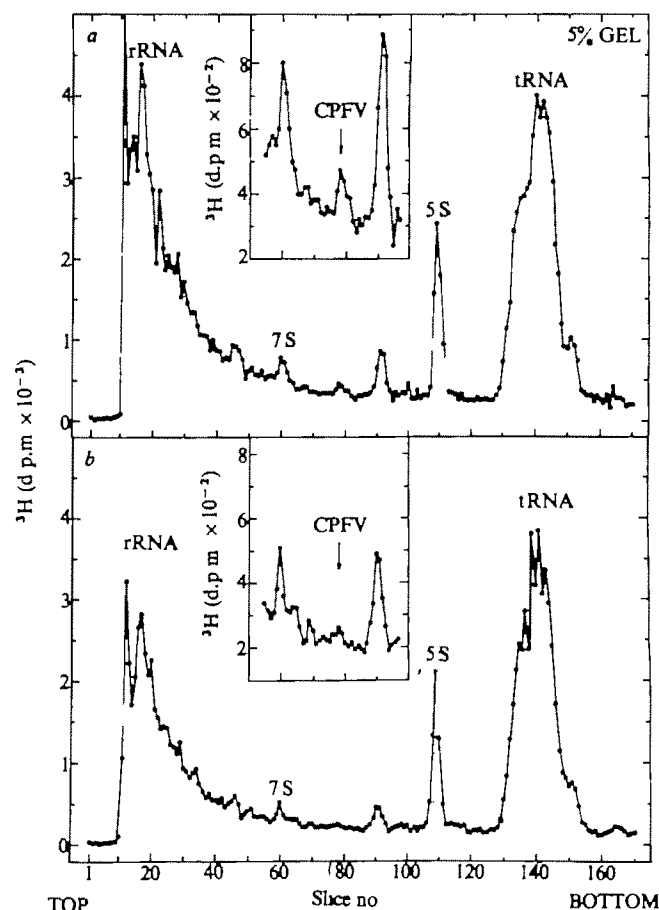


Fig. 1 Analysis of 2 M LiCl-soluble RNA from CPFV-infected tomato protoplasts in 5% polyacrylamide gel. Protoplasts were isolated from green leaves of the tomato cultivar 'Hilda 72' with the aid of Macerozyme R-10 and cellulase 'Onozuka R-10' at pH 7.0 and inoculated with 100 μ g purified CPFV in 10 ml glycine-KOH-buffered mannitol at pH 9 containing up to 5×10^7 protoplasts¹⁰. After inoculation, protoplasts were cultured at a cell density of 2×10^5 ml⁻¹ at 29 °C in the dark. ³H-Uridine (10 μ Ci ml⁻¹) was added at the beginning of the culture period. 48 h after inoculation one-third of the protoplasts was collected by centrifugation, shock-frozen with liquid nitrogen and stored at -80 °C until RNA extraction. To one-half of the remaining protoplast suspension α -amanitin (dissolved in culture medium) was added to give a final concentration of 50 μ g ml⁻¹. An equal volume of culture medium without α -amanitin was added to the other half of the protoplast suspension. 72 h after inoculation, that is, 24 h in the presence of α -amanitin, all protoplasts were collected and frozen. RNA was extracted from these samples with the aid of phenol-SDS and salt-fractionated with 2 M LiCl⁸. RNA soluble in 2 M LiCl was dissolved in 200 μ l buffer containing 8 M urea and aliquots of 50 μ l RNA were analysed by electrophoresis on 16 cm long cylindrical 5% polyacrylamide gels. 0.1 A₂₆₀ of purified CPFV was added as marker and the gels were run at 1 mA per gel for 12 h at 7 °C. After electrophoresis gels were stained with methylene blue, destained in 7% acetic acid, cut into 1 mm slices and counted for ³H radioactivity. **a**, RNA profile 72 h after inoculation and culture without α -amanitin. **b**, RNA profile 72 h after inoculation, but cultured in the presence of 50 μ g α -amanitin per ml culture medium during the period 48-72 h after inoculation. The control data of ³H-incorporation into RNA between 0 and 48 h are given in Table 1.

newly synthesised on viroid infection. Previous studies with actinomycin D, which inhibits DNA-dependent RNA transcription, applied to leaf strips⁶ and nuclei⁷ prepared from viroid-infected tomato leaves indicated that viroid replication was unlikely to proceed through an RNA-dependent RNA replicase.

Tomato protoplasts isolated from green leaves of the tomato cultivar 'Hilda 72' as previously described⁸ were used. They regenerate new cell walls after 3 days⁹ and divide within a week (unpublished results). Their general metabolic capacity is substantiated by their ability to produce complete tobacco mosaic virus (TMV) particles after infection with TMV RNA *in vitro*⁹. In these protoplasts viroid replication has been followed by ³H-uridine incorporation after infection *in vitro*¹⁰. Freshly

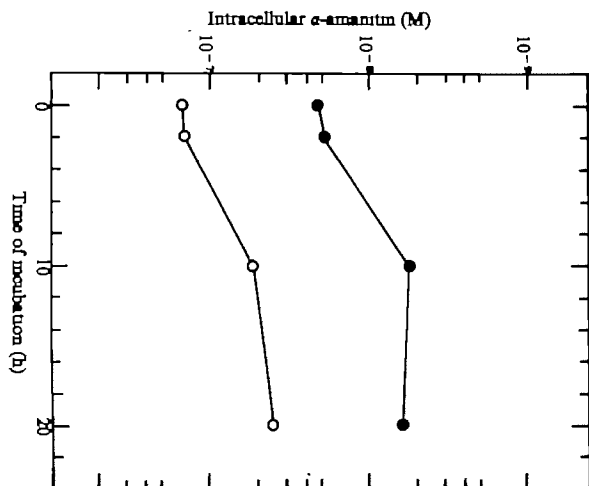


Fig. 2 Intracellular concentration of ³H-α-amanitin in tomato protoplasts. Protoplasts were isolated and cultured as described for Fig. 1. ³H-O-methyl-dehydroxymethyl-α-amanitin (specific activity 2 Ci mmol⁻¹) was added to protoplast suspensions to a final concentration of 5 µg ml⁻¹ (~10⁻⁶ M) (O) and 50 µg ml⁻¹ (~10⁻⁵ M) (●). At 0, 2, 10 and 20 h of incubation with ³H-α-amanitin aliquots were collected, washed four times with culture medium, digested with Soluene (Packard) and counted for ³H radioactivity. Quench corrections were performed in parallel experiments with equal numbers of protoplasts using ³H toluene as standard.

isolated protoplasts were inoculated with cucumber pale fruit viroid (CPFV)¹¹ and cultured as previously described¹⁰. Control experiments comparing the effect of α-amanitin on TMV replication (which proceeds through an RNA-dependent RNA-replicase) were carried out on protoplasts of the same batch after inoculation with TMV RNA according to our published procedure⁹. Biosynthesis of cellular RNA, TMV RNA and viroid was measured by incorporation of ³H uridine into these RNA species, which were separated on 2.5% and 5% polyacrylamide gels¹⁰ after extraction with phenol-SDS and salt fractionation⁸.

To discriminate clearly between the different DNA-dependent RNA polymerases possibly involved in viroid replication, we applied α-amanitin to our protoplast system. In eukaryotic cells α-amanitin selectively inhibits the DNA-dependent RNA polymerases II and III at low and high concentrations, respectively, whereas RNA polymerase I is not inhibited¹²⁻¹⁵. As shown in Table 1 α-amanitin has no appreciable effect on the biosynthesis of cellular RNA and viroid if present at a concentration of 10 µg ml⁻¹ during the period 48–72 h after inoculation, when viroid replication is detectably increased¹⁰. With 50 µg ml⁻¹ α-amanitin, viroid replication is inhibited to ~75%, whereas the biosynthesis of tRNA, 5S RNA, 7S RNA and rRNA is again not appreciably affected (Fig. 1 and Table 1). We wish to point out that our protoplasts are killed by this concen-

tration of α-amanitin if present throughout the total time of culture. Therefore, only the brief exposure of protoplasts to this drug described above gives meaningful results. The RNA profile of uninfected protoplasts, where the viroid peak is naturally lacking, shows a similar effect of α-amanitin on the cellular RNA species.

In view of the concentration-dependent specificity of α-amanitin inhibition of RNA polymerases the interpretation of such *in vivo* experiments depends on knowing the intracellular concentration of this inhibitor. We therefore determined its intracellular concentration in our experimental conditions using a ³H-labelled amanitin¹⁶. When tomato protoplasts were exposed to 10⁻⁵ M (50 µg ml⁻¹) and 10⁻⁶ M (5 µg ml⁻¹) ³H-α-amanitin for 20 h, an intracellular concentration of 2 × 10⁻⁸ M and 2 × 10⁻⁹ M, respectively, was already reached within the first 10 h incubation (Fig. 2). This clearly demonstrates that the intracellular concentration of α-amanitin is in the range of 10⁻⁸ M in the conditions in which viroid replication is inhibited, namely at 50 µg α-amanitin per ml culture medium. *In vitro* experiments with DNA-dependent RNA polymerases isolated from different sources¹⁴ including higher plants³ have shown that RNA polymerase II is, in fact, specifically inhibited at a concentration of 10⁻⁸ M α-amanitin, whereas inhibition of RNA polymerase III requires a 1,000-fold higher concentration¹⁵. Studies with animal^{17,18} and plant^{19,20} cells have shown that in comparable experimental conditions RNA polymerase II is also inhibited *in vivo*. As the intracellular concentration of α-amanitin which inhibits ³H uridine incorporation into viroid RNA in our protoplasts is in the range where the nuclear DNA-dependent RNA polymerase II is specifically inhibited, we suggest that this enzyme system is involved in viroid replication.

Control experiments using tomato protoplasts inoculated with TMV RNA revealed that replication and accumulation of complete TMV was not affected, when α-amanitin was present at a concentration of 50 µg ml⁻¹ during the period 24–48 h after inoculation, where TMV replication is known to increase logarithmically in our system (unpublished results). Therefore, the marked inhibition of viroid replication by α-amanitin is unlikely to be due to a secondary effect of this drug on cell metabolism in general.

For comparison, we also investigated the effect of actinomycin D on the DNA-dependent RNA metabolism in CPFV-infected protoplasts. As viroid replication in tomato protoplasts is known to increase to a detectable level 48–72 h after *in vitro* inoculation¹⁰, the inhibitor was applied so that it was present during this period. Actinomycin D at a concentration of 20 µg ml⁻¹ culture medium inhibits total RNA biosynthesis up to ~80% (Table 2) which agrees with previous protoplast work²¹. The detailed analysis showed that viroid replication is inhibited to about the same degree (~85%) as the synthesis of cellular RNA. Identical results were obtained in control experiments with uninfected tomato protoplasts, the RNA profile of which differs only by lacking the peak representing ³H-labelled viroid. From comparable results previously obtained with leaf disks⁶ and

Table 1 Effect of α-amanitin on RNA biosynthesis in viroid-infected tomato protoplasts

α-Amanitin µg ml ⁻¹	Total RNA		rRNA		7S RNA		5S RNA		tRNA		CPFV	
	d.p.m.	% inhibition	d.p.m.	% inhibition	d.p.m.	% inhibition	d.p.m.	% inhibition	d.p.m.	% inhibition	d.p.m.	% inhibition
0	821,808	—	286,416	—	1,421	—	12,222	—	150,307	—	412	—
10	920,029	0	292,796	0	1,161	18.3	11,532	5.7	137,549	8.5	343	16.8
Control 0–48 h	224,800		62,120		1,450		3,155		32,487		181	
0	1,779,988	—	318,818	—	1,581	—	5,437	—	38,346	—	726	—
50	1,597,237	10.3	312,531	1.9	1,277	19.2	6,230	0	36,234	5.5	177	75.6
Control 0–48 h	1,664,876		353,212		333		5,198		47,395		31	

The figures are derived from experiments as described for Fig. 1. D.p.m. values given for total RNA and for each of the prominent RNA species indicate the incorporation of ³H uridine into the RNA species during the period 48–72 h after inoculation, where α-amanitin was present. This net incorporation was calculated on the basis of the d.p.m. values of the ³H-incorporation into RNA between 0 and 48 h after inoculation (control 0–48 h).

Table 2 Effect of actinomycin D and cycloheximide on RNA biosynthesis in viroid-infected tomato protoplasts

Actinomycin D $\mu\text{g ml}^{-1}$	Cycloheximide $\mu\text{g ml}^{-1}$	Total RNA		rRNA		7S RNA		5S RNA		tRNA		CPFV	
		d.p.m.	% inhibition	d.p.m.	% inhibition	d.p.m.	% inhibition	d.p.m.	% inhibition	d.p.m.	% inhibition	d.p.m.	% inhibition
0	—	14,564,730	—	3,813,487	—	6,435	—	25,968	—	162,169	—	4,487	—
20	—	2,833,102	80.5	753,211	80.3	188	97.1	3,538	86.4	28,471	82.5	695	84.5
Control 0-48 h	—	5,646,928	—	1,438,744	—	1,335	—	12,334	—	138,961	—	108	—
—	0	224,900	—	20,514	—	367	—	4,556	—	60,517	—	300	—
—	1	169,240	24.8	16,325	20.4	278	24.2	3,238	28.9	26,515	56.2	126	58.0
—	50	75,120	66.6	9,763	52.4	0	100	593	87.0	5,025	91.7	0	100
Control 0-48 h	—	84,856	—	7,892	—	163	—	1,213	—	22,438	—	41	—

Protoplasts were inoculated with CPFV and cultured for 48 h as described for Fig. 1. At that time aliquots of the protoplast suspensions were collected by centrifugation and frozen. Actinomycin D or cycloheximide were added to remaining aliquots of the protoplast suspension to give the final concentrations as indicated. 72 h after inoculation, that is, 24 h in the presence of actinomycin D or cycloheximide, protoplasts were collected, RNA was extracted and analysed on 5% polyacrylamide gels. The data given show the [^3H]uridine incorporation into total RNA and into the different RNA species during the period 48-72 h after inoculation. This net incorporation was calculated on the basis of the d.p.m. values of the [^3H] incorporation into RNA between 0 and 48 h after inoculation (control 0-48 h).

nuclei⁷ from viroid-infected tomato leaves, the involvement of host DNA during viroid replication was inferred. However, as actinomycin D inhibits the biosynthesis of all RNA species including viroid RNA, a nonspecific secondary effect of this inhibitor cannot be ruled out completely. Therefore, we can exclude only the involvement of pre-existing RNA-dependent RNA replicases during viroid replication. These are actinomycin D resistant as demonstrated in several systems infected with conventional RNA plant viruses²¹⁻²³.

Finally, we studied the effect of cycloheximide on RNA synthesis to test the possible requirement of newly synthesised proteins for viroid replication. When cycloheximide is applied to CPFV-infected protoplasts in the range from 1 to 50 $\mu\text{g ml}^{-1}$ in exactly the same conditions as actinomycin D (Table 2), the biosynthesis of cellular and viroid RNA is strongly inhibited although cycloheximide is known to interfere specifically only with protein biosynthesis and not with nucleic acid transcription itself. Similar results are obtained with uninfected protoplasts. These results do not allow us to conclude that specific and newly synthesised proteins are a prerequisite for viroid replication, because cycloheximide exerts a nonspecific and rather general disturbance of cell metabolism.

The specific inhibition of [^3H] uridine incorporation into viroid RNA by an intracellular concentration of 10^{-6} M α -amanitin strongly suggests that DNA-dependent RNA polymerase II is involved in viroid replication. In these conditions the biosynthesis of the prominent cellular RNA species (rRNA, 7S RNA, 5S RNA and tRNA) is hardly affected. RNA polymerase II is located in the nucleoplasm and is responsible only for the transcription of heterogeneous nuclear RNA species from the corresponding sections of the cellular DNA genome^{13,15}. Our assumption that the nuclear RNA polymerase II is involved in viroid replication is supported by earlier findings^{24,25} that the bulk of viroid infectivity is closely associated with nuclei and chromatin. Our present inhibition studies could explain the identical location of RNA polymerase II and of the accumulated viroid RNA as one of its possible products. The sensitivity of viroid replication to actinomycin D agrees with this conclusion irrespective of whether it is directly affecting a possible viroid-specific DNA template or is inhibiting DNA-dependent RNA biosynthesis in general.

The DNA dependence of viroid RNA replication has previously been postulated from hybridisation experiments with [^{125}I]-labelled viroid RNA from which the existence of cellular DNA sequences complementary to viroid RNA in viroid-infected^{26,27} and even in healthy²⁷ host plant species was inferred. On the other hand, from a similar experimental approach viroid replication involving a complementary RNA was assumed²⁴. However, these conventional hybridisation experiments must be interpreted cautiously for the following reasons. The circularity and high degree of self-complementarity of the viroid molecule^{1,2}, require very specific experimental conditions to obtain reliable hybridisation data. Further-

more, the absence of any trace of contaminating cellular RNA species in the viroid probes used for hybridisation must be rigorously demonstrated by appropriate controls and by fingerprint analysis²⁹.

We emphasise that we do not regard the observed α -amanitin inhibition as definitive proof of direct replication of viroid RNA by the nuclear DNA-dependent RNA polymerase II. There are several possible mechanisms by which this enzyme system could be indirectly involved as a result of its central role in the formation of mRNAs in the host cell. There is also the remote possibility that viroid replication may proceed through a yet unknown pathway which is α -amanitin sensitive. If, however, DNA-dependent RNA polymerase II is directly involved it still remains to be determined whether viroid replication is based on derepression of a pre-existing viroid-specific DNA template or whether the infecting viroid molecule, because of its DNA-like secondary structure, is accepted directly as a template by DNA-dependent RNA polymerase II. The circularity and the unique secondary structure, which are a common feature of all viroids³⁰, are most probably of functional importance, and may add to the complexity of their replicating mechanism. For its elucidation, *in vitro* studies with appropriate enzymes, particularly of host plant origin, seem to be essential.

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Crystal structure of a eukaryotic initiator tRNA

OUR understanding of the molecular structure–function relationship in tRNA rests mainly on three types of information. First, on the common sequence patterns which have emerged from careful examination of many primary structures^{1–3}; second, a wide variety of spectral and other physical and chemical results must be accounted for by the molecular structure^{4–6}; and third, there is the detailed image of the yeast tRNA^{Phe} molecule independently determined and refined from two different—albeit similar—crystal forms^{7–10}. It is also clear, however, that the molecular model deduced from the yeast tRNA^{Phe} crystal structure cannot be easily reconciled with all structural requirements for function and is best considered a well-defined and stable canonical form of tRNA which is packed in an unusually well-ordered way in specific crystal lattices. Notwithstanding the enormous value of this canonical form in explaining the basic architectural features of tRNA, it is clearly important to image other crystalline tRNAs; particularly tRNAs that exhibit different functions (such as, initiators) or have significantly different covalent structures (for example, class III tRNAs)¹ or those that crystallise in different solvent conditions. We report here the initial results of the crystal structure determination of a eukaryotic initiator tRNA crystallised from a highly polar aqueous solvent^{11,12}. Its architecture is essentially the same as crystalline yeast tRNA^{Phe}, except for a small but significant difference in the position of the anticodon arm.

Our interpretation is based on a 4.5 Å electron density map prepared by the method of multiple isomorphous replacement (MIR) and augmented by direct methods¹³. The trace of the sugar–phosphate backbone is further constrained by four heavy-atom markers, each covalently linked to a specific residue and an elaborate network of crystallographic dyads upon which the

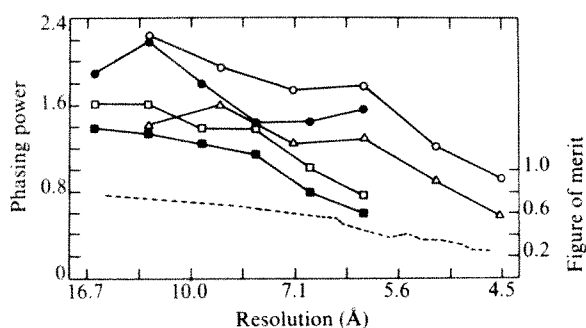


Fig. 1 Phasing power $(\sum |F_H|^2)^{1/2} / (\sum (|F_{PH}^{obs}| - |F_{PH}^{calc}|)^2)^{1/2}$ of five isomorphous derivatives plotted against resolution. They are gadolinium, lightly substituted, precession data (■); gadolinium, heavily substituted, precession data (□); gadolinium, oscillation data (△); pyridyl mercury acetate, precession data (●), and pyridyl mercury acetate, oscillation data (○). Figure of merit (---) is determined from MIR phasing³³ and has a mean value of 0.49 for all reflections to 4.5 Å resolution. F_H is the calculated heavy atom structure amplitude; $(|F_{PH}^{obs}| - |F_{PH}^{calc}|)$ is the 'lack of closure' error between observed and calculated isomorphous derivative structure amplitudes.

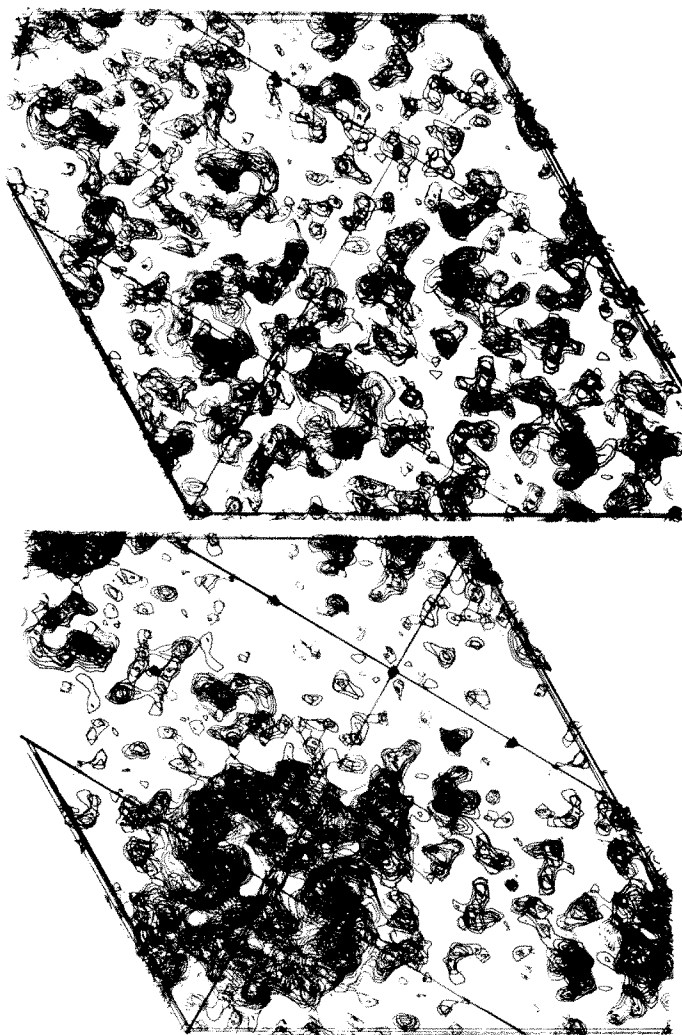


Fig. 2 Comparison of 10 sections of the 4.5 Å map of yeast tRNA^{Met} before (above) and after (below) the inclusion of 28 intense low resolution terms determined by matricial phase predictions. Both maps are contoured at the same intervals. Density cleared by this technique was ultimately shown to be noise, while density that was enhanced improved connectivity in the helical stems. Density on the dyad axes diminished.

molecule cannot intrude. The model presented here is based on a low resolution least-squares refinement procedure which assumes that the molecule is organised in four domains corresponding to the arms of the cloverleaf hydrogen-bonding pattern¹⁴.

The crystals have three important characteristics¹⁵. (1) They are grown from and stabilised in approximately 2M (NH₄)₂SO₄ containing 5 mM Mg²⁺ and 2 mM spermine and no organic solvent. The 2M ammonium ion may alter the structural role attributed to Mg²⁺ and spermine in crystalline yeast tRNA^{Phe} (refs 16–18). (2) Only 17.5% of the crystal volume is occupied by tRNA. Treating the remaining 82.5% as disordered solvent of uniform density was quite helpful in the structure analysis. (3) The crystal is poorly ordered, making accurate data collection very difficult and effectively impossible beyond 4 Å resolution. Data for the parent and three isomorphous heavy atom derivatives were collected initially to 6 Å by procession photography and extended to 4.0 Å by oscillation photographs in which a single crystal was devoted to each of ~30 overlapping 2.5° or 2° sectors. Figure 1 shows that there is useful phasing power in the best derivative to about 4.5 Å resolution. Schevitz *et al.*¹⁵ presented a 6 Å analysis of the yeast tRNA^{Met} crystal structure. Despite two heavy atom markers at iodine ⁵C₇₃ (5-bonding onto cytosine at residue 73) and osmium ^{5,6}C₃₈ the electron density could not be convincingly interpreted in terms of the molecular backbone. Even when the data for the parent structure and MIR

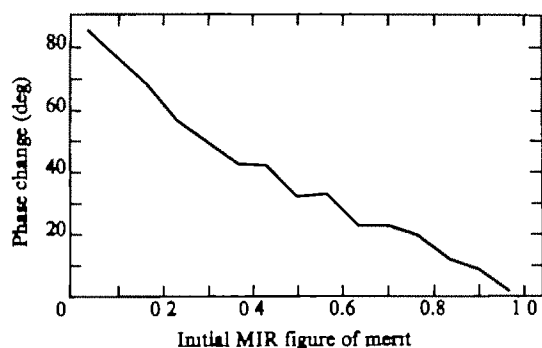


Fig. 3 Final phase change in degrees resulting from direct methods (see text) as a function of the original figure of merit determined by conventional MIR phasing. Phase information from both sources was merged by multiplying together their respective phase probability curves after each cycle of combined solvent levelling and density modification. Note that those reflections predicted to have a high reliability by MIR phasing (figure of merit near 1.0) remained closely tethered to their MIR phase while those with a low MIR reliability (figure of merit near 0) tend to be more easily shifted toward phases calculated by direct methods.

phases were extended to 4.5 Å resolution and a third heavy atom marker at iodine ^{127}I was used the map was still too 'noisy' to interpret confidently.

One particularly vexing deficiency in the MIR map was the inability to define the exact molecular boundary with

confidence. As over 80% of the unit cell volume was solvent this structure was particularly amenable to phase improvement by solvent levelling if the molecular boundary could be accurately established^{19,20}. The boundary was established by phasing the previously omitted very intense inner reflections ($d > 14$ Å). In the effort to secure high resolution data by extensively irradiating each crystal, these strong reflections were overexposed and far exceeded the dynamic range of even the most weakly exposed film. Even if relatively accurate amplitudes had been obtained for the parent and derivatives it is likely that the heavy atom contribution would not have been strong enough to provide accurate difference amplitudes to phase the very strongest of these reflections. To circumvent this problem the inner core of intense parent intensities was accurately remeasured and the phases of 28 of the very strongest reflections were determined by applying matricial methods to the amplitudes and MIR phases of 107 known reflections. The technique is similar to that used by Podjarny *et al.* to extend the phases of tricornic lysine²¹ and yeast tRNA^{Phe} (ref. 22) to a higher resolution than the starting phase set. Here the procedure was used to extend phases to a lower resolution than the MIR phase set. The details of this work will be published elsewhere¹³; however, it is evident from Fig. 2 that there was substantial enhancement of the contrast between the molecule and the solvent. The improved map enabled us to confidently define 65% of the unit cell as solvent. New phases were calculated from a map in which the noise in the 'solvent' was levelled to a uniform average value and negative regions in the molecule attenuated^{23,24}. These phases were merged with

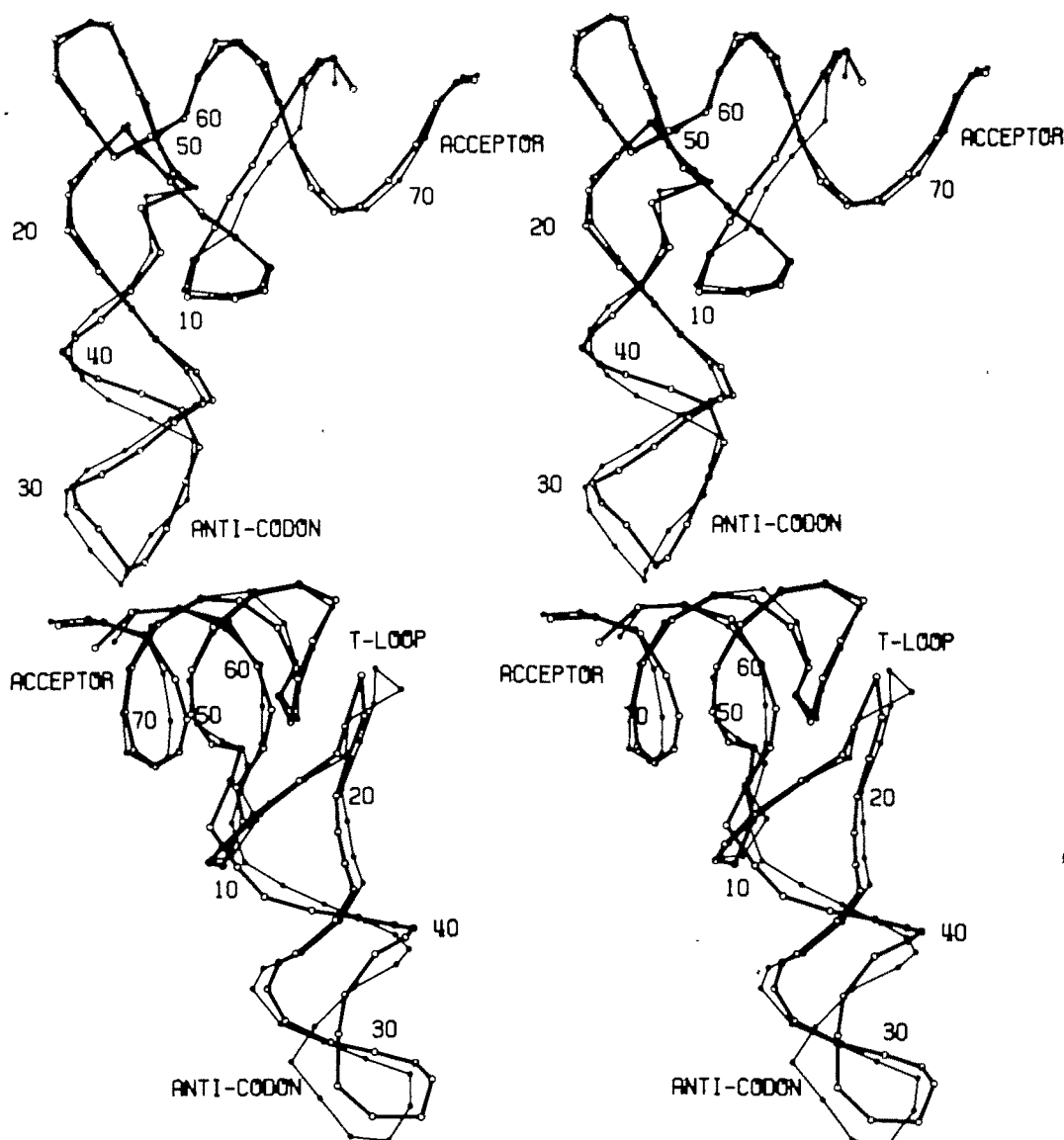


Fig. 4 Comparison of the yeast tRNA^{Met} (heavy line) and yeast tRNA^{Phe} (light line) structures in two stereo views. The phosphate backbone is shown with every tenth phosphate residue of yeast tRNA^{Met} numbered.

MIR phases and the 'density modification' procedure repeated for three cycles. Figure 3 shows that the phase improvement effected by density modification was most apparent for those reflections whose MIR phase was, in general, least accurate.

Four residues have been reacted stoichiometrically with covalently bonded heavy atoms. The heavy-atom markers were nicely distributed along the molecular backbone and provided a clear guide to the interpretation. (1) Iodine $^{57}\text{C}_{73}$: the third residue from the 3' terminus was labelled with iodine either by enzymatically rebuilding the 3' terminus with iodine ^{57}CTP and nucleotidyl transferase²⁵ or by directly iodinating the crystals²⁶. (2) iodine $^{57}\text{C}_{74}$: direct iodination also labels C_{74} which shows a small peak that cannot be refined, presumably because of disorder. (3) Iodine $^{57}\text{U}_8$: produced by direct iodination of the crystal²⁶. (4) Osmium $^{195}\text{C}_{38}$: direct reaction of the crystals with Os(VI) and pyridine²⁷ forming a 5,6-bispyridyl osmate diester^{28,29} with C_{38} (ref. 30).

Initially we interpreted the map with a conventional optical comparator and skeletal models, but it was found more convenient to use computerised 'static' molecular graphics³¹ to search for significantly different orientations of the stable helical substructures operating within the constraints imposed by the heavy atom markers and the crystallographic dyad axes upon which the model could not intrude. Subsequently, a residue-by-residue computerised fit of the entire model to the map was attempted in 'real time' at the Computer Graphics Laboratory at the University of North Carolina and this allowed us to rule out additional alternatives; however, we found the map was not sufficiently well resolved to impose a correspondence between phosphate, ribose and base moieties in the model and the peaks in the electron density.

The coordinates of the optimal fit were used as guides to position a tRNA^{Phe} model¹⁰ in the P6_422 unit cell. The root-mean-square deviation between the guide points and the tRNA^{Phe} model coordinates was 5.4 Å. This model was divided into four rigid groups corresponding to the helical arms and refined against observed intensities using a least squares procedure (CORELS) of Sussman *et al.*³² that 'restrained' the helical arms to remain within reasonable bonding distance of each other. The refinement was started at 12.5 Å and carried to 6 Å. The final crystallographic *R* factor between calculated and observed data to 6 Å was 42.3%; to 12 Å, 33%. These values indicate an essentially correct interpretation of a macromolecular crystal structure.

The molecular structure is shown in Fig. 4 contrasted with that of yeast tRNA^{Phe} . Except for a small region near the anticodon stem, the model accounts for virtually all the electron density in terms of a dominant sugar phosphate backbone. At this stage of the analysis the molecular architecture is generally similar to that of yeast tRNA^{Phe} .

The position of the anticodon arm in the crystal structure of yeast tRNA^{Met} differs significantly from the crystal structure of yeast tRNA^{Phe} (Fig. 4). Moreover, the refinement parameters show that although the anticodon arm converges to the position shown in Figure 4, it is the most poorly localised segment of the crystal structure. These observations suggest that the orientation of the anticodon arm is not rigidly fixed and the union of the D and anticodon stems may, as suggested previously^{18,34}, serve as a hinge point to allow alterations in the orientation of the anticodon during functional adjustments of the structure. Except for the possible stacking of a non Watson-Crick pair m^2G_{26} and A_{44} , no stabilising tertiary interaction can be invoked to ensure a particular orientation of the anticodon arm.

The molecules are packed with all 12 of the acceptor-T helices in the unit cell clustered around the 6_4 axis at an angle of approximately 20°. Pairs of neighbouring molecules that have opposing acceptor stems stack upon one another roughly in helical register. This explains the intense continuous helical diffraction pattern along c^* observed at approximately 3 Å resolution, well beyond the lattice transform¹⁵.

The anticodon arms project out roughly perpendicular to the 6_4 axes and contact neighbouring molecules at their anticodon

loop and the base of the D-stem. Thus many of the stabilising interactions involve the anticodon arm, which we believe to be extended on a flexible hinge. The fact that the lattice is connected through these hinged anticodon arms may be the reason that this (and possibly many other) tRNA crystals, although readily grown, are poorly ordered. The situation is reminiscent of the characteristically poorly ordered crystalline immunoglobulins³⁵—another macromolecule where flexibly hinged domains may be necessary for function.

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Note added in proof: Refinement with a more flexible model has reduced the crystallographic *R*-factor to 25% for all data to 4.0 Å resolution. Difference maps are clean and tertiary structural interactions are seen which can be correlated to those in crystalline yeast tRNA^{Phe} .

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A high molecular weight form of terminal deoxynucleotidyl transferase

CALF thymus terminal deoxynucleotidyl transferase (TdT) (EC 2.7.7.31) is a low molecular weight protein containing two peptides of MWs 24,000 and 8,000 in a native structure estimated to have a molecular weight of 32,000 (ref. 1). Use of antibody directed against homogeneous bovine transferase² for immunofluorescent cytology showed the normal thymus localisation to be cytoplasmic and nuclear, whereas bone marrow localisation is only nuclear³. Several human lymphoblastoid cell lines that contain terminal transferase activity also exhibit the nuclear location by immunofluorescence⁴. As cultured lymphoblastoid cell lines are readily labelled with radioactive amino acids we surveyed the peptides present in the immunoprecipitated transferase from these lines by fluorography of SDS gels. The results presented here show that a single 58,000-MW peptide is precipitated by monospecific rabbit antibody against bovine transferase. No peptides as small as those peptides present in calf thymus transferase were detected.

Our experiments used several lymphoblastoid cell lines. Line 8402 (ref. 5) contains >90% TdT⁺ cells and exhibits around 325 units of transferase per 10⁸ cells. Line 8392 (ref. 5), originating from the same patient, is completely negative by TdT immunofluorescence and enzyme assay. Line CEM-CCRF (ref. 6) contains about 19 units of enzyme per 10⁸ cells and has ~9% TdT⁺ cells. We cloned this line to produce CEM-clone 10 (>90% TdT⁺ cells, 280 units per 10⁸ cells) and CEM-clone 23 (<2% TdT⁺ cells, ~4 units per 10⁸ cells). All of these lines were grown in RPMI-1640 medium containing 10% fetal calf serum in spinner bottles at 37 °C. All have generation times of ~24 h in these conditions.

The human lymphoblastoid cells were labelled to high specific activity (about 60,000 c.p.m. per µg protein) by replacing the leucine and isoleucine in the medium with the corresponding ³H-amino acids. Cells were allowed to incorporate amino acids for 24 h and then extracts of the washed cells were prepared by sonication in 0.25 M potassium phosphate buffer at pH 7.5. The final cell extract is the supernatant fraction obtained after centrifugation at 100,000g for 1 h. Washed immunoprecipitates prepared from these cell extracts (see legend to Fig. 1) and separated on SDS-acrylamide gels produced the fluorograms shown in Fig. 1.

The results clearly demonstrate that a single radioactive protein is found in the immunoprecipitate from lines 8402 and CEM-clone 10 (Fig. 1, a' and b'). The MW of this peptide is estimated to be 58,000. No radioactive peptides were obtained from TdT-negative lines such as line 8392 (Fig. 1, c') and negative clones such as line CEM-clone 23 (Fig. 1, d'). Positive lines regularly contained the 58,000-MW peptide and we conclude that this is a high MW form of human TdT. Mouse line P-1798 and human line NALM-1 (also TdT⁺) also contain the 58,000-MW peptide (data not shown).

The specificity of each of the antibody reagents used was carefully checked, and no nonspecific contaminants were found. This is demonstrated by the absence of bands in the transferase negative lines (Fig. 1, c' and d'). Our first experiments (not shown here) used a secondary goat (IgG) anti-rabbit IgG for complete precipitation of the rabbit anti-transferase-transferase complex and a large excess of goat heavy chain was therefore present on the SDS gel near the TdT peptide band. Although common practice indicates that this excess of high MW peptides does not affect migration of lower MW species, we reduced the amount of goat heavy-chain peptide on the gel by using the F(ab')₂ of goat IgG as the secondary antibody in the experiments presented in Fig. 1. The position of the 58,000-MW peptide is unchanged on SDS gels of the F(ab')₂ precipitated complexes. The radioactive band migrates slightly more slowly than the rabbit heavy chain arising from the primary antibody (Fig. 1, a, b, c and d), and the goat heavy-chain fragments

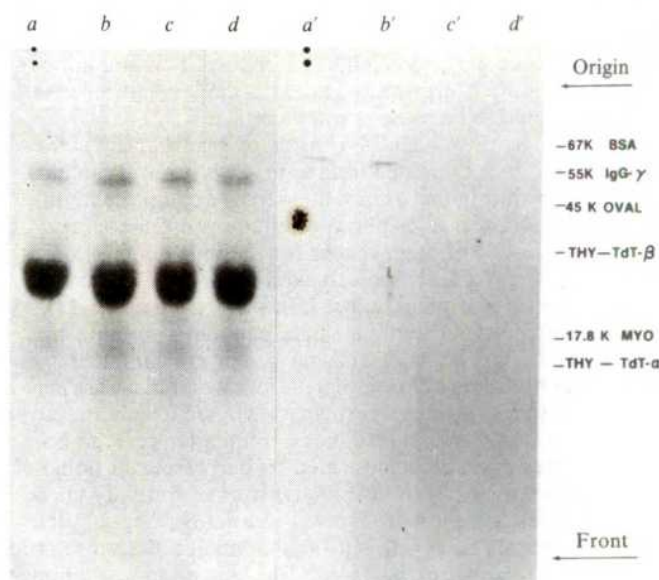


Fig. 1 Fluorogram of SDS-polyacrylamide gel of immunoprecipitated terminal transferase. Extracts of human lymphoblastoid cells labelled with ³H-leucine and ³H-isoleucine were precipitated with rabbit anti-bovine terminal transferase (0.36 mg ml⁻¹) purified by immunoabsorbent chromatography³ followed by F(ab')₂ goat anti-rabbit IgG (5 mg ml⁻¹). The immunoprecipitates were washed 4 times in Tris-Cl buffered saline, pH 7.2, and twice in Tris-Cl buffered saline containing 0.5% Triton X-100, 0.5% deoxycholate, 20 mM leucine, 20 mM isoleucine. The precipitates were resuspended for the final two washes by sonication. The washed precipitates were dissolved in sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.001% bromophenol blue, 5% 2-mercaptoethanol), heated for 1 h at 100 °C, and analysed on 16% SDS slab gels. Electrophoresis was carried out as described by Laemmli⁷. Gels were stained in Coomassie blue and processed for fluorography⁸. The position of MW markers and the α and β chains of calf thymus transferase are indicated by arrows. Lanes a, b, c, d, total protein in immunoprecipitates from line 8402, line CEM-clone 10, line 8392, and line CEM-clone 23, respectively (Coomassie stain). a', b', c', d', radioactivity from immunoprecipitates of line 8402, line CEM-clone 10, line 8392, and line CEM-clone 23, respectively. Peptide markers were bovine serum albumin (BSA, MW 67,000 67 K), human IgG (IgG, MW 55,000, γ chain), ovalbumin (OVAL, MW 45,000 45 K), calf thymus terminal transferase (THY-TdT-β and THY-TdT-α) and myoglobin (MYO, MW 17,800 17.8 K). The apparent MWs for TdT peptides in this gel system are 26,000 and 10,000.

migrate to the 24,000-MW region. Addition of pure bovine enzyme to the extract before addition of antibodies effectively competes with precipitation of radioactive protein. We conclude that the overwhelming majority of radioactivity in the immunoprecipitate has determinants related to the bovine enzyme.

The original work on rabbit anti-TdT demonstrated neutralisation of enzyme activity when the Ab-Ag mixture was assayed with d(pA)₅₀ and ³H-dGTP (ref. 2). A footnote suggested, however, that the immune precipitates could be demonstrated to contain enzyme activity. This was done by using an assay mixture containing d(pT)₃ and ³H-dATP to test for enzyme activity in the isolated immunoprecipitate. The small size of the initiator presumably permits diffusion into the immune complex and allows detection of enzyme activity at a level of around 10% of the free enzyme. We analysed enzyme activity in non-radioactive precipitates (requiring no background subtractions) and radioactive precipitates (which require rather large background subtraction) from line 8402 extracts and calculated that essentially all of the activity present in the original extract is present in the precipitate. We conclude that the 58,000-MW band on the SDS gels is responsible for the enzyme activity as no other bands are seen, and the precipitate is enzymatically active. The antibody procedure precipitates about 0.05–0.10 µg of protein from the crude extracts that contain

transferase activity, corresponding to 0.02–0.05% of the total cell protein added in the precipitation mixture. This amount of protein precipitate is also consistent with the level of enzyme protein estimated to be present in the extracts.

The existence of a high MW form of transferase in the malignant cell lines examined in this survey is a novel finding. Unfortunately this finding cannot be used to explain the multiplicity of forms observed by phosphocellulose chromatography of crude extracts^{9,10} of other tissues for the following reasons: Both line 8402 and CEM-clone 10 exhibit two distinct enzyme activity peaks on phosphocellulose (Hassur, Chang and F.J.B., unpublished results) but only a single peptide is seen on the fluorograms in Fig. 1; both enzyme peaks are inhibited by anti-TdT. The existence of several ionic forms of transferase in a single MW species requires an explanation based on amino acid changes or other ionic substitutions. Similarly, several isoelectric species can be demonstrated to be present in homogenous preparations of calf thymus transferase, but only the α and β peptides are present (Protzel and F.J.B., unpublished result). All species react with anti-TdT in microdiffusion tests. The immunoprecipitation procedures provide clean resolution of questions about peptide structure.

Our major objective in these experiments was to analyse a system exhibiting nuclear localisation of transferase. Having found a distinctly different form of transferase peptide in 'non-thymic' cells, we suggest that differentiation in the thymus may lead to peptide chain cleavage producing the 'thymic' form of enzyme. Of even greater speculative interest is the possibility that the high MW form, that form present in the nucleus, may be the form exhibiting important biological activity.

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Stopped visual motion

WHILE undertaking another experiment, we discovered a surprising and dramatic visual phenomenon. We were observing two gratings of high contrast and different spatial frequency (see Fig. 1 inset) while both gratings were rotating at a rate of 1 revolution per min. Both gratings were easily visible at the viewing distances used. The coarse grating was seen to revolve as expected; however, the fine grating appeared to revolve very slowly. This phenomenon has been shown to over 100 people¹ and all were startled by the observation. The individual reaction was to assert that the two gratings must be rotating at different physical speeds. This disbelief was readily corrected by removing the two gratings from their turntables and replacing each of them in the position of the other. Another entertaining variation of the observation is to observe the fine grating at the distance where it is perceived as a uniform disk; naturally, no movement can be seen at this distance. If one now advances slowly towards the grating it is initially seen as stationary; as one approaches closer it is seen to rotate progressively faster. At a particular distance it seems to rotate faster than the coarse grating. We have quantified these causal observations and show here that the

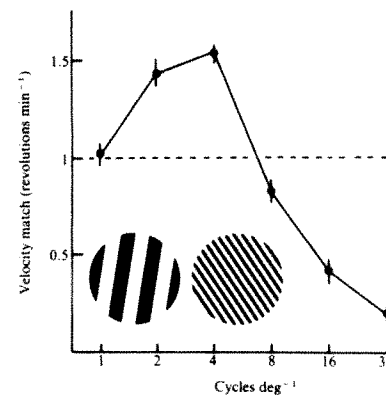


Fig. 1 Velocity match for gratings of different spatial frequencies—the dotted line is the true physical rate of revolution. The vertical bars are ± 1 s.e. ($n = 5$). The diameter of the disks was 10 cm.

perception of slow rotation movement is related to the spatial frequency and contrast of the pattern which is rotated.

A 1-cycle deg⁻¹ grating was used as reference and it was rotated at 1 revolution per min. A second grating was attached to a variable-speed turntable which could be controlled by the subject. In the first experiment the subject was requested to look fixedly at one or the other turntable alternately. He then adjusted the speed of rotation until the two disks were matched in velocity.

The results are shown in Fig. 1. The horizontal dotted line is the 1 cycle min⁻¹ reference velocity. It is clear that as the spatial frequency (fineness of the grating) is increased the apparent velocity rises, reaching a peak at 4 cycles deg⁻¹. Thereafter, the apparent velocity slows down rapidly.

Further quantitative observations established that the following variables affect the phenomenon. If the control grating is centred on the fovea and a grating of identical spatial frequency is viewed in the periphery of the visual field, the peripheral grating is seen to move much more slowly. In this experiment the subject looked with his fovea at a reference grating of 2 cycles deg⁻¹ with a velocity of 1 rotation per min. With reference to the foveal standard grating the velocity match of a grating of identical spatial frequency at the following eccentricities were: 5°: 1.4 ± 0.2 ; 10°: 2.2 ± 0.2 ; 15°: 3.2 ± 0.2 ; 20°: 3.7 ± 0.3 ; 25°: 5.4 ± 0.2 .

At very low contrasts (near threshold) the perceived speed of rotation also slows down or stops. However, at medium and high contrast no change in the rate of rotation is found as a function of contrast.

The above observations suggest two separate mechanisms for detecting rotation. In the first, the motion is actually seen; in the second, no motion is seen, but if the subject looks for some time he deduces that rotation must be occurring for he remembers that some time before, the grating was, for example, at 12 o'clock and now it is at 3 o'clock, and so on. When he cannot see the real motion, the observer is left with the strange impression that somehow the continuity of the flow of physiological time is lost.

Neurophysiological studies on single neurones of the visual system are now required to establish whether this singular deception of motion is due to an insensitivity of visual neurones to rotary disks containing high spatial frequencies.

F. W. CAMPBELL

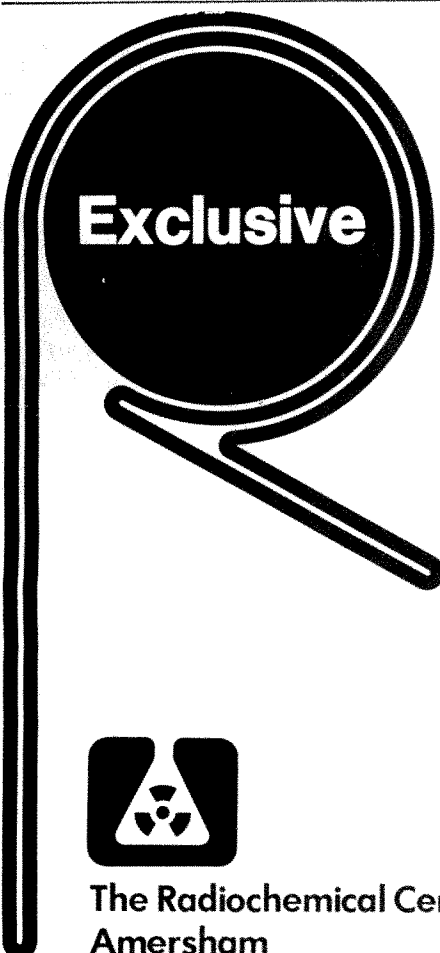
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1. Campbell, F. W. & Maffei, L. *J. Physiol., Lond.* **289** (in the press).


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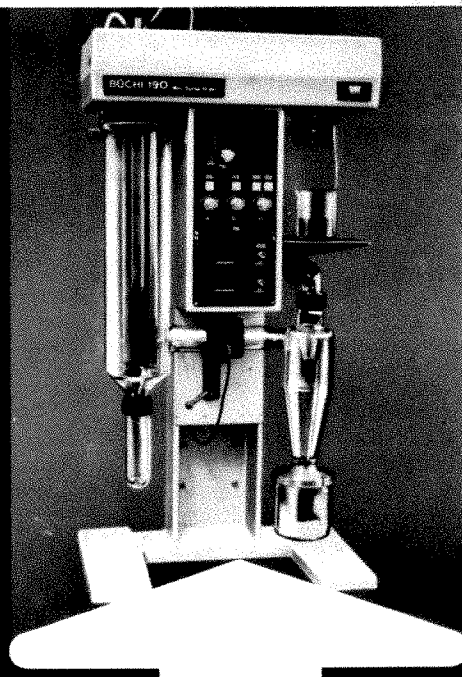
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A sociobiologist's new testament?

John Lawton

Behavioural Ecology: An Evolutionary Approach. By J. R. Krebs and N. B. Davies. Pp.494. (Blackwell Scientific: Oxford, 1978.) Paperback £8.50; hardback £18.

LARGE parts of this book are not really about ecology, and at least one chapter is not even about behaviour. Indeed, it might be more accurately (but still incorrectly) have been sub-titled *A Sociobiologist's New Testament*. The introduction says that it "takes modern population biology as its foundation and explores areas in which ethology and population biology overlap. Evolutionary thinking permeates the whole book", a statement of intent, which very clearly echoes E. O. Wilson's "Attempt to codify sociobiology into a branch of evolutionary biology and particularly modern population biology" (*Sociobiology. The New Synthesis* p4; Harvard University Press, 1975).

The text is divided into three sections, with an introduction (chapter 1) written by the editors. This chapter is an overview of ecology, natural selection and social behaviour, focusing particularly on the questions those who write later in the book will try to answer, and the tools with which they will answer them: optimality models, ecological stable strategies, and broad comparisons across species, together with brief summaries of the necessary genetics, the problems of altruism, inclusive fitness and group selection.

The first section contains four chapters, grouped round the theme "How animals harvest food, and how they avoid being eaten". Krebs discusses optimal foraging; Bertram the whys and wherefores of living in groups; Heinrich the economics of insect sociality, and Harvey and Greenwood anti-predator defence strategies, all topics that can properly be called behavioural ecology with the balance of the emphasis shifted towards one or the other of these words.

Part 2 (Sex, Mating and Signals) has five chapters with rather less unity. Maynard Smith under the heading "The Ecology of Sex" tackles the fascinating question of why organisms bother to reproduce sexually, with a brief look at parental care: excellent, no nonsense stuff which deserves (and has) a recent book to itself, but not

really ecology or even behaviour. Halliday in chapter 7 considers sexual selection and mate choice, followed by Parker who, as a colleague recently remarked, has the problem of mate choice "off pat" (for the uninitiated he studies dung flies). These two chapters complement one another well, as does Emlen's discussion of the evolution of cooperative breeding in birds. With less pleasing logic the section closes with a look at animal signals (by Dawkins and Krebs) under the pretext that the classical ethological work on signals was based on studies of sexual displays. The flavour of this whole section is very strongly behavioural, with only Emlen's chapter having much content that is strictly ecological, although this is intended as a signal of my own chauvinism rather than as a criticism.

The final section of the book tackles the problem of how an individual should deploy its behavioural options in space and time. Davies writes about territorial behaviour, and Partridge about habitat selection, both central themes in behavioural ecology. McCleery then carries us very firmly to the edges of modern behavioural theory with a chapter on how animals should make decisions about whether to mate, fight or feed, rather than what they should do within any one of these behavioural repertoires—the main concern of the rest of the book. Finally, Horn looks at life-history strategies, swinging us back to the mainstream of ecology with a chapter that contains some pleasing pot-shots at over-elaborate theory, some brilliantly simple insights, and almost no behaviour.

It ought, by now, to be clear that I found the book as a whole something of a mixed bag—an ecological, behavioural and sociobiological lucky dip—although like most lucky dips it is full of surprises and great fun to try. My feeling is that the editors have included just a bit too much. On the one hand there is material (for example, optimal foraging) that is certainly not sociobiology, and on the other, things that have precious little to do with mainstream ecology, or even behaviour. Perhaps we simply need a new pigeon-hole: but what should we label it?

With so many themes running

through it, it is difficult to provide one simple overview. Let me therefore close by developing three. As a teaching tool it will undoubtedly be valuable for students of all ages beyond first or second year undergraduate level. It is remarkably up to date, and covers well a host of complex ideas using hardly any mathematics (Parker's chapter is an exception in that he develops some formal models fairly extensively). Above all else it tends to avoid the glib phrase which hides a pit of uncertainty. Where there are problems it says so; and where there are exciting new roads to explore, it clearly signposts the way.

The book was presumably planned to complement R. M. May's *Theoretical Ecology*, which has the same publisher and format (for review see *Nature* 264, 138; 1976). Indeed, with the exception of Wilson's chapter in the latter, and Horn's chapter in the present book, there is almost no niche overlap between them. In combination they cover a massive area and might well be used together for advanced classes.

I suppose the real proof of a testament, old or new, is whether it will convert the hardened cynic: those who will argue that the whole edifice is but a harmless tautology. Throughout, behaviour is interpreted in terms of its survival value, what is in some sense 'optimal' or 'best'. And what is best? Obviously that which survives. The danger of slipping into this way of thinking is very real, because the sufficiently ingenious investigator can almost always dream up a convenient post-hoc Darwinian explanation for awkward observations, and without further testing, sit back contentedly having solved everything and nothing. Indeed there is more than a hint of this problem in several places throughout the book. For example, several authors make some very sweeping assumptions about the manner in which food 'must' be distributed in space and/or time, without actually measuring it, even though their original intention was to understand patterns of behaviour in relation to food supply. Even when ideas can be tested, they are difficult to refute because failure of the hypothesis can rather easily be attributed to "the rather artificial task with which [the animals] were faced" (p55); although

the artificiality of the situation can be conveniently ignored when the experiment 'fits' (for example, p44). However, and to be fair, a great deal of the experimentation and hypothesis testing is eminently reasonable. Indeed the editors have obviously anticipated most of the criticisms, and have been at pains both to draw attention to them, and avoid their worst excesses. What the book as a whole very clearly demonstrates is that if we accept the

central tenets of neo-Darwinism, and then use these tenets to generate interesting hypotheses and make predictions that can be independently tested, a great deal of behavioural ecology starts to make sense. At least, and on balance, it convinced this particular cynic. □

John Lawton is a Senior Lecturer in Ecology in the Department of Biology at the University of York, UK.

Unity in the sensory systems

The Unity of the Senses: Interrelations among the Modalities. By L. E. Marks. (Academic: New York, San Francisco and London, 1978.) \$17.50; £11.35.

IN 1974 Dr Marks published a book on psychophysics and followed this a year later with an article on synaesthesia. In the present book he combines these two interests with his interest in nineteenth-century poetry. The theme which serves as a link, provides a title for the book.

The first three quarters of the book leads to the conclusion that the sensory systems are more than discrete information channels, they demonstrate some unity. The evidence upon which this conclusion is based is grouped under four headings each of which is, for Dr Marks, a doctrine. To quote his rather ill-chosen words "Each chapter therefore amalgamates one of the doctrines with the evidence that highlights it."

The first of these four chapters which form the core of the book, is concerned with the way in which the various senses cooperate in the perception of size, form, space, time, motion and number. The second explores the possibility that there are supra-sensory qualities—brightness is an example—shared by the sensory systems. The third doctrine states that different senses have similar psychophysical properties. Generations of students reared on a diet of Fechner, Weber and Stevens would not disagree. The doctrine of neural correspondence completes the quartet. It states that there is a neural analogue for each of the preceding psychological doctrines. In fact the chapter is mainly concerned with the way in which the sensory systems code intensity, but it does include some interesting philosophical icing.

These four chapters lead to the final chapter of the first part of the book, which turns out to be a very brief discussion of the possibility that the similarities between the senses are best

understood if each sensory system is considered as a modality of a general primitive sense from which it has evolved. The remaining part of the book provides many examples of how the unity of the senses reveals itself in the language of the poet.

I found this a disappointing book. First, it is badly written. Nobody should use language as carelessly as does Dr Marks. Even his definition of synaesthesia, a central concept, lacks precision. Second, the book is shaped by the author's psychophysical interest and expertise. The senses are analy-

zers, but (surprise!) they demonstrate some degree of unity. Perhaps this can be explained by their evolution from a more primitive sense. But should these signs of unity cause surprise? True the senses are analysers but because of their input into the reticular formation they are also very much involved with the integrative mechanisms of the body. The greater the degree of specialisation of the analysers the more complex and the more important are the integrative mechanisms.

Coghill and the Herricks with their partial patterns, which are concerned primarily with the analysis of experience and action, and total patterns, which are synthetic and which maintain the integrity of the living body, would have approached the problem differently. Perhaps the influence of their sort of biology would have provided a more useful foundation than the influences which led to the psychologist's psychophysics.

J. R. Symons

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Chemical kinetics

Principles of Chemical Kinetics. By G. G. Hammes. Pp. 268. (Academic: New York, San Francisco and London, 1978.) \$19.50; £21.50.

THIS book, which is intended for graduate and senior undergraduate students, provides a general introduction to chemical kinetics covering reactions in the gas phase, reactions in solution and enzyme kinetics. In dealing with such a wide spread of topics in a relatively small book the author has had to be selective in the examples chosen, but he has generally been careful to avoid oversimplification. In cases where the principles are illustrated by quite simple examples the nature of possible complicating factors is also pointed out and references are provided to more detailed treatments. The short but well-chosen reference lists at the end of each chapter provide valuable sources of supplementary material and allow the reader to pursue individual topics in greater depth.

The development of the material in this book has been well planned with the reader being led naturally from one topic to another. The balance between the theoretical and empirical aspects of the subject is also carefully maintained with the author taking pains to point out the difference between the

theoretically achievable and the practically possible. Emphasis is given to relatively recent developments in experimental technique and to areas of particularly active current research interest in a way that successfully captures some of the author's own enthusiasm for the subject. Not surprising, in view of the author's own fields of interest, the sections on fast reactions in liquids and transient phase enzyme kinetics are especially valuable in this respect. A particularly useful feature of this book is the carefully thought-out problems at the end of each chapter that provide a challenging test of the reader's understanding of the material presented.

It is perhaps a measure of success of this book in stimulating the reader's interest, rather than a criticism, that there are several sections that I felt could have been given more space, although not at the expense of any of the other material. From my own prejudiced viewpoint I would have liked to have seen more space devoted to enzyme kinetics because of the successful way in which the author relates this subject to its background in chemical kinetics rather than treating it as a topic in isolation. I would have no hesitation in recommending this book most highly as a lucid and stimulating introduction to its subject.

K. F. Tipton

K. F. Tipton is Professor of Biochemistry at Trinity College, Dublin.

Sociobiology: Sense or Nonsense?

by Michael Ruse



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Sociobiology is one of the more contentious issues to have ruffled scientists this decade. Slanging matches have been conducted between leading biologists, pandemonium has broken out at conferences, the subject has been branded as a racist science, and the sociobiologists have been likened to reactionary political thinkers.

To evaluate this emotive topic, Michael Ruse, a philosopher of science, scientifically and philosophically analyses these controversies 'to see what case can be made for sociobiology and what case can be made against it'. He impartially discusses subjects such as animal and human altruism, racism, sexism, homosexuality, capitalism, falsifiability, and coherence. The current status and the future of sociobiology is assessed and the possible effects on the social sciences are considered.

The leading exponent of sociobiology, Edward Wilson, says that Ruse's book is the best written to date and is done so with unusual clarity. Indeed, the layman will find the book eminently readable and enlightening.

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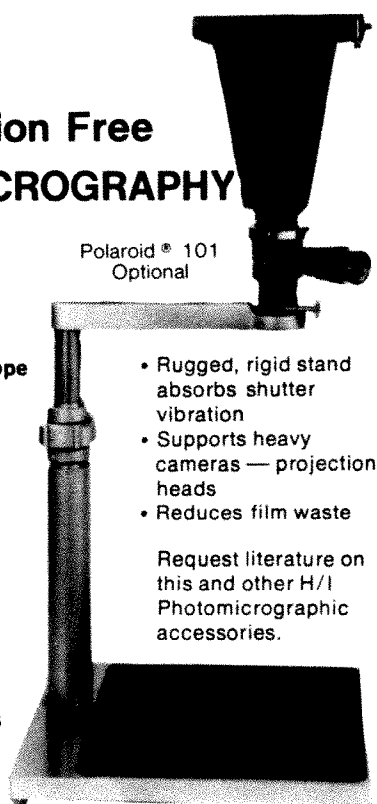
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Mycotoxins

Toxicology, Biochemistry and Pathology of Mycotoxins. Edited by K. Uraguchi and M. Yamazaki. Pp. 288. (Halsted/Wiley: New York and London; Kodansha: Tokyo, 1978.) £19.50.

"THE concept of mycotoxins is not yet widely appreciated" is a key statement in the preface to this book. To most laymen the notion that common moulds may produce potent animal toxins may be novel, but many industrial organisations involved in the production of human and animal foodstuffs are becoming well aware of mycotoxins. As is to be expected with a new branch of science which just happens to embrace one of the most carcinogenic substances, opinions vary from those who would attribute to mycotoxins several human and animal syndromes of unknown aetiology, to those who may have good commercial reasons to claim that there is little evidence that these substances have ever done any harm, particularly to humans.

The literature on the mycology, chemistry, biochemistry and pathology of mycotoxins has, in the past fifteen years or so, been evidence of one of the fastest growing facets of biological science. The first comprehensive review was included in the 1971-72 Academic Press series *The Microbial Toxins*. In the past year Marcel Dekker have published their three-volume *Encyclopedic Handbook* which, although it took several years to produce, is perhaps the most comprehensive current text. The present book is a useful and interesting addition to the review literature. It is unique in that it is written entirely by eight Japanese scientists, all of whom have published in the areas they review. It is natural, therefore, that there should be a thread of national emphasis through the book, in which the subject is treated mainly in the context of foodstuffs.

Western literature has tended to ascribe the origin of the recent wave of mycotoxin research to the 'Turkey X' syndrome in England during 1960. It is therefore salutary that the introduction to this book should emphasise the precedence of Japanese research into an epidemic of human acute cardiac beriberi in Japan between the two world wars. These studies led to the isolation of the yellowed rice toxins in the 1940s, though too late for direct verification of their involvement in the aetiology of cardiac beriberi—the epidemic by then having passed away, apparently, for good.

A notable feature of this book is the high standard of readability of the text. Several 1976 references are included, implying that the book can

claim to number among its 1,000 references most of the relevant studies available for review. It would be possible to instance a number of minor errors and omissions in the text but since mycotoxicology is expanding so quickly there is much to commend fairly rapid publication of a review-type book before it becomes too outdated.

The first chapter lists important mycotoxin-producing fungi and their significance concerning foodstuffs. The second chapter on the chemistry of mycotoxins is subdivided according to biosynthetic features, a treatment reminiscent of W. B. Turner's important 1971 book on fungal metabolites. The next three chapters are devoted to toxicology, the main topic of the book. There are sections on general and comparative toxicology. The fate, metabolism and mutagenicity of mycotoxins are discussed and cellular alterations, histopathology and carcinogenicity are liberally illustrated. As the chronic carcinogenic properties

of certain mycotoxins, notably aflatoxin, are of particular relevance to humans, the book concludes with a short chapter on traditional Japanese fermented foods, home-grown rice and imported peanuts and peanut products. Traditional foods are given a clean bill of health as the selection over many centuries of *Aspergillus* 'koji-moulds' for the preparation of miso, sake and soy sauce seems not to have included any capacity for aflatoxin biosynthesis. Further, the absence of aflatoxin from mouldy rice, otherwise contaminated with ochratoxin A and the aflatoxin precursor, sterigmatocystin, suggests that aflatoxin producing moulds are not indigenous to the relatively high latitudes in which Japan is situated.

This book is to be commended as providing a most useful oriental view of mycotoxicology.

P. G. Mantle

P. G. Mantle is Senior Tutor in Biochemistry at Imperial College, University of London, UK.

Physiology and pathology of the neutrophil

The Neutrophil: Function and Clinical Disorders. By S. J. Klebanoff and R. A. Clark. Pp. 810. (Elsevier/North Holland: Amsterdam, 1978.) \$148; Dfl.333.

IN recent years immunologists have focused their attention on macrophages and lymphocytes and minimised the importance of the neutrophil as a component of the cell-mediated defence system. This excellent book should help to redress the balance by stimulating interest in this fascinating cell by supplying a comprehensive account of contemporary knowledge of its physiology and pathology and a thorough citation of the literature.

In these days of specialisation a work as comprehensive as this, encompassing over 800 pages and possibly five times as many references, is generally tackled by a team of expert contributors. One of the strengths of this book is that the authors do not have vested interests in most of the contents and as a result the facts are presented in an unbiased manner without any attempt to influence readers' interpretation of the data. This has resulted in a highly informative exposition of current concepts that are expressed clearly and concisely. The mass of information that has been assembled is more suitable as a source

of reference than as an easily readable introduction to this cell.

A wide range of topics of cell biology and pathology have been systematically covered, including both normal and disordered structure, movement, phagocytosis, degranulation and antimicrobial systems of the cell as an entity, and its interaction as a component of the inflammatory process as a whole. The discussion of clinical disorders of neutrophil function covers the well known primary cellular defects such as Chronic Granulomatous Disease, myeloperoxidase deficiency and the Chediak-Higashi syndrome in addition to those abnormalities of function which occur as a consequence of abnormal humoral immunity or in association with systemic disease. There is little direct description of laboratory methodology, although this would be readily available from the literature references; and the rapidly expanding subject of leukapheresis and granulocyte transfusion is hardly touched upon.

The book is well produced, with numerous figures and diagrams. The index is extensive and comprehensive, as befits a reference volume. It will be of use to clinicians in the fields of infectious diseases, immunology, haematology and rheumatology, and an essential addition to the library of experimental biologists, particularly those with an interest in host defence in general and in the neutrophil in particular.

Anthony W. Segal

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Soil constituents

The Chemistry of Soil Constituents. Edited by D. J. Greenland and M. H. B. Hayes. Pp 469. (Wiley: London and Chichester, UK, 1978.) £26.

THIS enterprise is most welcome, as apart from the two encyclopaedic volumes (*Soil Components*, Springer: Berlin, 1975) edited by J. E. Gieseking, no advanced textbook of soil chemistry has been published for many years. The editors consider their subject falls naturally into two parts, the present volume *The Chemistry of Soil Constituents* covering static aspects, and a volume to be published shortly *The Chemistry of Soil Processes* covering dynamic aspects.

The introductory chapter (28 pages) by the editors places the topics of later chapters in the context of soil science as a whole. Chapter 2 (150 pages) entitled 'The Structures and Chemistry of Soil Clay Minerals' by the Rothamsted team of G. Brown, A. C. D. Newman, J. H. Rayner and A. H. Weir is a book in itself. Although clear accounts of the classical aluminosilicate clays already exist, for example, by Grim, this chapter treats the inorganic oxides and fibrous magnesium silicates more fully. Recent developments in two directions are particularly well described. Many soil clays consist of interstratified minerals in which different types of unit—kaolinite, smectite, and so on—alternate in a regular or irregular manner, and the account of the unravelling of these structures is very valuable. Soils developed from weathered volcanic ash commonly have such unusual features as a bulk density less than 0.85 g cm^{-3} . They contain the thread-like mineral imogolite and amorphous allophane; and the confused literature on these substances has been clarified. The excellent electron micrographs are a feature of this chapter. Much trouble has been taken to show complicated structures in two-dimensional diagrams, but this remains a problem, and I would like to have seen some stereopairs.

Chapter 3 (122 pages) entitled 'The Chemistry of Soil Organic Colloids' by M. H. B. Hayes and R. S. Swift deals with the chemical and physical properties of humified substances—not the materials from which they are derived, so lignin and polyphenols, for example, receive little mention. Those who have already decided that humus chemistry is a complex topic best left to its specialist devotees are unlikely to

change their opinion on reading this chapter. The authors rightly emphasise experimental methods of fractionating the humus moieties, and discuss how reliably they have been identified. The size, shape and weight of humus polymers are clearly described. Humic substances are distinguished in this account from "compounds belonging to recognisable classes, such as polysaccharides, polypeptides and altered lignins". The polysaccharides are thoroughly treated in a separate section, but the organic forms of nitrogen, sulphur and phosphorus receive no attention.

The rest of the book concentrates on surface phenomena. C. J. B. Mott and D. J. Greenland (chapter 4, 29 pages) describe the structure of the mineral surfaces and the determination of their area. G. W. Arnold (chapter 5, 44 pages) deals with surface charges and V. C. Farmer (chapter 6, 38 pages) with the structure of water on the surfaces. Together these chapters contain by far the most coherent account of soil surfaces yet assembled. Arnold's discussion of the point of

zero charge in soils is particularly illuminating. I wish he had treated the effect of Ca^{++} ions in ordering adjacent clay tactoids more thoroughly. The electrical double layer theory he so well describes does not account for this effect, on which the structure of most soils, and thus agriculture, depends. This latter section of the book cries out for a discussion of the affinity of the various surfaces for cations and anions, and a comprehensive account of cation and ligand exchange. These matters are to be treated in the second volume.

The writing throughout is clear and concise, and there are few serious errors. The index is good and the general standard of production high—so too is the price, though the scientific value per page is well above average. The book is unlikely to be replaced for at least a decade, so I rate it a good though expensive buy for all advanced students of soil chemistry.

P. H. Nye

P. H. Nye is Reader in Soil Science at the University of Oxford, UK.

Introductory oceanography

Introductory Dynamic Oceanography. By S. Pond and G. L. Pickard. Pp. 240. (Pergamon: Oxford, 1978.) Hardback £15; paperback £5.

ALTHOUGH a number of books published recently have given a general introduction to physical oceanography, a need has remained for a textbook providing a sound dynamical treatment, starting from first principles. This gap has been filled by Pond and Pickard's new book, which has evolved from a course of lectures given for a number of years at the University of British Columbia to students drawn from several disciplines. Its origin is reflected in the detailed explanations of many concepts which are frequently taken for granted in textbooks.

The book starts with a description of the properties of sea water and the basic physical laws relating to movements of water in the sea. There follows a clear explanation of the equations of continuity and motion, including a good discussion of the Coriolis effects. The influence of non-linear terms is given a separate chapter, which includes a discussion of the scales of terms in the equations of motion and introduces the role of the Rossby, Ekman and Richardson numbers in this context.

The chapter devoted to frictionless geostrophic flow gives a thorough treatment of the basic dynamics and its practical application to oceanographic data. The longest chapter in the book is that on currents with friction, which starts with the classical Ekman spiral theory and leads on to solutions applicable to the large scale wind-driven circulation of the oceans. The treatment of the boundary layer approach given in the last part of the chapter is more advanced than the rest but is well done.

A feature of particular interest is the inclusion of a chapter on numerical models. Without going into computational details, it gives a good account of the principles of modelling the ocean circulation and the treatment of boundary conditions, followed by a description and critical discussion of several individual models. The chapters on waves and tides are less detailed than the rest of the book and are largely qualitative. There are, however, other textbooks which deal with these topics.

The authors have succeeded in producing a textbook which will not only give the reader a basic understanding of physical oceanography but also bring him to the stage where he can appreciate advances being made at the frontiers of the subject.

K. F. Bowden

K. F. Bowden is Professor of Oceanography at the University of Liverpool, UK.

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STABLE ISOTOPE FACILITY DIRECTOR, CHILDREN'S NUTRITION LABORATORY

The United States Department of Agriculture has established a Children's Nutrition Laboratory under the Human Nutrition Center, Science and Education Administration. This laboratory is located within and administered under a cooperative agreement with Baylor College of Medicine and Texas Children's Hospital in Houston, Texas. The mission of the laboratory is the determination of nutrient requirements in infants and children during an age range which spans from conception to the adolescent. The emphasis is on normal nutrient requirements as a standard for the many applied programs in human nutrition of the Federal Government.

Because of the mandate to investigate nutritional requirements in normal children, it is determined to be essential that a Stable Isotope Facility be established as part of this national laboratory for childhood nutrition research. The Stable Isotope Facility will participate in intramural and extramural research projects which are designed to utilize the facility for stable isotope nutrient synthesis, nutrient isolation, and atomic mass ratio measurement and interpretation.

A Director of the Stable Isotope Facility is being sought to provide leadership in research development and operation of this national facility. This scientist should be an experienced biologist, physiological chemist or nutritionist who has demonstrated academic capacity and administrative experience in metabolic research utilizing mass spectroscopy. The Director of the division ideally should be a physician, however, this is an optional requirement.

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SENIOR BIOCHEMIST/ STAFF PHARMACIST

This new post will be in our Area Clinical Pharmacology Department. The successful applicant will work under the medical direction of the Area Clinical Pharmacologist and will develop and supervise assay methods for the investigation of drug based problems, phase one drug studies, clinical trials and the routine estimation of selected compounds.

Applicants should be graduates in Biochemistry, Chemistry, or Pharmacy, or their equivalent. They will have had at least four years' relevant post-graduate experience and be familiar with G.L.C. or H.P.L.C. techniques. The post involves laboratory management and supervision of the Biochemist, Research Pharmacist and Technician in post, and of a second Pharmacist, second Technician and part-time nurse to be recruited. Research and co-operation with the University Department of Clinical Pharmacology and Therapeutics will be encouraged.

Salary for this post is on the scale £5,451 per annum to £6,837 per annum. It is likely that new entrants to the Health Service will start on the minimum of the scale.

Application forms and job descriptions are available from the Personnel Department, 10 University Road, Leicester. Tel. Leicester 28464.

Closing date for completed applications is March 21. 9957(A)

UNIVERSITY OF THE WEST INDIES—JAMAICA

Applications are invited for three posts of

**SENIOR LECTURER/
LECTURER/
ASSISTANT LECTURER**

in the

DEPARTMENT OF PHYSIOLOGY

Appointees will be expected to participate in the development of the teaching and research programme of the Department. Particular emphasis will be placed on applicant's experience in the fields of neurophysiology, neuromuscular physiology, endocrinology and cardiovascular physiology. The Physiology Department teaches medical students preparing for the MBBS Stage 1 Part 1 Examination of the medical course, B.Sc. (Special) Physiology and Postgraduate Residents in the Faculty of Medicine.

Salary scales: non-medical, Senior Lecturer, J\$12,066 to J\$15,897 p.a.; Lecturer, J\$8,913 to J\$13,917 p.a.; Assistant Lecturer, J\$7,236 to J\$7,926 p.a.; medical, Senior Lecturer, J\$15,339 to J\$19,326 p.a.; Lecturer, J\$11,262 to J\$15,015 p.a.; Assistant Lecturer, J\$9,102 to J\$9,936 p.a. (£1 sterling = J\$3.42.) F.S.S.U. Study and Travel Grant. Unfurnished accommodation will be let by the University at a rental of 10% of salary or a housing allowance of 20% of salary will be paid. Family passages.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees should be sent as soon as possible direct to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica.

Applicants resident in the U.K. should also send one copy to the Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 9915(A)

UNIVERSITY OF OTAGO Dunedin, New Zealand

**VISITING
ASSISTANT LECTURER
IN CHEMISTRY**

Applications are invited for the position of Visiting Assistant Lecturer in the Department of Chemistry.

Applicants should hold a Ph.D. degree or its equivalent and have had some postdoctoral teaching or research experience in inorganic chemistry. The position would suit an applicant who wishes to take sabbatical leave, as ample opportunity will be given for research.

The position is for one year from August 1, 1979, although consideration would be given to an earlier starting date, or for tenure for less than one year.

Salary scale: NZ\$8,682 to NZ\$10,659 per annum.

Further particulars are available from the Association of Commonwealth Universities (Acpu), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on April 30, 1979. 9931(A)

POSITION AVAILABLE THE INTERNATIONAL POTATO CENTER

APARTADO 5969, LIMA, PERU

Announces a postdoctoral position in agronomy. Research duties will be concerned with optimizing potato production in tropical locations.

A candidate should have a Ph.D. degree in agronomy and be familiar with fertilizer, water and crop management strategies appropriate to optimizing crop yields. Field experience with potato production while desirable is not essential.

The position is for a three-year term and following an initial orientation in Peru, the successful candidate will be stationed in south-east Asia. The position offers an excellent opportunity to become familiar with international agriculture in developing countries in the tropics.

Please contact Dr S. Sadik, Head, Department of Plant Physiology, for application forms (air mail). 9990(A)

UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF BIOCHEMISTRY POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited from biochemists, molecular biologists or microbial geneticists to join a group investigating DNA repair and genetic recombination in *Escherichia coli*. The project is supported by the Medical Research Council until March 31, 1981. The work will involve a combination of biochemical and genetic techniques, and previous experience in nucleic acid biochemistry, molecular genetics, enzyme purification or DNA cloning would be advantageous.

Commencing salary will be within the scale £3,883 to £6,535 (under review) with full superannuation provision.

Applicants should send a curriculum vitae and the names of two referees to Dr P. T. Emmerson, Department of Biochemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU. 9948(A)

**THE AUSTRALIAN
NATIONAL UNIVERSITY
RESEARCH SCHOOL OF
BIOLOGICAL SCIENCES
DEPARTMENT OF
DEVELOPMENTAL BIOLOGY
POSTDOCTORAL FELLOWS
(two positions)**

The appointees will work with Professor B. E. S. Gunning on a programme of research on ultra-structural aspects of morphogenesis in plants, with emphasis on the roles of microtubules. Applicants should have experience of electron microscopy.

POSTDOCTORAL FELLOW

The appointee will work on problems of compartmental analysis in plant cells. Applicants should have experience in this and related fields of short distance transport in plants.

Appointments will be for two years. CLOSING DATE APRIL 15, 1979.

Salary on appointment will be in accordance with qualifications and experience, a fixed point within the range \$A15,786 to \$A20,606 per annum. Current exchange rates are \$A1 56p US\$1.13.

Reasonable appointment expenses are paid.

Superannuation benefits are available for applicants who are eligible to contribute. Assistance with finding accommodation is provided for an appointee from outside Canberra.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain further particulars from the Association of Commonwealth Universities (Acpts), 36 Gordon Square, London WC1H 0PF. 9980(A)

**UNIVERSITY OF GLASGOW
DEPARTMENT OF ASTRONOMY**

Applications are invited for a two year (S.R.C.)

**POSTDOCTORAL RESEARCH
ASSISTANTSHIP**

to collaborate with Dr J. C. Brown on developing the theory of interaction of energetic electrons with the solar atmosphere. The research will be oriented toward diagnosing hard X-ray and optical emissions from solar flares observed from NASA's SMM satellite. A background in theoretical physics/astrophysics would be highly desirable, and specific experience in plasma astrophysics or radiation theory/diagnostics advantageous.

Starting date will be between July 1 and December 1, 1979 and starting salary will be toward the lower end of the range £3,883 to £4,882 (under review) + U.S.S. Further details obtainable from Dr J. C. Brown, Department of Astronomy, University of Glasgow, G12 8QQ, Tel. 041-339-8855, Ext. 7183, to whom applications should be sent with curriculum vitae and names of at least 2 referees by May 1, 9979(P)

**THE UNIVERSITY OF
ALBERTA
FACULTY OF AGRICULTURE
AND FORESTRY
Academic Position
ASSISTANT PROFESSOR**

First degree in Veterinary Medicine and Ph.D. in Animal Science with broad interest and experience in Animal (Swine) Breeding and Genetics. Duties will include teaching at introductory and senior levels plus research and extension.

Appointment July 1, 1979 is subject to availability of funds.

Normal starting salary \$19,320 per annum (second step of Assistant Professor range of \$18,530 to \$24,059).

The University of Alberta is an equal opportunity employer.

Forward applications, including curriculum vitae, transcripts and names of three referees, by June 1, 1979 to:

Chairman
Department of Animal Science
Faculty of Agriculture and Forestry
The University of Alberta
Edmonton, Alberta
T6G 2E3. 9968(A)

**CARDIOTHORACIC
INSTITUTE
DEPARTMENT OF
MEDICINE
London University**

Medical Laboratory Scientific Officer required. Experience in immunology desirable. Work will mainly involve the laboratory investigations of immunological responses in patients with lung cancer. Further information and applications, giving details of age and experience to:

Mr R. A. Perkins, Secretary,
Cardiothoracic Institute, Fulham Road,
Brompton, London SW3 6HP, by
May 1, 1979 and quoting Ref. 01/79.
9967(A)

**UNIVERSITY OF
BIRMINGHAM
DEPARTMENT OF
CANCER STUDIES**

Applications are invited for a
LECTURESHP
(non-medical)

In the Department of Cancer Studies.

Candidates should have a higher degree and an established record of research in a field relevant to the study of the aetiology of cancer. The successful applicant will be expected to contribute to teaching programmes for both medical and science undergraduates and to play a role in supervising postgraduate students.

Salary will be in the range £3,883 to £7,754 plus superannuation.

Applications giving the names of three referees should be sent by March 30, 1979, to the Assistant Registrar, Medical School, Birmingham B15 2TJ. Further particulars may be obtained from Professor D. G. Harnden, the Department of Cancer Studies at the Medical School. 9926(A)

**THE UNIVERSITY
OF SYDNEY
PROFESSIONAL OFFICER,
RADIOCARBON DATING**

Applications are invited for a permanent position as Professional Officer in charge of an established radiocarbon laboratory. Appointee will be expected to maintain the consulting work of the laboratory and generate suitable research projects in radiocarbon dating.

Applicants should have a Ph.D. or equivalent research experience. Preference to someone with experience in natural radiocarbon assay using liquid-scintillation counting.

Salary within range for Professional Officer Grade II: \$A15,674 to \$A16,684 per annum. Appointment will commence as soon as possible.

Applications, with curriculum vitae, publications and names of up to three referees to the Registrar, University of Sydney, N.S.W. 2006, Australia, by April 2, 1979. Further details from Associate Professor R. B. Temple, School of Chemistry, in the University. 10010(A)

**IMPERIAL COLLEGE OF
SCIENCE AND TECHNOLOGY
LONDON
LECTURESHP
IN BIOCHEMISTRY**

A vacancy exists for a Lectureship in Biochemistry in the general area of Applied Microbial Biochemistry. Applications from those with interests in the expression of eukaryotic genes in *E. coli*.

For the potential production of medically or industrially useful proteins will be especially welcomed. The Department is fully equipped for the most modern techniques relevant to these interests.

Salary will be within the range £3,883 to £7,754 (under review), plus £450 London Allowance and is subject to U.S.S.

Applications, which should include a curriculum vitae, the names of three referees and a statement of future research plans, should be sent to Professor B. S. Hartley, F.R.S., Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ. 10009(A)



**UNIVERSITY OF ABERDEEN
UNIVERSITY OFFICE, REGENT WALK
ABERDEEN AB9 1FX
Tel: 0224-40241**

**DEPARTMENT OF BIO-MEDICAL PHYSICS &
BIO-ENGINEERING**

Research Assistant

Applications are invited for this post in a small group working on an evaluation of properties of short-term cultures of human tumour biopsy specimens with particular reference to their chemosensitivity. Applicants should have a degree in one of the biological sciences. Experience in either tissue culture or cancer research would be advantageous.

The appointment, funded by Cancer Research Campaign, is tenable until 31 October 1979, to commence as soon as possible. Extension for a further year is likely, subject to satisfactory progress.

Salary £3,883 to £4,382 per annum, on Range 1A Research and Analogous Staff Scale, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by March 30, 1979.

DEPARTMENT OF CHEMISTRY

Research Assistant

Applications are invited for the above post financed by the UK Atomic Energy Authority, under the supervision of Dr G. G. Cameron and concerned with methods of decomposing plastic and other polymeric items. The post is tenable until December 31, 1980 from a date to be arranged as soon as possible. Applicants should hold a university degree or equivalent, preferably at honours level in chemistry.

Salary within Range 1B Scale for Research and Analogous Staff, £3,384 to £4,382 per annum. Candidates interested in a part-time appointment will be considered.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by March 30, 1979.

DEPARTMENT OF GEOLOGY AND MINERALOGY

Postdoctoral Research Fellowship

Applications are invited from suitably qualified organic geochemists or clay mineralogists to work with Dr M. J. Fearson on the correlation of organic matter and clay mineral diagenesis in sediments from the North Sea Basin. The project involves the study of kerogen maturation and hydro-carbon generation during burial in relation to clay mineral transformations as a response to temperature increase and ion concentration in interstitial water.

The post is tenable for three years and is supported by the Natural Environment Research Council.

Salary £4,382 to £4,882 per annum on Research and Analogous Staff Range 1A Scale, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by March 30, 1979.

DEPARTMENT OF BIOCHEMISTRY

**Postdoctoral Research Fellowship/
Assistantship**

Applications are invited for Postdoctoral Research Fellowship to determine the amino acid sequences of pyruvate kinase; pre-doctoral applicants with suitable interests and experience will be considered. The project is a collaborative one with the protein crystallography group at the University of Bristol, and will lead to a detailed description of the molecular structure of pyruvate kinase. The successful applicant will work in collaboration with a pre-doctoral research assistant already in post, with Dr L. A. Fothergill's research group which is currently studying the structures of four other glycolytic enzymes.

The appointment is supported by the SRC and is available for a period of up to three years. Starting date by arrangement. Salary within Range 1A, £3,883 to £4,882 per annum, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by April 20, 1979. 10001(A)

free university amsterdam

The Faculty of Medicine, laboratory for Physiology invites applications for the position of a

(senior) research assistant

Applicants should have experience in experimental cardiovascular physiology. Ph.D. degree in Physiology or H.D. degree is required.

Additional information can be obtained from prof. dr. A.A. Knoop, phone 020 - 548 27 19

Candidates are expected to agree with the christian charter of the Free University. Candidates are requested to submit their applications, including a curriculum vitae and names and addresses of three referees, within one month after this announcement, to the Personnel Department, P.O. Box 7161, 1007 MC Amsterdam, the Netherlands, quoting reference no. 216-379. The Free University is situated at the De Boelelaan 1085, 1081 HV Amsterdam, the Netherlands.

9944(A)



AGRICULTURAL RESEARCH COUNCIL INSTITUTE OF ANIMAL PHYSIOLOGY Babraham, Cambridge CB2 4AT SCIENTIFIC OFFICER

required in the Endocrine Laboratory of the Department of Applied Biology. The research involves biochemical analytical methods, experience with such techniques and an interest in electronics would be an advantage. Applicants should have a pass degree, HNC or equivalent in a relevant biological science. Salary in scale £2,839 to £4,415 per annum, according to qualifications and relevant experience. Non-contributory superannuation scheme. Application form and further details may be obtained from the Secretary of the Institute Quote Ref. D.R.B. 2.

Closing date March 23rd 1979
9977(A)

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF IMMUNOLOGY LECTURESHIP IN RADIOBIOLOGY

Applications are invited for the post of LECTURER (non-medical) in RADIOBIOLOGY. Main teaching responsibilities are in the biological side of an interfaculty M.Sc. Course in Radiobiology and Medical Physics, taking 10-12 graduates per year. In addition there will be administrative responsibility in respect of the Faculty radioisotope counting service, and in advising staff and students on the design of projects involving the use of ionising radiations. Adequate time is available for pursuing its associated research in association with Departments in the Faculty or Hospital, depending on the successful applicant's research interests.

Salary on the scale £3,883 to £7,751 plus superannuation

Applications, giving the names of three referees, should be sent to the Assistant Registrar, The Medical School, Birmingham B15 2TT, by March 30, 1979.

Further particulars may be obtained from Professor J. Hardwicke in the Department of Immunology, The Medical School 9977(A)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF BIOCHEMISTRY RESEARCH ASSISTANT

Applications are invited from graduates in Biochemistry or related subjects to work on a project investigating the turnover of specific proteins in normal and abnormal muscle. The project is funded by the M.R.C. and is under the supervision of Dr R. J. Beynon.

The post will be tenable from April 1, 1979, at an initial salary of £3,384 per annum.

Applications, together with the names of two referees, should be received not later than March 30, 1979, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref. RV/514/N 9920(A)

DEPARTMENT OF GENETICS UNIVERSITY OF NEWCASTLE UPON TYNE RESEARCH ASSOCIATE Proteins of Animal Viruses

Applications are invited for the above post (salary scale £3,888 to £6,555) from candidates holding a Ph.D. degree in any of the following fields: virology, biochemistry, genetics or microbiology. The post is to be funded from a Wellcome Trust grant and is tenable for a two-year period (starting date negotiable).

The research will involve the development and assessment of new analytical methods for sequentially ordering peptides from several virus proteins isolated from different strains and mutants of the parainfluenza virus Newcastle Disease Virus (NDV). Work with NDV requires that the successful candidate should not normally come into contact with live birds.

Previous experience with animal viruses, protein or nucleic acid sequencing and tissue culture an advantage. Familiarity with aseptic technique essential.

Written applications including a full curriculum vitae together with the names and addresses of three academic referees should be sent to Dr A. C. R. Samson, Department of Genetics, Ridley Building, The University, Newcastle upon Tyne NE1 7RU.

Closing date for applications: April 6, 1979 9923(A)

QUEEN ELIZABETH COLLEGE Kensington (University of London) POSTDOCTORAL RESEARCH ASSISTANT IN THE DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a post to work on the biosynthesis of collagen in chondrocytes isolated from normal and arthritic cartilage. Particular attention will be paid to post-translational steps in collagen biosynthesis, and a knowledge of collagen chemistry or tissue culture techniques would be an advantage. The appointment will be for up to three years and will commence as soon as possible at starting salary of up to £4,408 per annum (plus London allowance). Further particulars may be obtained from, and applications in the form of a curriculum vitae including the names and addresses of two referees should be sent to, Dr R. G. Price, Biochemistry Department, Queen Elizabeth College, Campden Hill, London W8 7AH. 9963(A)

PORTSMOUTH POLYTECHNIC DEPARTMENT OF BIOLOGICAL SCIENCES 2 RESEARCH ASSISTANTS

required for D.O.E.-supported work on the introduced brown alga *Sargassum muticum*. One post will be concerned with investigating aspects of the alga's physiology and morphogenesis; the other post with monitoring the alga's distribution and evaluating clearance trials.

Applicants should hold, or be about to obtain, a good degree in a biological subject. The post will be for three years, and candidates may be able to register for a higher degree. Candidates should have SCUBA diving ability and a current driving licence.

Salary scale £2,589 to £2,700 per annum.

Application forms and further details from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth PO1 2QQ, to whom completed application forms should be returned by March 22, 1979 9940(A)

A.R.C. Animal Breeding Research Organisation BIOCHEMISTRY

A SCIENTIFIC OFFICER required to prepare and carry out radioimmunoassays and assist other experimental work, so with animals, as a member of small team studying the physiology of reproduction and lactation farm animals in the Department Physiological Genetics at Roslin near Edinburgh.

Candidates should have a pass degree, H.N.C. or equivalent biochemistry or biological science and preferably appropriate laboratory experience. Salary on scale £2,839 to £4,415 with start salary depending on qualifications and experience. Non-contributory superannuation.

Further information and application forms obtainable from the Secretary, A.R.C. Animal Breeding Research Organisation, W. Mains Road, Edinburgh EH9 3JF. Closing date March 24, 1979.

9948(A)

THE OPEN UNIVERSITY



Vice-Chancellor

The University is to appoint a Vice-Chancellor to take up office in the spring of 1980 to succeed Lord Par. Persons interested in the appointment are asked to write, in confidence, possible not later than the end of April 1979, to the Chairman of the Committee (Professor B. B. Boycott FRS), c/o The Secretary (JSVC), The Open University, Walton Hall, Milton Keynes, MK7 6AA.

Information about the University and the Office of Vice-Chancellor may be obtained from the Secretary of the University, Walton Hall, Milton Keynes, MK7 6AA. 9962(A)

Careers for Microbiologists..

on the fringe of the Lakes

Glaxo Operations UK Limited is the largest British pharmaceutical company in the UK and is responsible for the UK manufacture of Glaxo pharmaceuticals and their sale in this country. The Ulverston factory, involved in bulk phar-

maceutical production, has one of the country's largest fermentation works and is located in the southern part of the Lake District. The area is renowned for its natural unspoiled beauty and offers a wide range of recreational amenities.

STRAIN SELECTION

A graduate scientist is required to join a large multi-disciplinary Development Unit, which is responsible for finding new strains that will improve our fermentations. The Unit undertakes a wide range of microbial projects, including mutation and breeding studies, selection techniques, screening systems, culture preservation and seed stage development. You will have the opportunity

to contribute in a number of these areas and, therefore, should have a sound knowledge of microbiology or related disciplines, preferably with relevant post-graduate experience. Close collaboration with scientists of other disciplines within the Unit is necessary, and liaison with universities and research institutes will also be involved.

QUALITY ASSURANCE

This is an opportunity in the Microbiological Section to undertake microbiological control and investigational work, initially monitoring production processes and their environments. This will involve you in close liaison with the production function and other disciplines within Quality Assurance. There will be scope later to broaden

your activities into sterility testing and microbiological assay. Career prospects, for example into laboratory management, are good.

You should have a degree in microbiology, preferably with a professional qualification, and have had experience of quality control associated with sterile pharmaceutical production.

For both appointments, salaries will be according to qualifications and experience, together with an opportunity to participate in Glaxo Group profitability. There are the usual benefits associated with a major company, including relocation expenses, where appropriate, to this very pleasant

part of Cumbria.

Please write or telephone for an application form to: Richard Nelson, Personnel Department, Glaxo Operations UK Limited, Ulverston, Cumbria LA12 9DR. Tel: (0229) 52261, ext. 92, quoting ref. U/35.



10011(A)

CARDIOTHORACIC INSTITUTE

(Associated with the National Heart and Chest Hospitals)
University of London

Two appointments in asthma research are vacant. Appointees may be expected to undertake research on animal tissues *in Vitro* and *in Vivo*, as well as participating in clinical research projects. Preferred areas of interest would be smooth muscle pharmacology and second messenger systems.

Applicants should be at graduate or postdoctoral level, pharmacologists or biochemists. The appointment will be as research assistants on the appropriate salary scale for the University. Postdoctoral appointees may be nominated to lectureship; recent graduates would be encouraged to register for a higher degree.

Applications to be made to the Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP. 9966(A)

UNIVERSITY OF BRISTOL RESEARCH IN THEORETICAL CHEMISTRY

Applications are invited for a research position at either a postgraduate level (three years) or a postdoctoral level (two years) to carry out research in developing new methods in the field of *ab initio* molecular electronic structure calculations. The research project will be aimed at developing accurate, yet viable, methods for the calculation of wavefunctions and potential energy surfaces of molecules containing chemically interesting atoms with large numbers of electrons. The position is available from October 1979. For further details apply to: Dr G. G. Balint-Kurti, School of Chemistry, Bristol University, Bristol BS8 1TS. 10000(A)

UNIVERSITY OF LEICESTER DEPARTMENT OF PHYSICS RESEARCH ASSOCIATE IN CONDENSED MATTER PHYSICS

Applications are invited for a postdoctoral research associate to undertake a study of liquid semiconducting alloys using photoelectron spectroscopy. The project is part of a wider investigation of the properties of disordered systems being carried out in the Physics Department and supported by the Science Research Council.

The post is tenable initially until September 1981 and the salary is from £3,883 per annum. Applications with a curriculum vitae and the names of two referees should be sent to: Dr C. Norris, Department of Physics, University of Leicester, LE1 7RH. 9999(A)

UNIVERSITY OF NOTTINGHAM

SCHOOL OF AGRICULTURE
TEMPORARY LECTURESHIP
IN SOIL SCIENCE

There will be a vacancy for a Lecturer in Soil Science in the Department of Physiology and Environmental Studies. The appointment will be for two years from September 1, 1979, with a possible extension for the third year, and it will be made in the first three steps of the Lecturer scale.

The main emphasis in the Soil Science Section relates to physical and chemical aspects of soil/plant relationships and it is expected that the person appointed would strengthen these interests: research experience is desirable.

Further particulars and forms of application, returnable not later than March 23, 1979, from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Ref. No. 675. 10002(A)

UNIVERSITY OF NEWCASTLE UPON TYNE SCHOOL OF PHYSICS DEPARTMENT OF GEOPHYSICS AND PLANETARY PHYSICS Applications are invited for the post of RESEARCH ASSOCIATE

tenable for one year at a salary of £4,382. The person appointed will work with Dr D. W. Collinson in developing a new type of magnetometer for the measurement of the remanent magnetism of rocks and minerals. Applicants should have a degree in physics or equivalent qualifications, and an interest in instrumentation.

For further particulars write to Dr D. W. Collinson, Department of Geophysics and Planetary Physics, School of Physics, The University, Newcastle upon Tyne NE1 7RU. 9941(A)

UNIVERSITY OF LONDON GOLDSMITHS' COLLEGE New Cross, London SE14 6NW SCHOOL OF SCIENCE AND MATHEMATICS BIOLOGICAL SCIENCES DEPARTMENT

Applications are invited for a half-time

LECTURESHIP IN BOTANY

in the Biological Sciences Department from September 1, 1979 for a period of three years. The person appointed will be expected to provide teaching in the fields of cell biology and evolutionary biology. A good general background in botany would be an advantage.

Salary pro rata on the scale £4,575 by 10 increments to £7,032 per annum inclusive of London Allowance.

Write for further details to the Personnel Officer, to whom applications should be sent by March 30, 1979. 9949(A)

UNIVERSITY OF DUNDEE DEPARTMENT OF BIOLOGICAL SCIENCES Applications are invited for two LECTURESHIPS

in Microbiology in the above Department. No field of interest is excluded but it is hoped that one post will be filled by someone with an interest in microbial/molecular genetics.

The posts are permanent and superannuable under U.S.S. Salary scale (under review) £3,883 to £7,754 with entry at points appropriate to the ages, qualifications and experience of the successful candidates.

Applications (six copies, overseas applicants one copy) quoting Reference F/32/79J and naming three referees should be lodged by March 27, 1979, with The Secretary, The University, Dundee DD1 4HN, from whom further particulars are available. 9936(A)

SCOTTISH HORTICULTURAL RESEARCH INSTITUTE Invergowrie, Dundee DD2 5DA AGRICULTURAL RESEARCH COUNCIL POSTGRADUATE STUDENTSHIP

Applications are invited for a Studentship tenable for three years from October, 1979 in the Zoology Section to undertake research on the feeding behaviour of longidorid nematodes in relation to the histological changes induced in the roots of host plants.

Candidates should be qualified in nematology, zoology or botany. The minimum qualification is an Upper Second Class Honours degree and students graduating in 1979 will be eligible.

Application forms and further particulars are available from the Secretary of the Institute. The closing date for applications is March 31, 1979. 9978(F)

Firmenich

Many engaged in the manu-
industry of synthetic perfumes
opening for a

SENIOR TOXICOLOGIST

at its corporate headquarters in Geneva, Switzerland

Reporting directly to the corporate Management, the person appointed will assume the following major responsibilities:

- to represent FIRMENICH worldwide in matters of product safety,
- to provide a comprehensive advisory service to customers and government agencies, as well as to other companies in Firmenich's international Group,
- and, in general, to help formulate and implement Group policy for product safety, including the organization, supervision and interpretation of toxicity and dermatology tests carried out by independent laboratories.

The successful candidate will already have made a substantial impact in this field.

Ability to speak and write fluently in English as well as a good working knowledge of French are necessary, other languages being a further asset.

For more information please write in confidence to

Mr ERNEST K. WEBER, Director of Personnel

FIRMENICH SA - Département du Personnel
Case postale 238 - 1211 Genève 8

9925(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL (University of London) Denmark Hill, London SE5 8RX LIVER UNIT

Applications are invited for the post of
RESEARCH ASSISTANT

in this busy multi-disciplinary unit, to participate in a project investigating immune responses to liver antigens in Liver disease.

Candidates should have some post-degree experience, possibly an M.Sc., and should be able to work independently without close supervision.

Salary will be on the nationally agreed salary scales for research staff, according to age, qualifications and experience.

Please apply, giving full curriculum vitae and the names and addresses of two referees to the Secretary of the Medical School at the above address. Telephone 01-274 6222, ext. 2040 for further details.

Closing date: March 29, 1977.
9911(A)

CHELSEA COLLEGE University of London Basic Medical Sciences Group DEPARTMENT OF PHYSIOLOGY

There is an opportunity for a young person with an interest in electronics to develop this interest and apply it to physiological problems. The post is for a **GRADE 4 TECHNICIAN** in the Department of Physiology to take part in teaching and research.

We should be pleased to hear from suitably qualified or experienced and interested persons who should, in the first instance, contact Susan Holly, Department of Physiology, Manresa Road, London SW3 6LX (01-352 6421) for further details and application forms.

The salary scale for this post is £3,420 to £3,867 per annum (under review), including £465 London Allowance.
9965(A)

Regional Cytogenetics Laboratory EAST BIRMINGHAM HOSPITAL BASIC GRADE SCIENTIFIC OFFICER

Applications are invited for the new post of Scientific Officer. Experience in cytogenetics desirable but not essential. Salary range depending on experience and qualifications—£2,991 to £4,899 per annum.

Application forms and job descriptions obtainable from Regional Cytogenetics Laboratory, East Birmingham Hospital, Bordesley Green East, Birmingham B9 5ST. Tel No 021-772-4311, Ext. 4354.
9947(A)

UNIVERSITY OF KEELE DEPARTMENT OF COMMUNICATION AND NEUROSCIENCE

Applications invited for research studentships in postgraduate Department engaged in research into sensory brain mechanisms. The Department has several interdisciplinary research groups working in such fields as visual and auditory psychophysics, visual and auditory neurophysiology, speech, blindness and deafness. Both experimental and theoretical work is carried out and specialised electronic, optical, acoustic, physiological and computing facilities are available in well-equipped laboratories.

A limited number of studentships are likely to be available for suitably qualified candidates who wish to proceed to an M.Sc. or Ph.D. degree. Interested students with backgrounds in biology, electrical or electronic engineering, mathematics, physics, physiology or psychology are asked to write to:

Dr D. H. Foster,
Department of Communication and Neuroscience,
University of Keele,
Keele,
Staffordshire
ST5 5BG.
9984(A)

UNIVERSITY OF THE WEST INDIES—JAMAICA Applications are invited for the post of PROFESSOR OF ANATOMY in the FACULTY OF MEDICINE

The appointee will be expected to give leadership in the teaching and research activities of the Department of Anatomy which prepares students for the M.B., B.S. degree of the University of the West Indies, and in the teaching of Surgical Anatomy to postgraduate students of Surgery. In connection with the implementation of the new curriculum, experience in teaching integrated courses in the pre-clinical and clinical subjects will be an additional asset.

Salary in the range, J\$19,326 to J\$22,578 p.a. (Medical), J\$17,166 to J\$21,252 p.a. (Non-medical) (£1 sterling=J\$3.42) F.S.S.U. Unfurnished accommodation will be let by the University at a rental of 10% of salary or a housing allowance of 20% of salary will be paid Study and Travel Grant. Family passages.

Detailed applications (2 copies) with curriculum vitae and naming three referees should be sent direct as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica.

Applicants resident in the U.K. should also send one copy to the Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.
9912(A)

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF PATHOLOGY

Applications are invited for the office of

UNIVERSITY DEMONSTRATOR IN THE DEPARTMENT OF PATHOLOGY

Candidates should have a higher degree and appropriate research experience. The person appointed will be expected to demonstrate in general aspects of the pathological disciplines and to carry out research within the Division of Virology. Principal research interests of the Division are the biology of herpes and influenza viruses.

The initial appointment will be for three years from May 1, 1977. The pensionable stipend for a University Demonstrator will be on a scale of £4,133 rising by four annual increments to £5,129, with initial placing above the minimum where appropriate. The maximum tenure of a University Demonstratorship is five years. A grant is made towards removal expenses.

Candidates should send 12 copies of their application together with the names of not more than three referees to Mr G. R. ANDERSON, General Board Office, The Old Schools, Cambridge CB2 1TT, from whom further information can be obtained to arrive not later than March 28, 1977.
9919(A)

UNIVERSITY OF OXFORD NUFFIELD LABORATORY OF OPHTHALMOLOGY POSTDOCTORAL RESEARCH FELLOW and TECHNICIAN/RESEARCH ASSISTANT

required for three years for an investigation of the crosslink sites of collagen from normal and diseased sclera as part of a study of degenerative myopia, a common cause of blindness. Starting salaries up to £5,129 (postdoctoral) and £2,955 (technician grade 4). Experience of protein chemistry would be an advantage.

Further information from Dr J. J. Harding, Nuffield Laboratory of Ophthalmology, Walton Street, Oxford OX2 6AW to whom applications with curriculum vitae and the names of two referees should be submitted as soon as possible.
9935(A)

UNIVERSITY OF GLASGOW SENIOR LECTURER (POLLOCK LECTURER) IN CLINICAL PHARMACOLOGY

Applications are invited for the post of Senior Lecturer in the University Department of Materia Medica for a medical graduate with research and teaching experience in aspects of human pharmacology. It is expected that the successful applicant will establish and maintain an active research group. The present research interests of the department are cardiovascular and respiratory pharmacology and clinical aspects of pharmacokinetics and drug metabolism. Research interests outside these fields would not necessarily be a disadvantage. The department is responsible for teaching medicine and clinical pharmacology and therapeutics to medical students.

Salary will be fixed according to placement on the University scale for clinical teachers, maximum £12,068 (with consultant status).

Further details may be obtained from Professor John Reid, Department of Materia Medica, Stobhill General Hospital, Glasgow, telephone 041 55 0111.

Applications (12 copies), giving the names and addresses of three referees should be lodged with the Secretary of the University Court, (Room 18), University of Glasgow, Glasgow G1 8QQ, not later than March 30, 1977.

In reply please quote Ref No 9958(A)

UNIVERSITY OF DURHAM DEPARTMENT OF PHYSICS Applications are invited for a POSTDOCTORAL SENIOR RESEARCH ASSISTANTSHIP IN PHYSICS

for two years, starting as soon as possible after April 1, 1977, to work in collaboration with the Max Planck Institute for Radioastronomy in Bonn on the interpretation of the Galactic Continuum Radiation. Candidate should preferably have experience in cosmic rays, plasma physics or radio astronomy and an interest in computing.

Initial salary in the range £3,883 to £5,129 (under review), plus supernannuation.

Applicants (three copies) naming three referees should be sent by March 31, 1977, to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained.
10006(P)

UNIVERSITY OF GLASGOW SENIOR LECTURER (POLLOCK LECTURER) IN CLINICAL PHARMACOLOGY

Applications are invited for the post of Senior Lecturer in the University Department of Materia Medica for a medical graduate with research and teaching experience in aspects of human pharmacology. It is expected that the successful applicant will establish and maintain an active research group. The present research interests of the department are cardiovascular and clinical aspects of pharmacokinetics and drug metabolism. Research interests outside these fields would not necessarily be a disadvantage. The department is responsible for teaching medicine and clinical pharmacology and therapeutics to medical students.

Salary will be fixed according to placement on the University scale for clinical teachers, maximum £12,068 (with consultant status).

Further details may be obtained from Professor John Reid, Department of Materia Medica, Stobhill General Hospital Glasgow, telephone 041-558 0111.

Applications (12 copies), giving the names and addresses of three referees, should be lodged with the Secretary of the University Court, (Room 18), University of Glasgow, Glasgow G12 8QQ, not later than March 30, 1977.

In reply please quote Ref No 9975(A)
8375M

**SENIOR/ASSOCIATE
SCIENTISTS
IN EXPERIMENTAL
CELL MORPHOLOGY AND
CYTOGENETICS**

The W. Alton Jones Cell Science Center is interested in expanding its research program by the recruitment of two new staff members at the level of SENIOR or ASSOCIATE SCIENTIST (FULL OR ASSOCIATE PROFESSOR EQUIVALENT). The Center is an operational unit of the Tissue Culture Association and has an active program of research on a variety of topics. It is concerned, as well, with advanced training and education involving cell tissue and organ cultures. The laboratories are modern, spacious, and well equipped. The Center has affiliations with the University of Vermont, SUNY at Plattsburgh, and the University of Rhode Island.

Suitable candidates will have a Ph.D. or M.D. and have made significant contributions to their chosen field. They should currently hold positions of not less than Assistant Professor or equivalent. It is expected that the candidates' research will involve the use of cell tissue or organ culture. It is expected as well that the candidates will have an established track record in obtaining research support from the funding agencies.

For further information on the Center and its programs, contact the Office of the Director, W. Alton Jones Cell Science Center. Resumes and names of four references to the Chairperson, Search Committee, In Care of W. Alton Jones Cell Science Center, Old Barn Road, Lake Placid, New York 12946.

The W. Alton Jones Cell Science Center is an Affirmative Action/Equal Opportunity Employer. Applications from women and minority groups are encouraged. 9922(A)

**THE AUSTRALIAN
NATIONAL UNIVERSITY**

Applications are invited from suitably qualified persons for appointment to the following position.

**THE JOHN CURTIN SCHOOL
OF MEDICAL RESEARCH
DEPARTMENT OF
MICROBIOLOGY
RESEARCH FELLOW**

The appointee will work with Dr G. Air and Dr W. G. Laver on the molecular biology of influenza virus variants. The project involves selection of influenza virus variants with monoclonal antibodies, then immunological analysis of their proteins and nucleic acids.

The appointment, which is funded by a grant from the National Institutes of Health, U.S.A., is for one year in the first instance with probable extensions for a further two years. It is hoped that the successful applicant would be able to take up the appointment in the near future.

Salary: Commencing salary will be in accordance with qualifications within the range \$A15,786 to \$A17,716. Current exchange rates are \$A1:56p:US\$1.13.

Other Conditions: A limited grant towards travel and removal expenses may be made. Assistance with housing is provided for an appointee from outside Canberra.

The closing date for receipt of applications is April 5, 1979. They should be sent to the Registrar, P.O. Box 4, Canberra, ACT 2600, Australia. 9981(A)

**POSTDOCTORAL
RESEARCH POSITION**

Atmospheric scientists with research interests in the photochemical modeling of the troposphere and stratosphere, the evolution of planetary atmospheres, and/or atmospheric radiative transfer are invited to apply for postdoctoral positions at the University of Florida. Applicants should contact W. L. Chameides, Department of Physics and Astronomy, University of Florida, Gainesville, Florida 32611. The University of Florida is an equal opportunity/affirmative action employer. 9969(A)



Wellcome

Electrochemist

Department of Physical Chemistry

Our Physical Chemistry Department has an unusual opportunity for a young graduate with a degree in chemistry or related subject. The department uses many sophisticated techniques in studying molecular structures and provides a service to other research areas.

Reporting to a research scientist, the successful applicant will assist in the application of electrochemistry to chemical, biochemical and biological problems. Experience, although desirable, is likely to be less important than a keen interest in the subject. Where it is appropriate, extra-mural training will be encouraged.

The Wellcome Foundation Limited is a major British-owned international pharmaceutical organisation. Our Research Laboratories are located in beautiful parkland surroundings in Beckenham, Kent. Conditions of employment include 4 weeks' holiday, pension scheme, subsidised canteen, excellent sports and social facilities and generous assistance with relocation expenses, where appropriate.

Please write, quoting reference number U/4, to

P. C. Anderson, Personnel Assistant,
The Wellcome Research Laboratories,
Langley Court, Beckenham, Kent BR3 3BS.



10008(A)

**POSITION AVAILABLE
THE INTERNATIONAL POTATO
CENTER**

APARTADO 5969, LIMA, PERU

Announces a postdoctoral position in tropical agronomy. Research emphasis will be on developing agronomic practices for growing potatoes in the hot humid tropics.

A successful candidate would be expected to have a background in agronomy and soils. Duties would include assessment of selected genotypes for adaptation and management of plants grown from both tubers and true seed. Early concentration of work would be in selected sites in Peru with the possibility of associated work in central America and the Philippines.

Applicants must have a Ph.D. in agronomy. The assignment is for three years. A knowledge of potato culture and the Spanish language is not essential but desirable. Please contact Dr S. Sadik, Head of the Physiology Department, for application forms (air mail). 9992(A)

**HARVARD MEDICAL
SCHOOL
NEUROBIOLOGIST**

The Department of Neurobiology is seeking a new faculty member at the Assistant-Professor level to begin 1979 or 1980. Applications from women and minority people are encouraged. Please send curriculum vitae and a brief outline of research plans, and have three letters of reference sent to Search Committee, Department of Neurobiology, Harvard Medical School, 25 Shattuck Street, Boston MA 02115. 9946(A)

**A well-known
PHARMACEUTICAL GROUP
located in Paris requires a
RESEARCH SCIENTIST**

for its
Mass Spectrometer Department
who will be responsible for:

- Operating a gas chromatograph-mass spectrometer—data system. He will also assist non-specialist users to operate the system and interpret their results.
- Carrying out routine maintenance, performing minor repairs and maintaining adequate stocks of spare parts.
- Purchasing and preparation of gas chromatography columns (both packed and capillary), selecting the appropriate column for each analysis and assisting in the development of analytical methods.

Applicants should be suitably qualified and have at least 2 years experience of analysis of trace metabolites by gas chromatography. Experience with mass spectrometry would be an advantage, as well as an ability to operate complex equipment. Fluent French not required but appreciated. English would be an advantage.

Applications will be treated in the strictest confidence and should be sent directly to 'Nature', who will forward. 'Nature', c/o Advertisement Department, Box 9997, 3 Dyma Buildings, Holborn, London EC1N 2NR. 9997(A)

**UNIVERSITY OF DURHAM
DEPARTMENT OF
PHYSICS**

Applications are invited from graduates, preferably with Ph.D. in the field of High Energy Astrophysics, for a

**POSTDOCTORAL SENIOR
RESEARCH ASSISTANTSHIP**

tenable for three years from as soon as possible. The successful candidate will join a research group working in experimental GAMMA RAY ASTROPHYSICS and should have some experience with balloon borne experiments. Salary on National Research Range 1A £3,883 to £6,555 (under review) plus superannuation.

Applications (three copies) naming three referees should be sent by March 26, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. 9936(P)

**THE UNIVERSITY OF
MANCHESTER
LECTURESHIP IN
CHEMISTRY**

Applications are invited for the post of Lecturer in (Inorganic) Chemistry tenable from October 1, 1979. Preference may be given to applicants with research interests in the organometallic chemistry of the non-transition metals.

Initial salary within the range £3,883 to £4,831 per annum. Superannuation. Further particulars and application forms (returnable by May 1) from the Registrar, The University, Manchester M13 9PL. Quote reference 52/79/N. 10004(A)

THE OPEN UNIVERSITY
FACULTY OF SCIENCE



BIOLOGY TECHNICIANS

Biology Histology Technician

We require an experienced technician to take responsibility for organising and providing an histology service within the discipline. It is hoped that the post will also provide some photographic support.

Applicants should possess H.N.C. or equivalent qualification and at least six to ten years' experience in histology.

Salary on scale: £2,955—£3,402 p.a. (under review).

Ref: 3786

Biology Research Technician

The technician will be responsible for the day-to-day organisation of the Brain Research Group's main laboratory, and for assisting in the various research projects.

Applicants should possess H.N.C. or equivalent qualification and at least eight years' experience in biological or biochemical research laboratories.

Salary on scale: £2,955—£3,402 p.a. (under review).

Ref: 3787

Physiological Psychology Technician

The Science Faculty have a tenured vacancy in the Biology Discipline for a Technician to work with Dr. Geoff Eilon on investigations of the visual system. The technician will assume responsibility for the day-to-day running of a laboratory which uses electrophysiological recording and histological procedures to investigate visual projections in the avian forebrain. The experimental work involves the use of amplifiers, recorders, stimulators; programming and use of purpose built and general purpose microprocessor and general purpose computers; the use of a cryostat microtome and histological staining procedures; and the analysis and presentation of experimental data. The technician will be expected to contribute to all aspects of the research programme and become an integral member of the research group.

Applicants should have an H.N.C. or equivalent and 10-12 years' practical experience preferably in neurophysiological research.

Salary on scale: £3,654—£4,365 p.a. (under review).

Ref: 447

All three appointments are available with immediate effect.

Application forms and further particulars are available from The Recruitment Office (JD3786/3), The Open University, P.O. Box 75, Walton Hall, Milton Keynes, MK7 8AL or telephone Milton Keynes 63404; there is a 24 hour answering service on 63868.

PLEASE QUOTE THE REFERENCE NUMBER OF THE POST IN WHICH YOU ARE INTERESTED.

Closing date for applications: 23rd March.

9987(A)

Fife Health Board

Fife Area Laboratory

A vacancy has arisen for a

SENIOR GRADE BACTERIOLOGIST

in the Department of Medical Microbiology of Fife Area Laboratory. Candidates should be graduates with appropriate basic grade experience.

The Microbiology Department carries out diagnostic work for the hospitals, general practitioners and environmental health departments throughout Fife. The person appointed would be responsible to the Consultant Bacteriologist and should be able to assist in the preparation of reports on miscellaneous clinical specimens and in the supervision of the public health section. Experience in mycology and/or entomology would be desirable.

Further particulars and application forms available from the District Personnel Officer, East Fife District, Fife Health Board, Hayfield Road, Kirkcaldy (Telephone: Kirkcaldy 66141, Ext. 11) to whom they should be returned no later than March 31, 1979. The laboratory may be visited by arrangement with the Consultant Bacteriologist, Dr J. D. Barrie (Telephone: Kirkcaldy (0592) 61155, Ext. 350).

HEALTH CARE FOR A KINGDOM

9930(A)

UNIVERSITY OF ALBERTA DEPARTMENT OF ZOOLOGY EDMONTON, ALBERTA, CANADA

The Department will make tenurable appointments at the rank of Assistant Professor (minimally \$18,530.00 per annum, 1978-1979) on July 1, 1979. The Department well-developed Honours, Specialized and Graduate programmes, and present has 29 academic staff, graduate students, and 27 support staff. Applicants for the Assistant Professor positions must have a Ph.D. degree equivalent and should send a curriculum vitae, a brief statement of research objectives, a list of publications, the names of three referees to Dr F. Chia, Chairman, Department of Zoology, University of Alberta, Edmonton, Alberta, Canada T6G 2E9. deadline for receiving application May 1, 1979. The University of Alberta is an equal opportunity employer.

(1) Limnologist. Applicants with research interests in experimental quantitative limnology of lakes preferred. The person appointed expected to share in the teaching limnology and quantitative ecology to establish a strong research programme.

(2) Marine Invertebrate Zoologist. Individuals with research interests in any aspect of biology of marine invertebrates are invited to apply. Preference is given to applicants with work with molluscs or annelids. A person appointed is expected to share in the teaching of invertebrate zoology and marine biology and to establish a strong research programme.

9950(A)

UMIST APPOINTMENT OF POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Postdoctoral Research Assistantship, financed by the Department of Energy, to investigate the thermal decomposition of kerogen and related natural materials extracted from shales removed during exploratory oil-well drilling. Experience of analytical procedures such as thermal analysis, gas-liquid chromatography and mass spectrometry are desirable, though not essential.

The appointment will be for a maximum of two years and the work will be under the supervision of Mr J. M. Bather.

The salary will be on a range, being £4,382 per annum for a Ph.D. aged 26.

Applications, with curriculum vitae and the names of two referees, should be sent as soon as possible to Professor P. G. Ashmore, Department of Chemistry, UMIST, P.O. Box 88, Sackville Street, Manchester M60 1QD 9982(A).

UNIVERSITY OF SURREY DEPARTMENT OF BIOCHEMISTRY SENIOR LECTURER IN NUTRITION

Applications are invited for the full time position of Senior Lecturer in the Department of Biochemistry. The person appointed will contribute to the undergraduate and postgraduate teaching and research in the Department. Preference will be given to candidates with a particular interest in clinical nutrition.

Applications must be graduates with a higher degree and a good research record. A medical qualification is desirable but not essential.

Salary will be within the range £7,506 to £9,263 per annum, according to qualifications and experience. Superannuation will be under U.S. conditions.

Further particulars about the post may be obtained from the Academic Registrar (LFG), University of Surrey, Guildford, Surrey GU2 5XH, or telephone 71281, ext. 452. Application from men and women in the form of a curriculum vitae together with the names and addresses of three referees should be sent to the above by March 23, 1979. 9937(A)

UNIVERSITY OF LEEDS Applications are invited for the post of Trainee Technician in the Food Technology Laboratories of the Procter Department of Food Science. In the first instance the post involves working under supervision with a wide variety of food processing equipment and instrumentation, preparation of food products for clinical and assisting with the running of classes. Later training in mechanical construction of research apparatus; use of electronics could form a significant part of the evolving du further education on a day release basis will be arranged.

Salary will be on the scale £1 to £2,199 per annum (under review depending on age). Applications, giving age and details of school record should be sent to Professor David Robinson, Procter Department of Food Science, The University, Leeds LS2 9JT. 9976(A)

UNIVERSITY OF DUNDEE

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

There is an immediate vacancy in the above Department, based at Ninewells Hospital and Medical School, Dundee, for a

RESEARCH TECHNICIAN

to join a group engaged in a study of oncofetal proteins. Experience of protein purification techniques and radioimmunoassay is desirable though not essential.

The post is financed from an external grant and is available in the first instance for a period of three years, with a possibility of renewal.

An applicant with an appropriate H.N.C. or equivalent qualification and at least eight years' relevant experience would be eligible for appointment at a salary of up to £3,720 per annum (under review) but an appointment at a lower grade could be offered to a less well-qualified and experienced applicant.

Applications, giving details of qualifications and experience and the names of two referees should be sent to The Secretary, The University, Dundee DD1 4HN by March 22, 1979. Please quote Ref: EST/121/79J. 9929(A)

PLANT BREEDING INSTITUTE

Maris Lane, Trumpington
Cambridge CB2 2LQ

HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER

CYTOGENETICS DEPARTMENT

A research worker with experience in plant cytogenetics/cell biology is required for three years, commencing April 1, 1979, to participate in and be responsible for parts of a programme funded by the Ministry of Overseas Development to study the cytogenetics and cell biology of endo-perm development and grain quality in cereals with special attention to grain shrivelling in wheat-rye genotypes including triticale.

Candidates should have a 1st or upper 2nd class honours degree in an appropriate biological science and at least two years postgraduate experience in plant cytogenetics/cell biology or related research.

The appointment will be as Higher Scientific Officer (£4,101 to £5,448 per annum) or Senior Scientific Officer (£5,154 to £6,898 per annum). At least four years' relevant postgraduate experience is required for appointment as Senior Scientific Officer.

Applications with curriculum vitae and names and addresses of three referees should be sent to the Assistant Secretary (Establishment), quoting ref. CYT 47, by April 6, 1979. 9973(A)

THE UNIVERSITY OF LONDON

INSTITUTE OF OBSTETRICS AND GYNAECOLOGY AND

INSTITUTE OF NEUROLOGY

Postdoctoral post available for the study of endocrinological regulation of the reproductive system, with particular emphasis on the role of sex steroid. The successful candidate will be part of a research team working in new laboratories at the Institute of Obstetrics and Gynaecology, Hammersmith Hospital, Du Cane Road, W12, under the joint direction of Professor M. G. Elder and Dr L. Lim. Experience in molecular/cell biology, tissue culture and techniques associated with chromatin characterisation will be an advantage. Salary for this three-year appointment will be in the range of £3,883 to £4,882 per annum plus £450 London Allowance. Starting date can be negotiated (April to October 1979). Send applications with curriculum vitae to Dr L. Lim, Institute of Neurology, 33 Johns Mews, London WC1. 9988(A)

KUWAIT UNIVERSITY OF KUWAIT FACULTY OF MEDICINE

Medical Science and Clinical Appointments

Applications are invited for the following appointments in the Faculty of Medicine.

ANATOMY—Associate Professor. Candidates should have areas of specialisation in electron microscopy. The successful applicant's responsibilities will include directorship of the E.M. Unit, which is equipped with scanning and transmission microscopes. Assistant or Associate Professor. Preference will be given to candidates who have experience of teaching the anatomical disciplines to allied health students. The successful candidate will be responsible for co-ordinating allied health teaching in the Department of Anatomy.

COMMUNITY MEDICINE—Professor or Associate Professor in Epidemiology. Professor or Associate Professor in Public Health.

MEDICINE—Associate Professor. Candidates should be general physicians with a special interest in renal medicine and experience in haemodialysis, peritoneal dialysis and renal transplantation. Experience of renal morphology by light microscope would be an advantage.

MICROBIOLOGY—Professor or Associate Professor in Medical Bacteriology. Professor or Associate Professor in Clinical Bacteriology.

PATHOLOGY—Associate and Assistant Professors. Candidates should have experience in academic pathology, particularly in anatomic and histopathology. The successful candidates will have responsibilities in diagnostic pathology at the teaching hospital.

PHYSIOLOGY—Professor or Associate Professor in Neurophysiology.

Professor or Associate Professor in Endocrinology.

Professor or Associate Professor with specialisation in one of the following areas: Haematology, Gastrointestinal, Muscle or Cardiovascular.

SURGERY—Professor or Associate Professor in Urological Surgery.

Associate Professor in Traumatology and Burns.

Assistant Professor in Orthopaedics.

Applicants should possess a Ph.D. or equivalent higher medical qualification, and have conducted and published research in their field. Professors should have 14 years' experience, 4 as an associate professor or its equivalent, i.e. senior lecturer or reader. Associate professors should have 9 years' experience, 4 as an assistant professor or its equivalent, i.e. lecturer.

CONDITIONS OF APPOINTMENT

Salaries: Total monthly salaries will be within the following scales according to qualifications and experience (IKD—£1.80; US\$3.6 approx.).

Professors with clinical appointments: KD 755-860 (6 increments).

Professors medically or non-medically qualified with medical science appointments: KD 701-797 (6 increments).

Associate Professors with clinical appointments: KD 650-755 (6 increments).

Associate Professors medically qualified with medical science appointments: KD 605-701 (6 increments).

Associate Professors non-medically qualified: KD 575-665 (6 increments).

Assistant Professors with clinical appointments: KD 520-625 (6 increments).

Assistant Professors medically qualified with medical science appointments: KD 484-580 (6 increments).

Assistant Professors non-medically qualified: KD 460-550 (6 increments).

Clinical supplements: In addition to the above university salaries there will be a monthly clinical supplement paid by the Ministry of Public Health for medical school staff with clinical service commitments. These will be within the following scales:

Professor and Chairman: KD 200-250 (5 increments).

Professor: KD 150-200 (5 increments).

Associate Professor: KD 100-150 (5 increments).

Assistant Professor: KD 75.

Gratuity: There is a gratuity of one month's basic salary for each year employed which is payable on termination of contract.

Housing: Suitably furnished, air-conditioned accommodation, electricity and water are provided free of charge.

Medical Care: Free, comprehensive treatment is available in Kuwait under the State Health Service.

Travel: Airline tickets are provided from the country of recruitment for the appointee, spouse and up to 3 dependent children under 20 years. Thereafter return tickets are issued annually to the country of origin. On termination of contract, tickets are provided to the country of recruitment. A baggage and freight allowance is also provided. In addition, travel expenses for attending one scientific meeting a year are paid by the University.

Vacation: Sixty days paid annual leave as well as various official holidays.

Education: This is provided free in state schools where the instruction is in Arabic. Staff who have to send their children to non-Arabic schools in Kuwait will have the cost of up to a maximum of three met by the University.

Taxation: There is no income tax in Kuwait. Currency is freely transferable without restrictions.

Further details about these appointments and the method of application can be obtained from: The Dean, Faculty of Medicine, University of Kuwait, P.O. Box 5969, Kuwait.

Completed applications must be received by no later than April 25, 1979.

9993(A)

POSTDOCTORAL RESEARCH ASSOCIATE POSITION

To study role of poly(adenosine diphosphoribose) synthesis in eukaryotic DNA repair processes. Experience diphosphoribose) synthesis in eukaryotic DNA repair processes. Experience with DNA repair, nucleic acid and chromatin chemistry desirable. Send curriculum vitae and references to Dr N. A. Berger, Hematology-Oncology Division, Jewish Hospital, Washington University School of Medicine, 216 S. Kingshighway, St. Louis, Missouri, 63110.

Equal Opportunity Employer. 9860(A)

INSTITUTE OF OBSTETRICS AND GYNAECOLOGY MEDICAL LABORATORY SCIENTIFIC OFFICER

There is a vacancy at the Institute of Obstetrics and Gynaecology, Queen Charlotte's Maternity Hospital for a Medical Laboratory Scientific Officer for chemical investigation of lung surfactant in children with cystic fibrosis. The appointment will be for two years. Qualifications: Degree/H.N.C. in Chemistry or Biochemistry. Salary £3,243 to £3,720 per annum including London Weighting.

Applications with the names of 2 referees to:

Miss C. E. Parkinson,
Institute of Obstetrics and Gynaecology,
Queen Charlotte's Maternity Hospital,
Goldhawk Road,
London W6.

9986(A)

Scottish Institute of Agricultural Engineering

Head of Soils Section

Senior Scientific Officer/ Principal Scientific Officer (Ref. AG/18'B)

Re-advertisement

Applications are invited for the post of Senior Scientific Officer/Principal Scientific Officer to lead a team investigating the effects of agricultural machinery on the physical properties of field soils with particular reference to cultivations, zero-tillage and compaction.

Qualifications

A first or upper second class honours degree, and preferably a higher degree, or equivalent qualifications in a relevant branch of science or engineering. The applicant should have had considerable experience of research in soil physics or soil mechanics.

Salary Scales

Appointment in the grade of Senior Scientific Officer (£5,154-£6,898) or Principal Scientific Officer (£6,609-£8,461) according to qualifications and experience. Non-contributory superannuation.

Application forms and further information from the Secretary, Scottish Institute of Agricultural Engineering, Bush Estate, Penicuik, Midlothian, EH26 0PH, to whom applications should be submitted by April 2, 1979.

9917(A)

THE BRITISH INDUSTRIAL BIOLOGICAL RESEARCH ASSOCIATION

Histopathologist

The BIBRA Research Laboratories undertake research studies in the assessment of environmental chemicals for possible toxicity and are seeking a GRADUATE PATHOLOGIST/BIOLOGIST to strengthen their Pathology Department.

The position would involve the histopathological examination of tissues from safety evaluation studies and participation in fundamental research programmes designed to improve methodology and to give a better understanding of toxic effects.

The Department has modern facilities for histopathology, histochemistry and electron microscopy; the development of relevant research interests within these fields would be encouraged.

Applicants should be veterinary, medical or biology graduate with experience in pathology. Salary will be in accordance with age and experience.

Applications with curriculum vitae should be sent to:

**The Secretary
The British Industrial Biological
Research Association
Woodmansterne Road
Carshalton, Surrey SM5 4DS**

9964(A)

MEDICAL RESEARCH COUNCIL Clinical and Population Cytogenetics Unit Edinburgh

MOLECULAR OR CELL BIOLOGIST

A vacancy exists for a molecular or cell biologist to work with a small team studying the molecular organisation and function of human chromosomes. One area of interest involves the use of restriction enzymes in the study of DNA composition and variation in man and experience with relevant techniques, or in the handling of cultured mammalian cells and chromosomes, would be an advantage, but not essential. Candidates should have a Ph.D. or equivalent and the appointment will be made at an appropriate level on Medical Research Council scientific scales. Applications, including names of two referees, should be sent to the Administrative Officer, M.R.C. Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, by March 31, 1979.

9834(A)

THE UNIVERSITY OF CALGARY

Faculty of Medicine

DIVISION OF PHARMACOLOGY & THERAPEUTICS

Applications are invited for a position as an Assistant Professor commencing September 1, 1979.

Duties will include teaching in both the undergraduate and graduate educational programme.

Successful applicant must be an active researcher.

Salary and rank according to qualifications and experience.

Nominations and/or applications will be received by:

**Dr. S.H. Roth
Acting Head
Division of Pharmacology and Therapeutics
Faculty of Medicine
The University of Calgary
Calgary, Alberta
T2N 1N4**



9934(A)

THE UNIVERSITY OF MANCHESTER RESEARCH ASSOCIATE IN HIGH ENERGY PHYSICS

Applications are invited for the post of Research Associate with the experimental high energy physics group at the University of Manchester. The duties would be to work on the operation of an experiment on PETRA in the DESY Laboratory, Hamburg, and also to work on the analysis of the data obtained. It would be necessary to spend extended periods in Hamburg. Duties to start as soon as possible. Salary within the range £3,883 to £4,631 per annum. Superannuation. To make application (or to enquire further) please write to Professor P. G. Murphy, Department of Physics, The University, Manchester M13 9PL, including full curriculum vitae and the names of three referees (if applying without further enquiry). 9960(A)

UNIVERSITY OF THE WEST INDIES—BARBADOS Applications are invited for the post of LECTURER/ ASSISTANT LECTURER IN PHYSICS

The appointee will be expected to assume duty by September 1, 1979.

Salary scales: Lecturer, BDS\$19,071 to BDS\$29,799 p.a.; Assistant Lecturer, BDS\$15,480 to BDS\$16,974 p.a. (£1 sterling=BDS\$4.00.) F.S.S.U. Unfurnished accommodation will be let by the University at a rental of 10% of salary. A housing allowance of 20% of salary is payable to staff who make their own housing arrangements. Family passages. Study and Travel Grant.

Detailed applications (2 copies) with curriculum vitae and naming three referees should be sent direct as soon as possible to the Secretary, University of the West Indies, P.O. Box 64, Bridgetown, Barbados.

Applicants resident in the U.K. should also send one copy to the Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 9913(A)

UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF ZOOLOGY

The University invites applications for the post of
LECTURER

in the Department of Zoology, tenable from October 1, 1979. Candidates should possess a higher degree or equivalent research experience, and preference will be given to those with experience in invertebrate neurophysiology. Applicants with other qualifications, particularly in ecology or entomology may also be considered.

Salary will be at an appropriate point on the Lecturers' scale: £3,883 to £7,754 per annum, according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

Further particulars may be obtained from the Senior Assistant Registrar, The University, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU, with whom applications (three copies), together with the names and addresses of three referees, should be lodged not later than April 9, 1979. Please quote reference N. 10003(A)

BLOOD GROUP REFERENCE LABORATORY SCIENTIFIC OFFICER

A graduate is required to assist Dr C. M. Giles in reference work related to blood transfusion. This laboratory is recognised by the World Health Organisation as a Collaborating Laboratory for Reference and Research in Blood Grouping and many varied problems are investigated. Opportunities are available for study for a higher degree. Experience in the field of blood transfusion desirable.

Applications in writing, with curriculum vitae to Miss K. Lomas, Blood Group Reference Laboratory, Gatliff Road, off Ebury Bridge Road, London SW1W 8QJ. 9972(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL PEPTIDE SECTION (DEPARTMENT OF CHEMICAL PATHOLOGY) TECHNICIAN/SCIENTIFIC OFFICER

required in the Peptide Laboratory to take part in the synthesis of novel peptide hormone analogues. Applicant should possess H.N.C. or a degree in chemistry or biochemistry and have experience and/or interest in organic synthesis. The post is tenable for 1 months in the first instance, with possibility of further extension.

Salary within the range £3,646 to £4,199 placing dependent on qualifications and experience.

Application forms may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS quoting reference number 4/203/N. 9810(A)

UNIVERSITY OF OXFORD CLARENDON LABORATORY Temporary Teaching Appointment in Physics (Electronics)

Applications are invited for a five year appointment from October 1979, on the University Lecturer scale £3,883 to £8,257 per annum (under review), the duties being to lecture in electronics, assist with demonstrating and engage in advanced study and research. There are likely to be opportunities for the person appointed to assist also with college teaching. Further particulars may be obtained from Professor E. W. J. Mitchell, Clarendon Laboratory, Parks Road, Oxford OX1 3PU, to whom application should be sent by April 9. 9971(A)

UNIVERSITY OF SOUTHAMPTON INSTITUTE OF SOUND AND VIBRATION RESEARCH EXPERIMENTAL OFFICER

required by Industrial and Machinery Noise Group, to undertake design and testing of machinery components. The applicants should be graduate engineer with practical workshop and drawing office experience.

Salary scale £3,384 to £5,367 per annum (under review) plus U.S.S. benefits.

Applications, giving a brief curriculum vitae and the names of three referees, should be sent to D. A. S. Copland, The University, Southampton SO9 5NH, quoting reference 184/A/1. 9942(A)

RESEARCH ASSOCIATE PHYSIOLOGY AND BIOPHYSICS

Position available immediately concerning integrative studies of vascular smooth muscle and hypertension. Candidate must have Ph.D. or equivalent, and record of accomplishments in muscle structure or function or hypertension. Experience desirable in methods associated with one or more of the following: electrophysiology; microcirculatory hemodynamics. Send resume and names of three referees to: Dr William Halpern, Department of Physiology and Biophysics, University of Vermont, Burlington, Vermont 05405 U.S.A.

An Affirmative Action Employer. 9953(A)

UNIVERSITY COLLEGE DUBLIN

Applications are invited by the Governing Body of the College for the following full-time statutory appointment:

PROFESSORSHIP OF GEOLOGY

Prior to application, further information (including application procedure) should be obtained from the Secretary and Bursar, University College, Belfield, Dublin 4. Telephone enquiries: 693244, Ext. 431.

The closing date for receipt of completed applications is THURSDAY MARCH 29, 1979. 9921(A)

THE UNIVERSITY OF LEEDS

Department of Animal Physiology and Nutrition

Applications are invited for the post of LECTURER in the above Department, available from October 1, 1979. The appointment will be made for a fixed term period of three years. Applicants must have experience in either the nutrition or physiology of farm livestock.

Salary on the scale for lecturers £3,883 to £7,754 (under review), according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 61/5/D. Closing date for applications May 14, 1979.

9928(A)

UNIVERSITY COLLEGE OF NORTH WALES, BANGOR

Applications are invited for the post of

RESEARCH ASSISTANT

in the College's Department of Zoology, to assist in the experimental investigation of foraging behaviour in marine invertebrate predators.

The appointment, funded by the N.E.R.C. and commencing September 1, 1979, will be for three years, and will be at an initial salary of £3,384 per annum.

Applications (two copies), giving details of qualifications and experience, together with the names and addresses of three referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, from whom further particulars may be obtained.

Closing date for applications: March 26, 1979.

9939(A)

UNIVERSITY OF CAMBRIDGE ANIMAL PRODUCTIVITY/ NUTRITION

Applications invited for a University Demonstratorship in Animal Productivity/Nutrition in the Department of Applied Biology. Qualifications—good Honours degree, research experience in some aspect of animal productivity related to nutrition and competence in biometry. Salary in scale £4,133 to £5,129. Pensionable under U.S.S. Limited contribution to removal expenses. Further details from and applications including full personal particulars, list of publications and names and addresses of not more than three referees to the Secretary, Faculty of Biology 'A', Appointments Committee, Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX not later than March 31, 1979.

9974(A)

UNIVERSITY OF EDINBURGH DEPARTMENT OF ZOOLOGY LECTURER

(Sub-Department of Parasitology)

Applications are invited for the post of Lecturer in the Department of Zoology (Sub-Department of Parasitology). Candidates must be prepared to teach helminthology. Salary scale £3,883 to £7,754 per annum (under review), with placement according to qualifications and age, and with superannuation benefit.

The successful candidate will be expected to take up duty on October 1, 1979.

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, with whom applications (six copies), giving the name of two referees, should be lodged not later than April 10, 1979. Please quote Reference 1028.

9998(A)

POSITION AVAILABLE THE INTERNATIONAL POTATO CENTER

APARTADO 5949, LIMA, PERU

Announces a postdoctoral position in the utilization of true seed for the production of consumer potatoes. Research will involve studies of pollination and fruit development, harvesting, seed extraction, dormancy and germination.

The utilization of true seed as a means of propagating consumer potatoes is a relatively new and challenging field. The successful candidate must be innovative in research and have a sound botanical background. A knowledge of embryogenesis and seed physiology as part of a Ph.D. background would be essential.

The position is for a period of three years. A knowledge of Spanish is desirable but not essential. Please contact Dr S. Sadik, Head, Department of Plant Physiology, for application forms (air mail).

9991(A)

GUY'S HOSPITAL MEDICAL SCHOOL (University of London) JUNIOR LECTURER

in Biochemistry required as soon as possible.

Applicants should have a Ph.D. in Biochemistry. Duties include teaching biochemistry to Medical, Dental and B.Sc. students, and Chemistry to Preliminary students, and the successful applicant will be expected to initiate and carry out a research project of his own choosing.

The appointment will be for three years in the first instance. Salary range £4,655 to £5,604 plus £450 London Allowance and Superannuation. Further details may be obtained from Professor A. T. Diplock (01-407 7600, Ext. 2365). Application forms obtainable from the Dean, Guy's Hospital Medical School, London Bridge, London SE1 9RT. The closing date for applications will be April 6, 1979.

9955(A)

INDUSTRIAL TOXICOLOGIST

Bio-Research Laboratories Ltd. is the major contract research company in Canada and has immediate openings for industrial toxicologists who will become part of a multidisciplinary team of scientists involved in the evaluation in laboratory animals of the toxicological effects of drugs, food additives, pesticides and other industrial chemicals. In general, the responsibilities of the toxicologists will include design, supervision, interpretation and reporting of a variety of toxicological research including teratology, reproduction, subacute, chronic and carcinogenicity studies involving laboratory rodents, dogs and subhuman primates.

Prospective candidates should have a background in veterinary medicine, pharmacology, physiology or biochemistry, preferably with postgraduate training at the Ph.D. level and a minimum of two years' experience in industrial toxicology.

Our new modern laboratories are located in pleasant surroundings in a western suburb of Montreal. Salary and other benefits will be commensurate with training and experience. Please forward detailed résumé, including references and salary history and requirements to:

Dr. B. G. Procter,
Director of Toxicology,
Bio-Research Laboratories Ltd.,
87 Senneville Road,
Senneville, Quebec, Canada.
H9X 3R3

BIO-RESEARCH LABORATORIES LTD

9970(A)

FELLOWSHIPS and STUDENTSHIPS

As a pharmaceutical Research Institute, we are a subsidiary of the worldwide SANDOZ-Group. Our objective is the discovery of new approaches to the therapy and prophylaxis of infectious diseases.

Currently, we offer a

POSTDOCTORAL FELLOWSHIP

in our Department of Bacteriology. This opening is concerned with the influence of antibiotics on phagocytosis, and the efficacy of antibiotics against bacteria within phagocytes.

The successful candidate will find excellent working conditions in well equipped laboratories. The appointment will be for an initial 2-year period. We offer an attractive salary. Travel expenses from and to the country of origin will be paid.

We invite applications from **biochemists**, **cell-biologists** or **microbiologists**. Experience in antibiotic research will be helpful but is not essential.

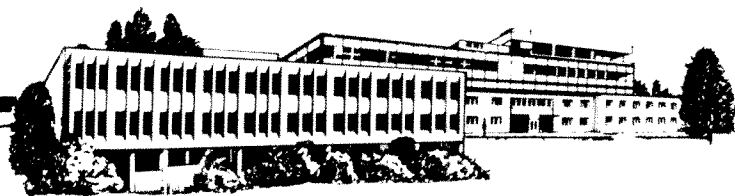
Please send application including curriculum vitae and credentials to



Dr. N. Zacherl, Personnel Manager,
SANDOZ Forschungsinstitut,
A-1235 Wien, Brunner Strasse 59,
Austria.

9918(A)

FELLOWSHIPS and STUDENTSHIPS



Industrial Fellowship

Organic Chemistry

Merck Sharp & Dohme Research Laboratories invite applications for an Industrial Fellowship, tenable for two years at their Development Laboratories at Hoddesdon, Hertfordshire.

The Organic Chemistry Department carries out a wide range of assignments concerned with the syntheses of compounds for clinical and pharmacological investigations and also for medicinal chemistry research programmes.

The position provides a unique opportunity for a young post-graduate chemist to gain experience in the pharmaceutical industry of working from small scale bench experiments through to pilot scale preparations.

The successful candidate will have a research degree in Organic Chemistry (probably Ph.D.) with some experience in physical-organic chemistry.

Further details and an application form may be obtained from: The Personnel Manager.



Merck Sharp & Dohme Limited,
Hertford Road, Hoddesdon, Herts EN11 9BU.
Tel: Hoddesdon 67272 ext 377.

9994(E)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF EARTH SCIENCES

O.D.M. RESEARCH STUDENTSHIPS IN GEOPHYSICS

Applications are invited for two Ph.D. research studentships, funded by the Ministry of Overseas Development, to undertake the regional gravity survey of western and southern Sudan. Certain seismological investigations will also be carried out in conjunction with the survey. The projects are to investigate the structure of the Jebel Marra volcanic centre and the surrounding Precambrian basement which is mainly obscured by more recent sediments.

Applicants should have a good background knowledge of geophysics at undergraduate or M.Sc. level and possess full driving licences.

The studentships are for three years starting October 1, 1979. Requests for further information and applications, including a full curriculum vitae and the names of two referees should be made to Dr G. W. Stuart, Department of Earth Sciences, The University of Leeds, Leeds LS2 9JT.

9938(F)

UNIVERSITY OF NOTTINGHAM

and

Tropical Products Institute

RESEARCH STUDENTSHIP

Silage from Tropical Fish

Applications are invited from those holding at least a 2(i) Honours B.Sc. degree or an M.Sc. degree, in biochemistry, chemistry, animal science or food science, for a T.P.I. Research Studentship tenable at the University of Nottingham.

The successful applicant will be based in the Food Science Laboratories of the Department of Applied Biochemistry and Nutrition, Sutton Bonington, and will be required to work full-time on a programme for the development and nutritional testing of silage from tropical fish and register for a research degree of the University of Nottingham. During the tenure of the studentship the appointee, who will be selected following an interview, will probably be required to spend a period of time overseas.

Those interested should write, in the first instance to:

The Secretary,
University of Nottingham,
School of Agriculture,
Sutton Bonington,
Loughborough,
Leicestershire LE12 5RD.
Telephone: Kegworth 2386

10005(F)

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF CERAMICS, GLASSES AND POLYMERS

Research Studentships in the Physics and Chemistry of Materials

The Department expects to be able to offer a number of Research Studentships this year over a wide range of topics in the science and technology of materials.

Two C.A.S.E. studentships, a Harwell-sponsored studentship and an independently financed studentship are already available. Further C.A.S.E. studentships which may become available shortly are for work in association with a number of major companies in the chemical, electrical and materials manufacturing industries. They are likely to be of interest to graduates in physics, chemistry, materials science and, in one case, applied mathematics or theoretical physics.

Further information from the Head of Department, Professor J. P. Roberts, Department of Ceramics, Glasses and Polymers, "Elmfield", Northumberland Road, Sheffield S10 2TZ.

Quote Ref. 255/ . 9937(F)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY

Applications are invited for two POSTDOCTORAL RESEARCH FELLOWSHIPS

in BIOCHEMICAL ENDOCRINOLOGY supported by the Cancer Research Campaign and The Wellcome Trust to work with Dr B. A. Cooke on the hormonal control of normal and neoplastic Leydig cells. Experience of cell culture and hormone receptors would be an advantage.

Commencing salary within range £3,909 to £4,655 plus £450 London Allowance.

Further information from the School Secretary, R.F.H.S.M., 8 Hunte Street, London WC1N 1BP (or telephone 01-837 5385, ext. 54), to whom applications should be submitted as soon as possible, quoting reference CRC/W. 9924(A)

NEW ZEALAND UNIVERSITY OF CANTERBURY

Christchurch

Applications are invited for a UNIVERSITY POSTDOCTORAL FELLOWSHIP

in the

DEPARTMENT OF CHEMISTRY

Applicants must possess a Ph.D. or equivalent degree, preferably followed by some research experience. Applicants should state the field of research in which they would intend to work during the period of the Fellowship.

Applications close on April 30, 1979. The emolument will be NZ\$8,500 per annum. Fellowships are tenable for one year.

Further particulars and Conditions of Appointment may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordo Square, London WC1H 0PF. 9961(E)

CITY OF LONDON POLYTECHNIC

DEPARTMENT OF GEOLOGY

SIR JOHN CASS'S FOUNDATION

RESEARCH FELLOWSHIP IN GEOLOGY

A newly created postdoctoral Fellowship funded by the Sir John Cass' Foundation is being offered in the field of Sedimentology. This 3-year post will be available from September 1 1979, with a salary in the range of £3,747 to £4,203, plus £474 (London Allowance).

Applications (giving age, qualifications, experience and intended research programme, together with the name and addresses of two referees) should be made as soon as possible to Dr R. R. Skelhorn, Department of Geology, City of London Polytechnic, Walburgh House, Bigland Street, London E1 2NG. 9914(E)

IMPERIAL CANCER RESEARCH FUND

RESEARCH FELLOWSHIP

A Postdoctoral Fellow required for an appointment for three years. The work will be on the mechanisms by which host cells control the expression of tumour-inducing viruses. Familiarity with nucleic acid hybridisation and allied techniques would be an advantage but is not essential. Salary with entry according to qualifications and experience within range £5,332 to £6,530, inc. L.A.

Further information from Dr John Wyke (01-242 0200, ext. 267). Applications with curriculum vitae and names of two referees, should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX by April 13, 1979 quoting ref. 141/142/79. 9951(E)

RESEARCH FELLOWSHIP IMPERIAL CANCER RESEARCH FUND

A postdoctoral medicinal chemist is required to join a multi-disciplinary group working on the synthesis and evaluation of potentially selective antitumour agents. The project encompasses both the induction of differentiation and also the exploitation of the characteristic biochemical properties of certain specific tumours to achieve selective toxicity. The preferred candidate would be primarily a good synthetic chemist who has had previous experience of working in the field of biologically active compounds.

Appointment will be for three years. Salary with entry according to qualifications and experience within the range £5,332 to £6,530 inc L.A.

Further information from Dr A. M. Creighton (01-242 0200, Ext. 284). Applications with curriculum vitae and names of two referees should be sent to The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, quoting reference 139/140/79 by April 13, 1979. 9952(E)

FEBS FELLOWSHIPS

FEBS Fellowships are to support short-term (not longer than two months) visits by members of any FEBS Constituent Society to laboratories in another country in the FEBS area for the purpose of carrying out experiments with special techniques or other forms of scientific collaboration or advanced training, and especially to support developments arising at short notice.

Fellowships will not be granted to attend courses, symposia, meetings or congresses.

Fellowships will cover both travel and subsistence.

Information and details of making an application may be obtained from:

Professor G. Dirheimer,
FEBS Fellowships Officer,
Institut de Biologie Moléculaire et Cellulaire du CNRS,
15 rue Descartes,
67084 Strasbourg Cedex,
France. 9933(E)

UNIVERSITY OF YORK POSTDOCTORAL FELLOWSHIP

An M.R.C. Fellowship is available for a joint project between the Cancer Research Unit and the Department of Chemistry. The research topic will be concerned with studying the metabolic activation of aflatoxins, a group of carcinogenic human food contaminants. The proposed studies will concern the reasons for species differences in susceptibility to these compounds. Applicants should have a sound background in foreign compound metabolism and preferably some experience in identification of metabolites.

The appointment will be for three years from June 1, 1979 or as soon as possible thereafter and the salary will be within Range 1A of the scales for research staff, starting at £3,883 per annum (under review), plus USS.

Three copies of applications, with curriculum vitae and the names of two referees, should be sent by April 6, 1979 to the Registrar, University of York, Heslington, York YO1 5DD. Informal enquiries to Dr Colin Garner, Cancer Research Unit. Please quote reference number 11/6099. 9959(E)

WORKSHOPS

WORKSHOP:

Wetlands and Estuarine
Processes and Water Quality
Modelling

Sponsor: U.S. Army Corps of
Engineers, Waterways Experiment
Station.

Date: June 18-20, 1979, New
Orleans, La., U.S.A.

Abstracts to be submitted
before March 16, 1979, to:
Dr Peter Hamilton, Science
Applications, Inc., 4900
Water's Edge Drive, Suite
255, Raleigh NC 27606,
U.S.A. 9996(V)

SCHOLARSHIPS

THE AUSTRALIAN NATIONAL UNIVERSITY SCHOLARSHIPS FOR Ph.D. DEGREE COURSES

Persons who hold, or expect to hold, a bachelor degree with at least upper second-class honours or equivalent from a recognised University and who have a capacity for research, are invited to apply for Australian National University Ph.D. Scholarships, tenable over a wide range of subjects in the Humanities and the Physical, Medical, Chemical, Biological, Earth and Social Sciences. Scholarships are available in any of the departments or units of the Institute of Advanced Studies which consists of Research Schools of Physical Sciences, Biological Sciences, Social Sciences, Pacific Studies, Chemistry, Earth Sciences and the John Curtin School of Medical Research, of the School of General Studies (Faculties of Arts, Asian Studies, Economics, Law and Science); or in one of the University Centres.

Scholarship Benefits: The basic stipend payable is \$4,700 per annum (tax free) with additional allowances for dependants and housing assistance for married scholars. In addition, return economy-standard air fares and a grant towards removal expenses are normally provided. (The latter will not be provided for Australian citizens overseas who are eligible for Commonwealth Government Research Awards.)

Tenure: Scholarships are normally tenable for three years and may be taken up at any time of the year.

There is no set closing date, but applicants from outside Australia are advised to apply at least six months before they expect to be available to take up a scholarship, if offered.

Full particulars and application forms are available from the Registrar, The Australian National University, P.O. Box 4, Canberra, A.C.T., 2601, or from the Association of Commonwealth Universities (Apsu), 36 Gordon Square, London WC1H 0PF. 9985(E)

COURSES, CONFERENCES and SYMPOSIA

NOW AVAILABLE PITTSBURGH CONFERENCE ON BL LAC OBJECTS

ED. BY ARTHUR M. WOLFE

Proceedings of a Conference on BL LAC Objects held at the

Department of Physics and Astronomy
University of Pittsburgh
Pittsburgh, Pennsylvania 15260

APRIL 24-26, 1978

The price of a single copy of these proceedings is \$10.00. Copies may be obtained by sending a check or money order to:

BL LAC Conference
Department of Physics and Astronomy
University of Pittsburgh
Pittsburgh, PA 15260

Make out remittances to "University of Pittsburgh—BL LAC Meeting"
9996(C)

A Comprehensive International Two-day Conference NEW TECHNOLOGIES IN THE HEALTH CARE INDUSTRY San Francisco—May 21-22 London —June 11-12

This market-oriented conference for the corporate executive in planning and research and development will focus on future market possibilities.

Brochure available from the organisers: ROBERT S. FIRST, INC., 19a Avenue Marix, 1050 Brussels. Telephone: (02)512 50 63. Telex: 22859. 10007(C)

UNIVERSITY OF DURHAM DEPARTMENT OF GEOLOGICAL SCIENCES CONOCO ADVANCED COURSE STUDENTSHIP IN GEOPHYSICS

Candidates intending to seek a career in the oil industry are invited to apply for the above studentship (equivalent to a N.E.R.C. Studentship) to attend the one-year M.Sc. Advanced Course in Geophysics from October 1979.

Candidates should hold, or expect to obtain in the Summer of 1979, at least a 2(i) Honours degree. Preference is given to candidates with single or joint honours in Geology. An adequate background in Mathematics, preferably to first year university level, is required.

Applications should be submitted before April 30, giving full academic qualifications and the names and addresses of at least two referees, to Dr. R. E. Long, Department of Geological Sciences, University of Durham, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars of the course and the studentship may be obtained. 9989(D)

Please mention
Nature
when replying to
these advertisements

FEBS ADVANCED COURSES

No. 65. "The Control of Glycogen Metabolism"
Brussels, Belgium,
5-7 September 1979

The Course, organised by H. De Wulf, H. G. Hers, L. Hue and G. van de Werve, will cover the properties of enzymes involved in glycogen metabolism as well as aspects specific to muscle, liver, adipocytes and leucocytes. It will include lectures by invited speakers and presentation of posters by registered participants. A limited number of these posters will be subjected to general discussion. Registration fee: about \$50. For further information, contact Professor H. G. Hers, Université Catholique de Louvain and International Institute of Cellular and Molecular Pathology, UCL 75.39-75 avenue Hippocrate, B-1200 Brussels, Belgium. The deadline for applications and abstracts is May 15, 1979.

No. 67. "Recombinant DNA"
Zagreb, Yugoslavia,
15-27 October 1979

A Laboratory Course for 25 participants. The experimental program will include: In vitro recombination of DNA segments with plasmid and viral vectors. Cloning in *Escherichia coli* and *Bacillus subtilis*. Selection and screening of cloned recombinant DNA. Characterization of cloned recombinant DNA. Registration fee \$100. Applications (by April 30, 1979) and info: Dr Vjera Zaga, Dept. of Experimental Biology and Medicine, "Ruder Boskovic" Institute, P.O.B. 1016, 41001 Zagreb, Yugoslavia.

No. 64. "Modern Methods of DNA Sequencing"
Gliwice, Poland,
5-10 November 1979

This Course for 15 students aims to disseminate within European research laboratories the two basic methods of DNA sequencing: the Maxam-Gilbert technique and Sanger's inhibitor chain-termination technique. Advanced studies will cover sequence organization of DNAs from various sources. Further information and application forms (to be returned by May 31, 1979) are available from the organizers Dr M. Chomay/Dr S. Szala, Dept. of Tumour Biology, Institute of Oncology, 44-100 Gliwice, Poland. 9932(D)

COURSES, CONFERENCES AND SYMPOSIA (Continued)

FIFTH EMBO ANNUAL SYMPOSIUM NUCLEIC ACID-PROTEIN INTERACTIONS

October 12–15 inclusive

EMBL Heidelberg

In addition to members of the Organising Committee invited speakers include:

J. Abelson (La Jolla); T. Bickle (Basel); H. Bujard (Heidelberg); P. Chambon (Strasbourg); R. Flavell (Amsterdam); C. Guthrie (San Francisco); S. Harrison (Harvard); C. Helene (Orléans); H. Hoffmann-Berling (Heidelberg); A. Landy (Brown); A. McPherson (Pennsylvania); M. Ptashne (Harvard); M. T. Record (Madison); H. Saedler (Freiburg); A. Schmitz (Geneva); J. Sperling (Rehovot); G. Stöffler (Berlin); F. W. Studier (Brookhaven); E. Vinuela (Madrid); J. C. Wang (Harvard); W. Wintermeyer (Munich); C. Yanofsky (Stanford).

The eight Plenary Sessions will cover Model Systems; Viruses; Chromatin; Polymerases; Regulatory Signals on DNA; Cleaving, Processing and Modifying Nucleic Acids; Ribosomes and tRNA; New Frontiers.

The Symposium will be held at the European Molecular Biology Laboratory Heidelberg with registration on Thursday, October 11. Plenary sessions will be held in the mornings and afternoons of October 12–15 inclusive. The Symposium banquet will be held on the evening of October 15. Poster sessions will be arranged; applicants wishing to present a poster should submit a title and an abstract of 1–2 pages. Participants will be accommodated in two hotels on downtown Heidelberg, and transportation to the meeting will be arranged.

The registration fee is DM 80.00 and includes daily lunches and the banquet but neither hotel charges nor evening meals.

Applications including a curriculum vitae should be sent to Dr. J. Tooze, Executive Secretary, European Molecular Biology Organisation, Postfach 1022.40, 69 Heidelberg 1, FRG, by May 31, 1979. Since the number of participants is limited to 200 the Organising Committee will notify those who have been accepted in August.

Organising Committee: D. Crothers (Yale); J.-P. Ebel (Strasbourg); A. Klug (Cambridge); C. Kurland (Uppsala, Chairman); J. Miller (Geneva); H. Schaller (Heidelberg); J. Tooze (Heidelberg, Secretary); C. Weissmann (Zürich). 9943(M)

THE NEW YORK ACADEMY OF SCIENCES

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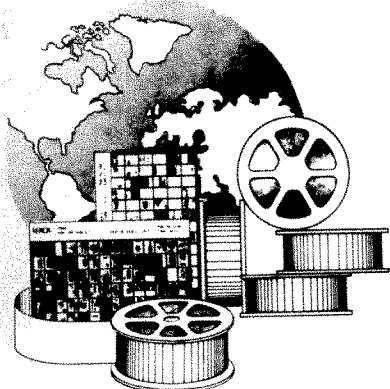
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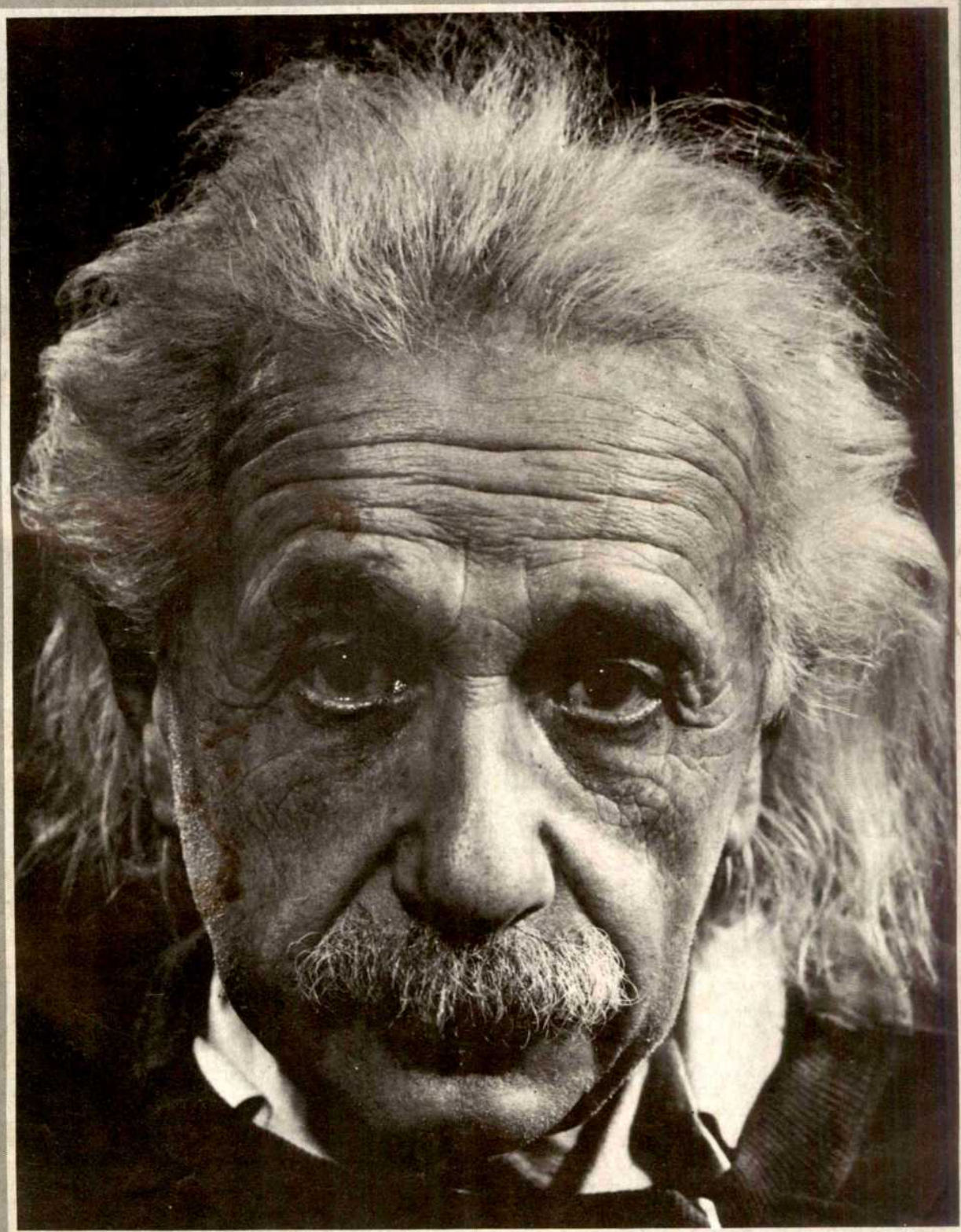
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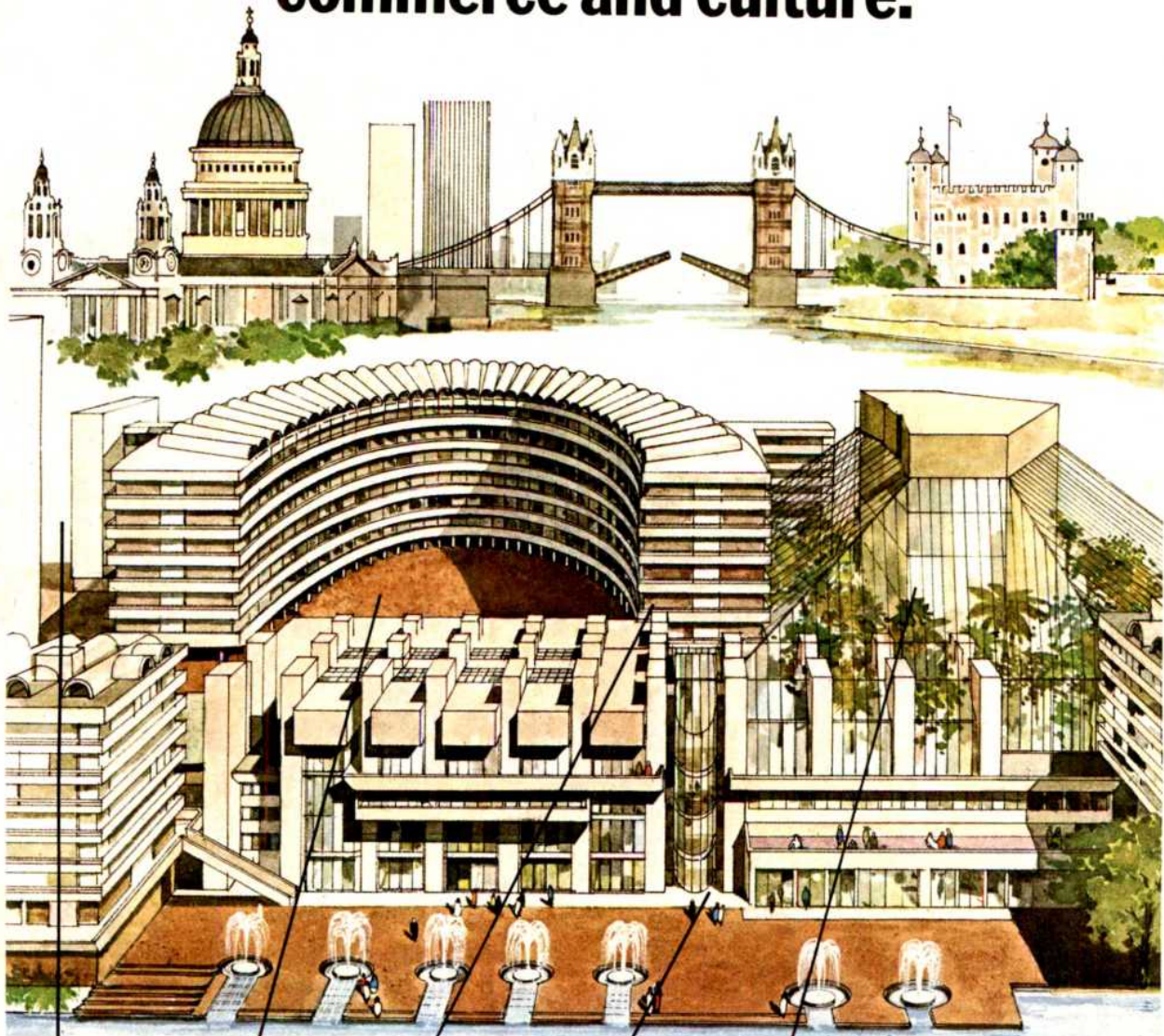
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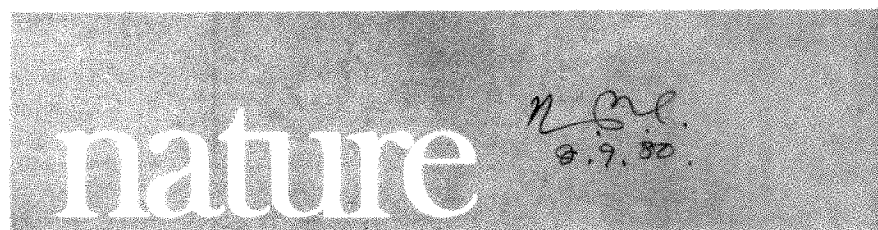
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In celebration of the Einstein centenary, *News & Views* reports on the unified field theory (p209) and reproduces an early Eddington account of the general theory of relativity (p213); Kenneth Brecher compiles some quotations by or about Einstein (p215); and the review section looks at various facets of Einstein's world (p285).

(Photo: Philippe Halsman)

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15 March 1979

The machinery works—does it produce the goods?

IN 1972 the British government published a White Paper 'Framework for Government Research and Development' which recommended re-structuring of financial support for R & D. The White Paper sprang largely from a report written by Lord Rothschild, and proposed, amongst other things, that support for applied R & D, whether conducted within or outside government departments should be controlled by the customer/contractor principle: departments as customers define their needs; contractors advise on the possibility of meeting these needs and undertake the work; and customer and contractor keep in close contact to ensure that objectives remain obtainable at a reasonable cost.

Of course, much departmental in-house R & D had always run along these general lines, but the controversial element of the Rothschild recommendations was that a substantial amount of work supported by Research Councils, particularly in their own establishments, should no longer be funded through the Science Vote (and thus ultimately by the Department of Education and Science) but be paid for by customer departments; research councils would continue to provide management for such activities, but the customers would control their direction. Customer departments were expected to acquire a Chief Scientist who would strengthen the department's ability to commission research widely. Chief Scientists would play an active role in research councils and also in the newly-formed Advisory Board for the Research Councils (ABRC) which took on a role of supervising the councils. The reorganisation could be seen as a corrective to a tendency for work in research council establishments to cover roughly the fields of interest of government departments without any obvious way for departments to express this interest and guide research, beyond gentle persuasion.

Has it worked? The Lord Privy Seal, quaintly entrusted with overseeing research and development policy co-ordination, has just issued, with a little help from his friends, a review of the R & D machinery as it now stands (Cmd 7499; £1.25). The answer, no great surprise, is that whilst there are still defects and weaknesses in the system, there is no call for further wholesale change in the arrangements.

The areas still needing attention, says the report, include the following. First, there is a danger that research councils (and, it could have been added, individual establishments such as the Institute of Geological Sciences) become so loaded with commissioned research that they lose independence to pursue their own ideas. The report sounds a particular warning over the Agricultural Research Council, which receives more than half its income from the Ministry of Agriculture, Fisheries and Food for contract work. Second, the report points out that the Department of Health has until recently had difficulties in fulfilling its role as informed customer in biomedical research, owing to the conflicting demands of health and social services research on the limited expertise available in the department. Third,

ABRC has, up to now, been too preoccupied with the Rothschild reorganisation and then with allocation of diminishing resources to devote enough time to "more general problems which arise from, or affect, the scientific activities of the government, research councils or universities". It is to be 'invited' to review its working methods to free more time for broader questions.

The machinery works tolerably well. But what of the product? It is not unreasonable to ask not only whether the right sort of bureaucratic connections are now being made, but also whether these are bearing fruit in terms of research results of real value. On this the report is silent—two paragraphs, entitled 'The Dissemination and Utilisation of Results' comprise only generalities. Of course it is much more difficult to reach objective judgements on whether customers and the nation are really profiting from the new arrangements than it is just to verify that the arrangements meet general approval. Of course some of the timescales involved are too long for answers to be given yet. But without a dispassionate and rigorous assessment (and more than the department's point of view would have to be taken into account), it is impossible to be sure that all the time and effort actually bears fruit. The report claims to "try to assess whether the scientific activities financed by the government are now properly related to national need." It does not do this; someone should.

Less publicised amongst Rothschild's recommendation was that scientists in government should be enabled to play a larger role in working out departmental needs and in policy formulation by improving their managerial skills. This was to be achieved by courses in management training, competitive transfer of scientists to the Administration Group and the stimulation of staff interchange with universities and industry. Here the report valuably notes that there has been miserable failure. Few civil service scientists are interested in jumping to administration and few are prepared to do even a short time outside the service—the inter-change unit was disbanded last year.

It is difficult to know what is wrong. Scientists are often to be heard grumbling that they don't get as many chances as administrators—"always on tap, never on top". Yet there are always apparently reasons why they never take the plunge. If they stay in science, however, many lose their enthusiasm and spend an unhappy ten or twenty years up to retirement.

Individuals have to recognise that no switch in career can be made without at least temporary inconvenience and struggle. Government has to recognise that private industry offers financial inducements to people it wants to move, and that it cannot expect much success without offering the same. A review of these manpower problems of the scientific civil service, under the chairmanship of Dr Martin Holdgate of the Department of the Environment, is just about to start to cover this ground yet again. □

Science exchanges at stake in SALT talks, says adviser

A FAILURE to agree on the terms of a strategic arms limitation treaty between the United States and the USSR could jeopardise the future of scientific exchanges between the two countries, Dr Frank Press, director of the Office of Science and Technology and science adviser to President Carter, said last week.

In a speech designed to encourage support in the scientific community for the second Strategic Arms Limitation Treaty (SALT 2), the terms of which are expected to be agreed shortly, Dr Press told a meeting organised by the State Department that if SALT 2 succeeds, other forms of cooperation—such as scientific exchange—could succeed. But if the agreement failed, it was difficult to see much chance for other kinds of cooperation to succeed.

"I am not saying we should sign SALT—any SALT agreement—just or even primarily to insure the future of scientific cooperation and exchange with the Soviet Union. What I am saying is that it is a clear side-benefit of SALT", he said.

Dr Press's remarks came as the administration is preparing a campaign to convince members of Congress,

some of whom have already expressed their opposition, to ratify the treaty once the terms are agreed by both sides.

Scientists had an important role to play in the debate on SALT, since before the Senate and the public could judge the treaty, they would have to understand it, he said. "Your views will carry special weight in the public mind on an issue like this. I urge you to play an active role and share your expertise and understanding of SALT with friends and colleagues."

An illustration of the type of issue involved in the forthcoming debate arose last week when Senator Henry Jackson announced that he was not going to support SALT 2, partly because he felt that the loss of US monitoring posts in Iran made the terms of the proposed treaty impossible to verify.

Earlier in the week, however, Mr Herbert Scoville, formerly deputy director responsible for scientific affairs in the Central Intelligence Agency, disputed this claim. He said that redundancy in the current monitoring system, as well as other verification systems such as satellites, was adequate

to ensure compliance, even with the loss of the Iran stations (much of the equipment from which is now being moved to Turkey).

Some scientists have expressed concern that, although they agree with Dr Press on the need to participate in wide public discussion of the technical aspects of the proposed SALT agreement, it will be difficult to do this adequately when much of the necessary information on, for example, monitoring techniques remains classified.

"I am quite prepared to encourage debate on the treaty provided that we can get the information on which such discussions can be based. But if we cannot get the necessary information, then I would be very doubtful about taking it up," Mr William Carey, executive officer of the American Association for the Advancement of Science, said last week.

Others are concerned that, in this eagerness to make the proposed treaty acceptable to a wide constituency in the Senate, President Carter is prepared to give the go-ahead to a range of new military technologies, thus doing little to curb the overall arms race.

David Dickson

Europe plans to predict earthquakes

EUROPE is to embark on a programme of earthquake prediction involving the collection of data on the ground and from satellites. A preliminary outline for the programme was drawn up by scientists attending a seminar organised by the European Space Agency and the Parliamentary Assembly of the Council of Europe in Strasbourg last week.

The programme is intended to monitor the Alpine-Mediterranean belt which runs from Turkey through Italy and Spain to the Azores. It is initially proposed to run from 1980-91 by which time a fully operational system of data-collecting stations should be in existence.

The possibility of an earthquake prediction programme for Europe was first raised last October at the Parliamentary Assembly of the Council of Europe by the chairman of the Assembly's Committee on Science and Technology, who comes from the Friuli region of Italy which suffered severe earthquakes in May 1976. He felt that because several of the member states of the Council of Europe are prone to earthquakes, efforts to predict them should be coordinated. Support from other parliamentarians was sufficient to warrant a detailed debate and the set-

ting up of last week's seminar.

The idea is to augment and co-ordinate earthquake prediction programmes already existing in individual states. The ground-based programme will involve collecting geophysical, geodesic, astronomical, geochemical and other data from 150 fixed stations and several mobile ones. Existing stations will be redefined to form part of a network and new ones will be built.

The space-based programme will involve imaging from satellites and aircraft, including laser-ranging and, later on, satellite-assisted very long base interferometry. Future geostationary satellites will be equipped with relevant imaging instruments. One of the aims is to draw a new seismic map of Europe.

The seminar decided that the European-Mediterranean Seismological Centre (EMSC) at Strasbourg should collect, analyse and interpret the data. It also recommended that a European Association for Earthquake Prediction be set up to run the programme, although initial responsibility will probably rest with the working party on geodynamics of the Parliamentary Assembly of the Council of Europe in conjunction with the European Space



Earthquake in Italy: a stimulus for the programme.

Agency, the European Seismology Commission and the Europe-Mediterranean Seismology Centre.

No estimate of the cost has yet been made. Rather than a pool of money being available for the programme as a whole, ground-based projects will probably be paid for by the state in whose territory they are and space-based projects will probably be built and paid for under ESA's new and optional Earth-oriented research programme.

Judy Redfearn

OTA faces another summer of uncertainty



David Dickson discusses the continuing troubles of the Office of Technology Assessment, whose third director in six years resigned earlier this month

LAST Thursday the twelve US Congressmen who make up the board of the Congress' Office of Technology Assessment met for the second time in eighteen months to discuss how to choose a new director for the agency.

The meeting was made necessary by the surprise resignation of OTA's current director Dr Russell Peterson (above), who took on the position last January. A chemist by training, Dr Peterson spent 26 years with the Du Pont Company, heading its research and development division until he was elected governor of the State of Delaware in 1968. In 1973 he was made chairman of the Council on Environmental Quality, which he left to set up a citizen's lobby group New Directions before moving to OTA.

Stating that his varied experiences in private and public life have "led me to prefer an advocacy role rather than an advisory one," Dr Peterson announced that he had accepted an invitation to become the president of the National Audubon Society, a body of 400,000 members whose main role is to protect the bird life of America.

In his 13-month occupancy at OTA, Dr Peterson has done much to put the agency on its feet, following a shaky start plagued by internal and external political wrangles. By streamlining day-to-day management, introducing clear hierarchies of responsibility, and other such moves, he has restored both OTA's reputation and its morale.

However it is no secret that Dr Peterson's strong views, both about how OTA should be run—he demanded, and got, complete authority to hire and fire staff—and on its role with respect to Congress, have themselves caused considerable friction (his resignation came not long after a particularly bitter board meeting in January).

Given that the aspects of Dr Peterson's personality which have exacerbated this friction are those that have helped place OTA on a more stable and effective footing, his departure has once again raised the

question of whether OTA—however desirable its objectives—can in fact function in the way that it was intended to do by Congress.

The Office of Technology Assessment was established by statute as an advisory arm of Congress in 1972. Its function is to help legislators anticipate and plan for the long-term consequences of technological applications, and to examine the many ways in which technology affects peoples' lives.

Few challenge the need for such analysis. And although the quality of the agency's reports have varied enormously, some—such as those on solar energy, on medical technology, and on a proposed coal slurry pipeline—have been widely praised, and played an important role in the development of legislation.

The big stumbling block has therefore not been whether, but how, such studies should be carried out. Under the first chairman, Mr Emilio Q Daddario—himself a former Congressman—OTA stayed close to the political process. This meant good contact with the political community; but the price was fierce political infighting that frequently spilled into the press, and consequently drained credibility from the agency's reports.

Under Dr Peterson's leadership the agency has, some feel, gone to the other extreme. By refusing to accept direct political pressure, he has eliminated many festering sores; but the criticism is that he has spent too much time telling Congress what it should be interested in, rather than listening to its demands.

Dr Peterson himself has strong, if controversial, views about technology assessment and its goals. "If we are to ensure change for the better, we must become skilled and successful architects of change. In today's interdependent world, that requires a holistic perspective—a comprehensive, world-wide, long-range view," he said.

The most obvious manifestation of this "holistic" approach has been an ambitious priority-setting exercise which

he set in motion last year. Over 5,000 people were asked to suggest critical technological issues facing the US and the world; to 1,530 topics suggested for study were added a further 2,875 extracted from the published literature. Synthesis and elimination by OTA staff led to a final list of 30 ranked items.

Few complained about the outcome. Of the final list, seven items were immediately selected by the board as areas for study, and three more subsequently added; these included alternative national energy futures, regulation and technological innovation, and the effects of nuclear war.

However a certain amount of resentment was caused by the fact that the exercise was initiated by Dr Peterson largely on his own initiative, and that by placing heavy emphasis on the exercise, he had shifted away from what they see as OTA's prime responsibility, namely responding to demands relating to specific legislation.

The issue of short-term versus long-term studies is endemic to discussions over the role of OTA, and is far from satisfactory resolution. And the need to maintain a balanced strategy places heavy responsibility on the shoulders of the director. The consensus in Washington seems to be that the new director needs the political skills and sensitivities of Daddario, the vision and managerial capabilities of Peterson, and a professional reputation respected in the scientific and technical community.

The task of finding a successor is therefore a daunting one. So much so that some are questioning whether OTA might not benefit from some structural change; for example, it could be more formally separated from political decisions by reducing the involvement of the 12 board members and increasing the independence of the director (a possible model might be Congress' General Accounting Office).

Many would welcome such a move, possibly with the idea of a scientific Brookings Institute in mind. Others, however, see it as a betrayal of the ideals that established OTA as a research tool forming part of, not a commentator on, the political process.

As usual in such cases, the last word may rest with those who hold the purse strings. Appropriations committees in both House and Senate are looking closely at OTA's budget request for 1980; possible cuts of \$2 to \$3 million (in a request of \$11.2 million), are being rumoured, and the final decision may well reflect Congress' reaction to the choice of a new director. It promises to be another long, hot summer for OTA. □

How the CIA backed research on mind control

David Dickson reports on recent revelations about the Central Intelligence Agency's support for university research

IN THE late 1950s and early 1960s, the US Central Intelligence Agency mounted a comprehensive and well-planned effort to encourage university scientists to carry out research that would, the agency hoped, lead directly to techniques for controlling the human mind.

According to previously classified documents, the agency was responsible for initiating or supporting research that led subsequently to important contributions in various fields of behavioural science, including sociolinguistics, psychotherapy and personality assessment.

And in an effort to legitimise its interest and obtain access to leading investigators, the agency financed a funding organisation that provided grants to a number of prominent members of the scientific community—including Carl Rogers, B. F. Skinner and Professor Hans Eysenck—even though the researchers were frequently unaware of the true source of their support.

The details of the CIA's involvement in these fields, described as helping to "liberate the behavioural sciences from the world of rats and cheese", are provided in a book which has just been published in the US by Mr John Marks, a former Senate aide and State Department official. (The book will appear in the UK later this year.)

Using documents obtained through the Freedom of Information Act, Mr Marks has put together a detailed picture of how the agency used the academic community in its search for the holy grail: a technique that would provide sufficient manipulation of the human mind to eliminate uncertainty from the intelligence business.

Many of the more bizarre, farcical or outrageous aspects of this pursuit have since received wide publicity. These range from experiments that switched the East coast academic community on to LSD, to the construction of a bugged San Francisco brothel (and included a scheme to make Fidel Castro's beard fall out).

But behind these there was, as Mr Marks shows, a closely considered plan for deriving, through the extension of conventional research techniques and the often unknowing cooperation of university scientists, the type of information that the CIA could use for controlling the behaviour of its agents and others.

Much of this research was financed through an organisation known as the Society for the Investigation of Human

Ecology, set up at Cornell University in 1955 under the guidance of Dr Harry Wolff, professor of neurology and psychiatry, who was later to become president of the American Neurological Association.

Dr Wolff's aim, which received considerable sympathy in various academic circles of the time, was to provide a framework for the multi-disciplinary study of man in relation to his environment. The CIA's interest was sufficient for it to provide 90% of the society's funding until, having separated from Cornell in 1957 to obtain "more established stature in the research community", the organisation was folded in the mid-1960s.

Some of the research funded through the society had a direct and obvious relation to the agency's interests. It provided Wolff himself with a research grant of \$74,000 to study the factors that cause men to defect from their countries and cooperate with a foreign government.

In other cases, the concerns were more basic. Professor Charles Osgood, for example, who had asked the CIA to support his research into the way people in different societies express similar feelings, received \$192,975 over five years through the society. He later completed his influential work on cross-cultural meaning on a grant from the National Institute of Mental Health.

In some cases, the CIA used research grants explicitly to build up the prestige of the Human Ecology Society. Thus the society provided \$26,000 to Professor Eysenck of London University to support his work on motivation; the agency accepted that the research would have "no immediate relevance to agency needs", but the society took funding credit for nine of his publications in its 1963 report.

"It's news to me", said Professor Eysenck. Often the research workers

were unaware of the source of the majority of the society's funds; some were told that the money came from rich New York doctors or Texas millionaires. "I don't like secret involvement of any kind. I can't see why it could not have been open and above board", says Professor B. F. Skinner, who received \$5,000 towards the research leading to his book *Freedom and Dignity*.

But as well as providing academic respectability, funding of research through the society allowed the CIA legitimately to approach "anyone in the academic community", as one source put it. Thus in a move that Mr Marks describes as "turning scholars into spies", the society provided \$15,000 towards a tour of the Soviet Union by ten prominent psychologists (only one of whom knew of the agency's involvement), and CIA officials debriefed the group on its return.

Support for university behavioural research was curtailed in the mid-1960s. However, in contradiction of testimony later given to Senate committees, the research itself was continued, although with a greater emphasis on more direct ways of influencing behaviour, such as inserting electrodes in the brain. (At one point, agency scientists considered the potential of genetic manipulation: "The rest of the world didn't ask until 1976 the type of questions we were facing in 1965", one agency source is quoted as saying.)

Mr Marks admits that much of the research which the CIA sponsored led to important contributions to accepted academic knowledge—possibly because the agency was free to take imaginative leaps denied to more conservative (or more democratic) agencies. But he is uncompromising in criticising the ethical basis under which many of the experiments were carried out. (This was not always the agency's fault; some scientists had to be restrained from taking their experiments on, for example, sensory deprivation to their "terminal" conclusion.)

"We as a country can defend ourselves without sending our own scientists—mad or otherwise—into a hidden war that violates our basic ethical and constitutional principles. . . . The time has come for the United States to lead by example by voluntarily renouncing secret government behavioural research", Mr Marks writes in his conclusion. □



The Search for the 'Manchurian Candidate': The CIA and Mind Control by John Marks (Times Books, New York, \$9.95). The British edition will be published by Penguin.

UK atomic authority 'was a great mistake'—Benn

THE creation of the United Kingdom Atomic Energy Authority in 1955 "hived off policy formation as well as execution". It was, in retrospect, "a great mistake", the UK Secretary of State for Energy, Mr Tony Benn, said last week.

In a *Nature* interview with Mr Benn and his chief scientist, Sir Hermann Bondi, Mr Benn criticised the constitution of the UKAEA—under which research on waste management was relatively neglected for a long period. "If—in the creation of the Authority—the government had hived off Harwell and Aldermaston as research establishments, but kept the leading figures in nuclear policy within government, their relationship with the minister would have been very much closer than it is", he said.

Mr Benn has been minister in charge of the Authority from 1966 to 1970 and from 1975 to now. He believes "we have suffered" from its political autonomy.

In fact Sir John Hill, chairman of the United Kingdom Atomic Energy Authority, sought an interview with Mr Benn last week to discuss how to improve the flow of information between the Authority and the minister. After the meeting, Mr Benn announced that he would be sending a letter to Sir John outlining "a new list of areas of legitimate ministerial interest".

"It's not a question of control", said Mr Benn "it's a question of the integration of this very high technology with the democratic process. There are some very serious defects, but they don't arise from wickedness. They arise from the nature of the problem.

"Sir John came and said 'I tell you everything. What's wrong?'; but actually the problem of observing, watching, and connecting all these issues that a minister has, is quite different from the role of a manager of an authority. I am interested in all sorts of things—like, for example, what was that explosion in Russia in 1958? What happened to the 200 tonnes of uranium lost in the Mediterranean? I am interested in the movement of plutonium nitrates from Dounreay to Windscale, the real role of fusion, and all the areas relevant to the International Fuel Cycle Evaluation Programme. I try to preside over a range of issues as intelligently as I can, and need to keep informed on them all."

A second institutional problem, said Benn, is that in an agency set up to develop nuclear power the consequential effects of the technology "have tended to become subsidiaries to the agency's prime purpose". In practice,



Tony Benn: seeking more information on nuclear power

however, the effects have turned out to be very important.

"Where you fail to institutionalise basic conflicts that ought to exist in political discussion, then what happens is that the conflicts are solved at the Deputy Secretary level instead of at the Cabinet level. You must institutionalise the control of nuclear power to the point where all real arguments come to the top."

"The issues are enormous", said Mr Benn. He lists among them "the flow of information, the role of secrecy" in all high technology industries—including oil and gas—not just nuclear power. Also civil liberties, "where when you centralise, you then find the package deal includes a degree of intervention in civil liberties which is potentially unacceptable. All these things need to be brought out."

"In the end—however complicated a technology is—the public have the right in a democratic society to say no, as we've seen in Austria and very nearly saw in Switzerland. There is no escape from the ultimate provision of consent. The job of a minister is not to mystify, or to over-simplify, but to clarify, so that people know what they are doing. And you have to do it much earlier in the case of high technology."

"If it's a case of a road—do you want the M3 to go through Aldershot or not?—then you have a planning inquiry; but in the case of fast breeders, or nuclear power, or any other high technology, decisions will be taken that pre-empt things that will not happen for 10 or 20 years."

"The role of a minister as a public interrogator, who goes on asking questions, and then publishes the questions and invites the debate, is essential—otherwise you wake up one day and find that all the key decisions were taken 10 years ago. Then people get frustrated, they get violent, they get pessimistic, they get cynical. That is destructive of the whole democratic process, and it undermines the technology on which you have spent billions

and billions of pounds."

"This is my main interest in politics: how to get democratic control established effectively over areas where high skill, high complexity, high security, make the normal operations of democracy very very difficult."

Sir Hermann Bondi thought that the history of nuclear waste management "was an extremely interesting case". What the UKAEA had failed to foresee, said Sir Hermann, was not the need for the management of wastes, "but that there should be public anxiety about it".

In a sense the issue fell between two stools: "it fell into that gap between the institution and the politicians, so that neither saw that there would be an issue on which the public would become excited. The institution felt that all that the public were worried about was getting the power out of the plug cheaply, and they felt that technically the waste problem was soluble, so that there was no hurry yet to solve it."

Mr Benn illustrated the point with a story about cufflinks. "Have you had the British Nuclear fuel cufflinks?" he asked Sir Hermann. Sir Hermann had not. "I was sent a set of cufflinks by BNFL" said Benn "which is an ordinary set of cufflinks, but on the side where you would have a piece of jade or whatever, is a little brown, glass thing, and the card with them said 'These cufflinks represent how much vitrified waste you would generate through household usage in a year if all electricity were generated by nuclear power'. This was to show how small the problem is. They ignored the fact that they have got to last 20,000 years, and that even 40 million cufflinks make a pile." The idea that waste could be tackled by "the PR techniques of cufflinks" was "absolutely fantastic. I've never worn them because they are to me such a precious example of the wrong way of presenting a problem to people."

Robert Walgate

news in brief

SRC action puts new pressure on short-term contract workers: The Science Research Council has proposed a shift in funding emphasis from staff to equipment in a letter, written, "as a matter of urgency", to senior administrators at universities, colleges and polytechnics. Expressing concern about possible expenditure cuts arising from "the present industrial troubles", the council considers that the best way it can help research at present is to encourage applications for equipment. The council says "we cannot make additional major commitments to research grants which involve substantial expenditure over a period of several years such as those in which the provision of staff plays an important part". It urges speedy application before the next grant closing date of 1 April. [Brian Oakley, SRC secretary, says it is only partly a worry about the economic climate that has caused the shift in emphasis. "Good applications depend on good people and using the money for a three-year grant splurge will not bring in the young people that are necessary to swell the number of good applications."] Research workers on short-term contracts have expressed dismay at the SRC's action. "By spending money in this way, the SRC ignores the proven quality of senior researchers as shown by successive grant renewals through the highly competitive SRC grant selection process. Instead, the council is adopting a cynical policy of recruitment of younger workers who are cheaper and less knowledgeable about the system", says Robert Golub of the committee for long-term non-tenured faculty at Sussex University.



Levich appointed to New York chair: Professor Benjamin Levich (left), the Soviet dissident who was allowed to leave last year after a long campaign on his behalf by western scientists, has been appointed to the Albert Einstein chair in science at the City College of the City University of New York. Professor Levich has already become affiliated to Tel Aviv University in Israel. Dr Robert J. Kibbee, chancellor of City University, said last week that the appointment "helps to im-

plement an earlier agreement of cooperation reached between City University and Israeli universities." City College hopes to establish with Tel Aviv University a joint institute for applied chemical physics under Professor Levich's leadership.

Congolese geneticist still detained: This month marks the second anniversary of the arrest of Pascal Lissouba, agricultural geneticist and former Prime Minister of the Congo. Since his arrest and summary trial in March 1977, Professor Lissouba has been held under harsh conditions in the prison at Ouessou in the northern Congo. He is known to be seriously ill with kidney disease.

The Congolese Labour Party—theoretically the ruling body—is to hold its Third Extraordinary Congress later this month, and it is hoped that certain political detainees may be released in an amnesty for the occasion. The Congolese government, however, categorically denies having any political prisoners at all, and maintains that Professor Lissouba was involved in the assassination of President Nguabi in 1977.

Detailed enquiries by Amnesty International (whose charter precludes intervention in cases of political violence)

failed to find any substance in this charge—indeed, Professor Lissouba was adopted as an Amnesty International "Prisoner of the Month".

Professor Lissouba's arrest and imprisonment are almost certainly due to his political career during the 1960s. Although he had resigned the Premiership in 1966, after a disagreement with the then President, Massamba-Debat, and withdrew finally from politics in 1969, in order to devote himself to his work in cell biology, he was still, apparently, considered a focus of potential political opposition.

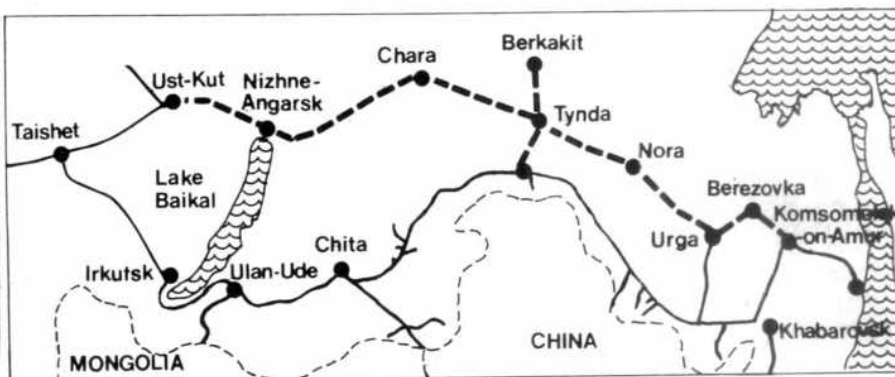
In 1976, when the military government was facing a general strike, Professor Lissouba was placed under house arrest for a time and deprived of his position as Professor of Cellular Biology at Brazzaville University. At the time of his arrest, he was under a restraining order forbidding him to leave the country.

Dow fights ban on 2, 4, 5-T: The Dow Chemical Company and 10 other plaintiffs are seeking an injunction to prevent the US Environmental Protection Agency enforcing its ban on the sale of the herbicides 2, 4, 5-T and Wilvex. They argue that the ban is based on a "seriously flawed study" which linked miscarriages in Oregon with herbicide spraying in nearby forests. They have asked a district court judge in Michigan, the chemical company's home state, to declare the EPA's emergency suspension orders "arbitrary and capricious", and to set them aside. The EPA has agreed to hold hearings requested by Dow and five other chemical manufacturers and users to consider lifting the emergency ban.

UKAEA responds to environmentalists' demands: The UK Atomic Energy Authority has agreed to let Friends of the Earth publish a critique of the Inhaber report on alternative energy in its magazine ATOM, subject to review by Professor F. R. Farmer, the AEA's health and safety adviser (1 March 1979). The AEA will also give FOE space to criticise Lord Rothschild's Dimpleby lecture on risk and will circulate a notice with its release of Rothschild's lecture saying that a contrary view is available.

California law on nuclear waste "unconstitutional": A federal judge in California has ruled that the state's law prohibiting the construction of nuclear power plants until a waste disposal system has been approved by the federal government is unconstitutional. The law has meant the virtual abandonment of plans for further nuclear power stations in California. Uncertainty over methods for disposing of nuclear waste has also led to similar moratoria in other states. Judge William B. Enright, however, ruled that the state law disrupted national plans for the development of nuclear energy, and that such preemption of the federal government was unconstitutional.

GMAG must cover industrial research, says the minister: Early fears that the widespread use of genetic engineering might be hazardous if organisms containing recombinant DNA were to escape "may have been mis-stated", Mrs Shirley Williams, UK Secretary of State for Education and Science told the House of Commons Select on Science and Technology last week. Nevertheless she does not think that this would justify moving "as far as NIH" in relaxing guidelines. In particular she feels that industrial research must come under GMAG's remit even though its inclusion means that some members must leave during discussions of a commercial nature. "If we said that no one could withdraw then we would have no industrial members or a lack of cooperation from industry".



Baikal railway raises environmental questions

"We are setting aside vast resources for the construction of the Baikal-Amur Mainline Railway" said Mr Kosygin, earlier this month. "Its commissioning will enable the rich natural resources of the trans-Baikal area, southern Yakutia, the Far East and Western Siberia to be more intensively exploited".

BAM—the Baikal-Amur Mainline—is due for completion in 1983. It has considerable symbolic importance to the Soviet planners. Its 3,000 km of through track (plus spur lines to major coal and ore deposits) will help to open up Siberia by providing a northern alternative to the existing Trans-Siberian route.

BAM was based on almost 13 years of surveying, before construction was begun in 1974—a survey which made extensive use of satellite photos, incidentally fulfilling Five Year Plan directives that the "exploration of near-Earth space" should be used "in the interests of the national economy".

Numerous research bodies were drawn into the planning of the project. A major role was played by the various institutes of the Siberian branch of the Soviet Academy of Sciences and the Far Eastern Science Centre. The Institute of Siberian and Far Eastern Geography has mapped the plant distribution of the BAM zone, classifying the various natural regions and determining the plant resources and the role of plants in erosion protection.

The same institute has also made a detailed survey of meteorological conditions, especially avalanches, in the

Kodar and Udokan ranges, while the Institute of Geology and Geophysics of the Siberian branch of the Academy has been working on tectonic movements. Preliminary results have been somewhat surprising. In the region of Lake Baikal, the rate of vertical movement of the crust appears to exceed that in the Caucasus, increasing northwards, so that Irkutsk is rising at 8 mm per year, and Nizhne-Angarsk (a station on BAM) at 19-20 mm.

Since the BAM zone is so highly seismic, the Institute of the Earth's Crust of the Siberian branch of the Academy developed a network of automatic seismic stations. So far, some 3,500 weak tremors have been registered and the system is shortly to be extended. Using aerial and satellite photography and computer simulation, a team from Alma-Ata are mapping sites of potential mud torrents in the future BAM industrial zone.

In addition to this seismic expertise, there have been several less expected spin-offs from BAM. One of the most interesting occurred on the section between the Baikal and Severomuisk tunnels, where the incursion of modern man turned up traces of primitive man, who, it would seem, first appeared in the area some 180,000 years ago.

Apart from problems directly connected with construction, however, the main direction of research has been ecology and conservation. From the beginning, BAM was planned with considerable respect for the environment. At times, the precautions seemed almost ludicrous, as when construction workers

were forbidden to use pesticides against the swarms of mosquitos which ruin the Siberian spring.

For the most part, though, the ecological planning was serious and well-founded. Round the northern shore of Lake Baikal, the original plan called for either a shore tunnel or else for the track to be laid along the actual shore of the lake. In the interests of conservation, however, it was finally decided to tunnel through the solid rock, to protect the lake from debris.

A vast nature reserve has been set aside in the BAM zone, including coniferous and mixed forest, mountains, rivers, lakes and alpine meadows. Fauna thus protected include wild elk, reindeer, black stork, white tailed sea-eagle, sable, brown bear, ermine and lynx. New legislation will ensure adequate crossing points for migrating animals to traverse BAM.

Planning is going forward, too, for the ecology of the future settlements of the area. Industrial complexes will be surrounded by green belts. According to Academician Brezhnev, Director of the Plant Breeding Institute of the All-Union Academy of Agricultural Sciences, "specialised farms" are to be set up along the route, which will produce fresh produce all the year round for the new towns. Sociological problems of life in remote settlements, and estimates of requirements in housing, education and recreation facilities are well under way.

However, perhaps the most unlikely spin-off from BAM is the tacit acceptance, in mass-media reports, that the seismic problems of the zone are due to plate tectonics. Until recently, Soviet geologists have been reluctant to commit themselves publicly to plates, due to the opposition of the prestigious Academician Belousov. Recent TASS releases, however, accept plate tectonics as an accepted feature of the BAM zone. Indeed, TASS reported last January that, owing to plate movements, by the end of the century, BAM will be some 50 cm longer than planned.

Vera Rich



The Baikal-Amur railway: route to the wealth of Siberia

Multiple aimpoints not the answer

The Carter administration is expected to decide next month which of a variety of possible basing modes it will adopt for the deployment of its new generation of intercontinental missiles, the MX. Richard Garwin here discusses how the alternatives relate to the current agreement on strategic arms limitation being negotiated with the USSR.

WIDELY heard around Washington these days is the acronym MAP, for Multiple Aimpoint System (also known as "MPS", for Multiple Protective Shelter). MAP has emerged as this year's leading contender to the endemic problem of the vulnerability of US intercontinental Minuteman missiles. But among the difficulties which MAP poses could be its impact on the structure and progress of SALT, the Strategic Arms Limitation Talks.

At present the United States deploys 1,000 Minuteman missiles in silos; a little over half contain three re-entry vehicles (RVs) per missile, the remainder only one. Minuteman silos are concentrated into six wings at separate locations in the United States. This concentration is in itself a weakness of the Minuteman force, for unless the United States chose to launch a planned strike it would be possible for the Soviet Union to create for a limited period an environment in which there was no point in launching missiles. Around one megaton detonated every minute above the atmosphere in the vicinity of each Minuteman wing would reliably 'pin-down' the missiles, which are fragile during the boost phase.

Such an attack, started perhaps with submarine launched missiles of short flight time and continued with land-based missiles, could not continue indefinitely. At some stage a direct attack would have to be made on the silos themselves. It is widely recognised now that Minuteman is not vulnerable to a short-duration attack unless enemy RVs are so accurate that no more than two (and preferably one) need be used against each silo. Even the low airburst required to produce sufficient overpressures on the ground to destroy a Minuteman silo throws a large amount of debris into the air, and causes problems with the penetration of a second RV to the target region.

The current interest in vulnerability arises from the improved accuracy demonstrated by Soviet missile tests. If the Soviet Union indeed deploys

2,600 or so RVs against Minuteman, with no systematic errors in targeting, and if the operational aspects of timing, command and control can be resolved, as well as the problem of retargeting backup missiles to replace those which have failed to emerge from the silo or have failed in boost, then the Soviet Union should be able to destroy a large fraction of the Minuteman force at some time in the 1980s—supposing Minuteman is not launched under attack or on warning of attack. Why it would want to spend 2,600 megaton-range RVs to destroy 2,000 submegaton-range RVs is a question which has not been given a compelling answer. The Soviet Union would reduce its own RV numbers (already below those of the US) by a larger fraction than would the United States, and would have expended three to four times as many megatons as it had destroyed. The US would still possess a large fraction of its 4,960 Poseidon warheads on nuclear submarines.

If the Soviet Union should choose to do this, however, MAP is an eminently rational proposal to counter Minuteman vulnerability. Grant the Soviet Union perfect reliability of its missiles and re-entry vehicles, essentially perfect accuracy and perfect knowledge of the locations of our land-based missiles, and the desire to destroy the US inter-continental ballistic missile force (ICBM). Grant that the US ICBMs would remain in their silos until after an attack, rather than be launched under attack or on warning. Then in principle one could preserve a substantial fraction of an ICBM force by siting individual missiles deceptively in a number of 'aimpoints' substantially larger than the number of RVs which the Soviet Union might bring to bear in an attack. A figure of 5,000–6,000 aimpoints has been discussed, and this could preserve more than half the US ICBMs against a Soviet force of 3,000 re-entry vehicles.

The number of US missiles actually secreted can be chosen to satisfy US force requirements, as can the size of the missile. One might imagine 1,000 single-RV missiles, each with an RV comparable with that on Minuteman III, or alternatively, a force of 200 large missiles in the same 6,000 shelters, each with 10 or 11 RVs of the same type. The choice of missile is strongly influenced by SALT considerations. The keystone of SALT II is equal numerical limits on ICBMs, and particularly on those containing multiple re-entry vehicles, and so the possibility of doing MAP under SALT leads one to consider very large mis-

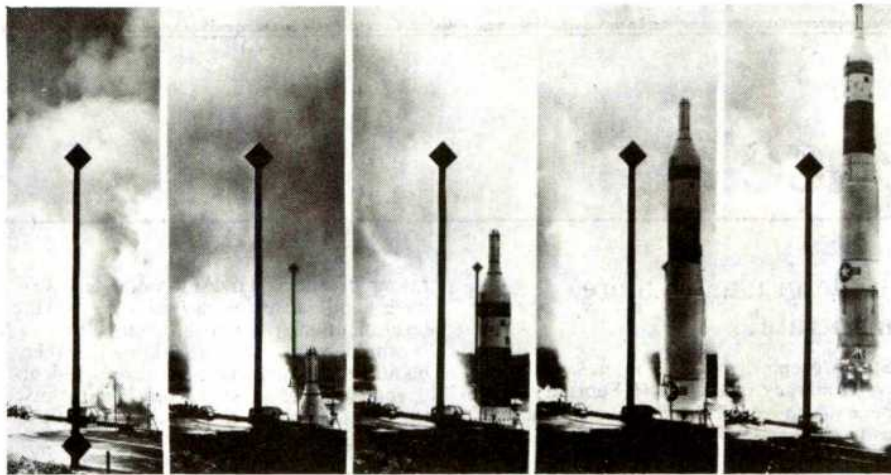
siles in order to have the largest throw-weight possible.

A typical breakdown of costs would be \$5 billion for missile R&D; \$3 billion for missile procurement (200 of 11 RV each); and \$20 billion for construction and maintenance of aimpoints. Because the cost of MAP is very largely in aimpoints, there is every incentive to reduce their cost—although that conflicts with the desire to have a large missile. On the other hand, a MAP-deployed ICBM force could be expanded greatly at little extra cost. With missile procurement a small fraction of total cost, there would be substantial interest in tripling or quadrupling the total missile throw-weight after deployment. This would be hard to resist unless constrained either by policy or by SALT.

Invulnerability of MAP is only guaranteed if the Soviet RVs remain fewer than the number which MAP is designed to counter. Even with achievable accuracies and reliabilities, a Soviet force of two RVs per MAP shelter could destroy a very large fraction of missiles, and the projection of such capabilities might lead to an early Soviet conclusion that MAP will be as vulnerable against the evolving Soviet force as is Minuteman now.

The MAP system would be even more vulnerable if the Soviet Union could learn which MAP shelters contained real missiles. Present discussions largely focus on shelters which contain either real missiles or decoys which look very realistic. Operational personnel probably must themselves be kept in ignorance of which shelters contain real missiles. When a missile is returned for service, for instance, its 29 decoys would also be returned.

The real problems with MAP are twofold. First, I believe that by the time MAP can be fully deployed, about ten years from now, the Soviet Union will readily have accuracies on the order of 100 m, allowing RV yields to be as low as 50 kilotons for effective destruction of MAP shelters. Such accuracy would come about not simply by improvement of inertial guidance systems, but also by capitalising on the availability of satellites (or ground-based radio transmitters) which will provide 10 m positional accuracy in three dimensions world-wide every 0.1 seconds. Thus it would be very difficult to argue that the Soviet Union, bent upon imperilling the land-based ICBM force, could not threaten MAP at modest cost. Further, the Soviet Union could readily stay ahead of the deployment of additional aimpoints if these aimpoints were sufficiently costly to



Underground missile sites are always vulnerable; in-shore submarines could be the answer



accommodate large missiles—probably around \$2 million per aim-point.

A second problem concerns leaving room for MAP within SALT (as demanded by Chairman of the Joint Chiefs General David Jones and by Secretary of Defense Harold Brown), for it would permit the Soviet Union to do likewise. Some may worry about the number of missiles the Soviet Union has deployed in its (hypothetical) MAP fields; I think the problem is a different one. The Soviet Union could manufacture a large number of MAP missiles, keeping them in store until it wanted to abrogate SALT. It could then deploy them within weeks into hardened MAP shelters already in existence and provided with good command, control, and communications. If a durable, accurate, commandable ICBM force does indeed convey a political advantage—as many critics of SALT assert, and hardly anyone is willing to challenge—the Soviet Union could in this way have seized an advantage unavailable at present.

For these reasons, I personally believe that we should not allow in SALT II the possibility of deployment of MAP, and that the US should not go ahead with MAP under SALT.

One basing mode for ICBMs free of pin-down constraints, and not vulnerable to improved accuracy and further fractionation of the throw-weight of Soviet ICBMs, would be to carry 6,000-mile range missiles horizontally outside the hull of many small submarines deployed close to US shores. Each submarine might weigh 350 tons and carry some 350 tons of missiles. At least 400,000 square miles is available within 200-300 miles of US shores,

far too large an area to be barraged by Soviet nuclear weapons. The proximity to the shore allows efficient use of submarine time even if the maximum speed were only 5 knots, for which a 30-kW propulsion plant would serve.

Available in the nearer term is the air-launched ICBM. Large military cargo aircraft would be stationed routinely, one or two at a time, at sparsely-equipped bases in the Central US. Each would contain one or more modern ICBMs which are launched by being dragged out of the aircraft by parachute, and fired within seconds.

Survival of the aircraft would be assured by rapid takeoff in response to indications by infrared warning satellites of attack. The aircraft could also be launched if this warning system were disrupted or destroyed, flying to one of a large number of dispersed military or civilian bases within the United States. Such a system could be made more secure if it were based on advanced short-take-off aircraft.

Because the missile would be launched from aircraft at altitude and speed, a substantial increase in number of RVs would be available in comparison with surface or submarine launch. Compared to conventional bombers, the military cargo aircraft would be much simpler and would have a higher readiness rate, thereby making the system cheaper for a given number of alert missiles. Unlike MAP, the system would preserve a very large fraction of the deployed missiles against the assumed threat. In the past, such concepts have encountered cost difficulties; it remains to be seen whether the management techniques used by the US Air Force in holding down

operational cost for silo-based systems can be applied to a new-generation ICBM and its aircraft.

Finally, because Minuteman silos are so numerous and considerably 'harder' than an RV they could in fact be defended at an affordable cost. This is not true of unique targets such as command centres, nor targets such as cities against which dozens of nuclear warheads could be used. Effective nuclear defence of Minuteman silos could be achieved by burying a nuclear explosive about 1 km north of each silo and detonating it under the control of an upward-looking mini-radar a few km further north. A non-nuclear defence which is receiving some attention uses similar mini-radars north of each silo to predict the arrival time and point of each RV at an imaginary screen some 1 km north of a Minuteman silo. At this point the RV would be met by a flight of 10,000-40,000 mini-rockets (about 500 g each) flying at a speed of 1,000 m/sec from a launching silo near the Minuteman silo. Because the radars look at the threat RVs from the side and not from the nose, and particularly because they see them at 2 km distance rather than 100-1,000 km range, they can be very small and cheap. Although they are more vulnerable to attack than a Minuteman silo when operating, the radars can be made effectively immune by the use of additional radars in mini-silos to be put into service after the operating radars are destroyed. Such a system might serve to prolong the life of Minuteman silos during the awkward period from 1982, when the Defence Department assesses vulnerability to be likely, to the completion of full-scale deployment of some new ICBM basing mode in 1988.

In conclusion, I believe that MAP is a non-starter. It is far too expensive, far too late in remedying Minuteman vulnerability (if such be important), essentially incompatible with SALT, and burdened with environmental problems. While the possibility of defending existing Minuteman silos suffers from lack of appeal to advanced technologists, it deserves more and urgent attention. In my opinion, the basing of a new generation of encapsulated ICBMs on near-ocean submersibles is also very attractive, and it may have some appeal in the modernisation of the strategic nuclear force of the United Kingdom. The more familiar concept of air launched ICBMs, though perhaps more expensive than the mini-subs, may be available sooner as an effective counter to Minuteman vulnerability.

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correspondence

Smallpox research at Birmingham

SIR,—We took note of your editorial (11 January, page 75) concerning Professor Shooter's report and related aspects of the smallpox case in Birmingham.

It may seem surprising to your readership that, to date, various statements and opinions concerning this report have gone unchallenged by Professor Bedson's colleagues. We, who are members of the Department of Medical Microbiology, should like to record that this does not imply acquiescence or agreement with public statements concerning the report nor indeed, with the content, interpretation and conclusions of the report. The Shooter report is not yet public and there is pending a prosecution from the Health and Safety Executive; on this account, it seemed improper to make public comment.

We think, however, that in certain areas of your editorial the reader is entitled to some factual information. For example, with respect to Dr Mark Darlow's statement that many academics still believe that "what was good enough for Pasteur is good enough for me", we feel that most academics would wish to know which of their fellows supported this contention; and later, with respect to Dr Darlow's comment: "Bedson knew he was backing a lame horse and that it would stumble sooner or later. It stumbled sooner," we should like to enquire what evidence led Dr Darlow to this conclusion and, further, was his conclusion based on personal knowledge of Professor Bedson and his department or on his own interpretation of the report?

It does not seem to accord with the opinion of grant-giving bodies who were supporting the work, nor with that of his academic colleagues in the Department of Medical Microbiology. One minor point—your editorial properly refers to Dr Darlow by his correct professional designation; we feel disappointed that Dr Darlow did not see fit to accord the late Professor Bedson a similar courtesy.

Finally, your extensive editorial, which we felt to be a very reasonable assessment of the Shooter report is, *de facto*, based only on the findings of the report and must, of necessity, depend on the assumption that the report is a correct and reasonable account. In our opinion, it is not a correct and reasonable account, and quite simply is unfair to Professor Bedson and his professional reputation. We hope for the courtesy of your columns at a later date (when the Shooter report is public) to communicate to the academic readership our personal reservations and criticisms.

Yours faithfully,

G. R. B. SKINNER
A. BUCHAN

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Undergraduates more numerate?

SIR,—Joseph Schwartz' interesting article on numeracy in Britain (1 February, page 344) gives obvious cause for thought as to the basis on which one can make any statement concerning supposed changes in numeracy over the years. So far as university entrants are concerned one hears many subjective views expressed, mostly of a pessimistic kind, but seldom are any hard facts adduced in support of these views. In the absence of any long-term research study having been carried out over the last decade or two it is hard to reach any conclusions that would satisfy the stringent requirements of a research publication, but our own experiences with biological sciences students at Leicester may provide a pointer, at least so far as one identifiable group of 18-year-olds is concerned.

In common with many other schools of biological sciences we find it necessary to teach a limited degree of basic algebra and statistics to our entrants, most particularly to those (over half) who have not read A-level mathematics. We have taught such a course to our first years since the inception of the school in 1968 and at least since 1971 the format, content and method of assessment of the course (mostly by computer marked multiple choice tests with questions drawn from a largely unvarying data bank) have remained unchanged. The average A-level attainment of our entrants, and the proportion having A-level maths have also not changed significantly during this period.

Until some three years ago one could safely predict that some 10–15% of our entrants would be almost entirely unable to cope with even the simple course which we give. At the other end, the numbers gaining a very high mark would never exceed 20%, and was often much lower. Each of the last three years, however, has seen a clear improvement over any previous year, either in the numbers failing, the numbers gaining a distinction, or both.

Other than an improvement effected three years ago in the physical arrangements for our problem classes (which could be significant) those of us who lecture in this course are not aware of any change in our teaching technique in the last three years, and indeed with the rise in the proportion of entrants who have taken "modern maths" at school a certain communication problem is beginning to be evident.

These observations clearly cannot be taken as definitive evidence for a rise in numeracy amongst our entrants, even though my subjective judgement would be that our current entrants are much less afraid of numerical work than their predecessors of, say, seven or eight years ago.

They most certainly, however, do not lend any credence to the notion of a decline in numeracy in the group concerned, and in areas such as computing and numerical biology in which we give

rather detailed courses at Leicester, we have good cause for satisfaction with the level of interest and attainment found. It would be interesting to know if other institutions which have admitted students over a number of years and have assessed their numeracy in some way have had similar experiences.

Yours faithfully,

A. J. ROWE

Department of Biochemistry,
University of Leicester, UK

Scientists should press for Freedom of Information

SIR,—As a public member of the all-party Parliamentary Freedom of Information Campaign (FOIC), I should like to draw the attention of the scientific community to the important private member's bill of Mr Clement Freud MP, called an Official Information Bill, which has been given an unopposed second reading (*Hansard*, Vol 960, No 38, 19 January), and is now in its committee stage.

The main purpose of this measure is to repeal the notorious "catch all" Section 2 of the discredited Official Secrets Act of 1911, and to replace it by a form of Freedom of Information legislation. This latter is broadly in accordance with the obligation of the United Kingdom as a signatory to the European Convention on Human Rights and Fundamental Freedoms, and with especial reference to Article 10 (freedom of expression) of the Convention. Thus, the Council of Europe (Report No 4195) calls upon "the governments of member states which have not yet done so, to introduce a system of freedom of information, ie access to government files, comprising the right to seek and receive information from government agencies and departments, the right to inspect and correct personal files, the right to privacy and the right to rapid action before the courts in these matters."

On behalf of the FOIC, I therefore urge all members of the scientific community to write to their MPs to support the Official Information Bill during its next stages in Parliament; and to make representations through their unions to similar effect. (Freedom of Information legislation is official TUC policy.)

And finally, I appeal to those members of the scientific community living in countries which have already introduced Freedom of Information into statute law, to impress upon their British colleagues, in their various contacts with them, the importance of such legislation both for the advancement of science (and the public interest) in the United Kingdom and for the broader aims of effective international cooperation.

Yours faithfully,

STANLEY ALDERSON

Cambridge, UK.

news and views

LEAD is an extremely useful metal and men have mined and smelted it for over 8,000 years. The enormous demand for the metal in classical times produced widespread and generally fatal plumbism among the slaves and prisoners who usually mined it. Occupational plumbism was for centuries a serious menace among lead workers, potters and in lead pigment mills (see Dickens 'A Star in the East' in *The Uncommercial Traveller*); however the general public was not exempt from the ravages of this disease. Widespread and frequently fatal colic epidemics, usually caused by lead-adulterated wines ravaged many parts of Europe until the 19th century (see *TIBS* 2, 147; 1977).

Environmental lead poisoning of comparable severity is fortunately rare today (but see 'Thai village finds tragedy on a road paved with lead [slag]', *New York Times*, July 5; 1977), but the levels of lead in the environment as well as in the bodies of inhabitants of industrial countries have

Living with lead

from Josef Eisinger

been rising along with the exponential growth of lead production and are now one or more orders of magnitude greater than the 'natural' levels. This has evoked considerable concern among the public as well as governmental health authorities grappling with the task of defining safe limits for environmental lead exposure.

The most important biochemical interactions of inorganic lead are characterised by the ions' propensity for binding to sulphhydryl groups which leads to the inhibition of many different enzyme systems. As a result the early symptoms of chronic lead intoxication are variable and nonspecific and the

problem of relating exposure to disease (for example, effects on the nervous system) is a difficult one and is, because of its economic ramifications, often controversial.

A recent report (*Investigations into lead from motor vehicles*, Chamberlain *et al.*, Harwell, HMSO; 1978) illustrates one approach to improving our understanding. It presents the results of a painstaking experimental study of the pathways by which lead enters and leaves our bodies and what is the size and fate of the fraction which stays behind.

Since the study, which employs the tracer ^{203}Pb ($T_{1/2}=52\text{ h}$) as well as stable lead, is primarily concerned with emission from motor vehicles it includes ambient measurements of the concentrations and size distributions of lead aerosols near roadways. These are found to depend upon driving and weather conditions, with air lead levels near motorways ranging up to $10\text{ }\mu\text{g m}^{-3}$, about the same as in the vicinity of lead smelters. Air levels in busy London sites are broadly speaking about half of this value and inside a car the levels are only slightly lower. From the data given and some reasonable assumptions the amount of lead emitted along a road carrying an average of 1,000 cars per hour is $100\text{ g m}^{-1}\text{ d}^{-1}$, some 10% of which is deposited within 100 m of the road.

When such lead laden air is inhaled, approximately a third is deposited in the lung although this fraction is considerably higher for very small aerosol particles ($<0.05\text{ }\mu\text{m}$) and there appears to be a significant variation among the subjects who participated in these experiments. Clearance rates from the lung follow complex kinetics, depend on particle size and chemical composition and again show individual variability. About a day after a person is injected with or inhales ^{203}Pb , his blood lead level reaches a maximum and the total amount in his blood is approximately half of the dose given.

The efficiency with which lead compounds are taken up from the gut is also found to vary considerably from subject to subject (for example 2–26% for PbS) and depends on the chemical composition, with even nominally insoluble compounds (for example PbCrO_4) being taken up quite efficiently, presumably because of the high stomach acidity. A full stomach is found to offer a measure of protection:



This woodprint from Agricola's *De re metallica* (Basle, 1556) shows the smelter foreman eating from a tub of butter which, the caption explains, is a specific remedy against the poisonous lead fumes issuing from the smelter behind him.

A 12-hour fast increased the efficiency with which PbCl_2 is taken up from 7 to 45%. This appears to bear out the traditional advice offered to lead workers and potters by the earliest miners' physicians, to eat lots of fatty foods and soups (Stockhausen *Libellus de lithargyrii* . . . Goslar, 1656, see figure).

Chamberlain *et al.* (*op. cit.*) perform a great service by presenting a synthesis of their results and those of other investigators in the field. This should be useful in constructing kinetic models for the lead transport within the body. The factor relating blood levels (in $\mu\text{g dl}^{-1}$) to ambient air levels (in $\mu\text{g m}^{-3}$) for instance, is found to range from 0.5 to 2, depending on the investigator and/or the methodology employed.

The most significant conclusion from the point of view of environmental policy may be that at $1 \mu\text{g m}^{-3}$, the average air level in British cities, the ratio of air lead to other lead intake is low, between 8 and 20%, and is in substantial agreement with the conclusion of another recent study (*Lead pollution in Birmingham*, HMSO, 1978). The importance of local conditions can be seen from the finding that the mean lead level in the blood of mothers and children in North Wales is doubled if their houses have lead, as opposed to copper piping (Elwood *et al.* *Lancet* **i**, 1363; 1978). This suggests that the greatest lead intake among the general population in Britain is caused by lead piping in soft-water areas.

A somewhat different approach to the problem of gauging the severity of environmental lead disease makes use of biological indicators of lead toxicity. While the blood lead level has been used traditionally for this purpose it suffers from serious drawbacks in that such assays are slow, expensive, require skilled personnel, have poor reproducibility and are prone to contamination errors. In view of the complex and variable kinetics of lead transport with-

in the body the blood level is moreover an indicator of uncertain validity for long term lead exposure. While elevated porphyrin levels have long been known to be associated with lead disease (Watson *et al. Pediatrics* **12**, 40; 1958) the zinc protoporphyrin (ZPP) level in blood has been demonstrated recently to be a superior indicator to blood lead, in that it was found to correlate better with the signs and symptoms of chronic lead disease and has, as will be seen below, numerous practical advantages as well. (Eisinger *et al. J. Env. Path. Tox.* **1**, 897; 1978; Grandjean *et al. Scand. J. clin. Lab. Invest.* **38**, 669; 1978).

The enzyme ferrochelatase inserts iron into protoporphyrin IX to form haem and is inhibited by lead ions at the site of erythropoiesis. When this occurs (or if there is a shortage of the substrate Fe^{2+} as in iron deficiency anaemia) zinc ions, which are available in the bone marrow are substituted for iron to form ZPP which is then incorporated in the globin (Lamola *et al. Science* **186**, 936; 1974). The resulting impairment of the blood's oxygen carrying ability is negligible since the haemoglobin of even seriously lead poisoned persons contains less than 1% of ZPP-globin. What makes this assay for lead toxicity so useful is that (1) ZPP levels in blood can be measured fluorometrically since ZPP fluoresces while the haem does not, and (2) ZPP-globin once formed, remains in the erythrocyte for the life time of the cell (~ 120 d) so that ZPP level in blood is an indicator with a 4-month integration time (Lamola *et al. J. Lab. clin. Med.* **56**, 1528; 1975). This is particularly important where exposure is chronic but intermittent.

In the US the ZPP assay has been recommended for the screening of children for lead exposure by the Center for Disease Control, with the test's response to iron deficiency anaemia being considered an additional advan-

tage. Recently the US Occupational Safety and Health Administration while retaining standard limits for environmental and blood lead levels, recommended ZPP assays for the medical surveillance of lead exposed workers (*Fed. Reg.* **43**, No. 220; 1978).

ZPP levels in blood have a considerable dynamic range. The blood of city and country dwellers typically register 50 and $20 \mu\text{g dl}^{-1}$ respectively while that of lead workers can range up to $1,000 \mu\text{g dl}^{-1}$ (Lilis *et al. Environ. Res.* **14**, 255; 1977). Central nervous system dysfunction, probably the most serious but elusive aspect of lead disease, has been shown to be significantly correlated with the blood ZPP levels (Valciukas *et al. Science* **201**, 465; 1978).

The ZPP assay is not only a meaningful indicator of lead disease but a particularly convenient one, because of the development of a portable instrument. This dedicated front face fluorometer, called a haematofluorometer, requires only a drop of blood, generally obtained by finger puncture (Blumberg *et al. J. Lab. clin. Med.* **89**, 712; 1977). The blood is smeared on a glass cover slip which is inserted in the instrument, and a digital display gives the ZPP level in convenient units within seconds. Since haematofluorometers have become commercially available, ZPP levels can now be measured quickly, accurately and at a small fraction of the cost of a blood lead determination. One may hope that ZPP assays will be useful not only for the screening of potentially exposed persons, but that they will make feasible large scale epidemiological studies of the general population to improve our understanding of the relationship between lead exposure and disease. $\square$

Josef Eisinger, a scientist at Bell Laboratories, Murray Hill, New Jersey is at present a visiting Guggenheim Fellow at The Physiological Laboratory, Downing Street, Cambridge.

A hundred years ago

We are greatly disappointed and much surprised to learn that the application of the Scottish Meteorological Society for assistance to establish an observatory on Ben Nevis has been rejected both by the Meteorological Council, with its yearly 15,000*l.*, and the Government Grant Committee, with its 5,000*l.* The former had other matters to attend to, the latter handed the application over to the Council of the Royal Society, labelled "highly commendable." We have recently shown our readers with what heartiness and cosmopolitanism such nationally beneficial undertakings are managed in France; yet in this disgracefully wealthy country, with 15,000*l.* a year expressly devoted to meteorology, a plan of promoting meteorological research that would lead to results of the highest consequence must collapse for want of a paltry 500*l.* to start it. It would be a

shame if so really national an enterprise were to depend entirely on private subscription.

From *Nature* **19**, 13 March, 445; 1879.

A REMARKABLE phenomenon is reported from Neufchatel. On February 10 the Lake of Neufchatel suddenly assumed a motion like the sea with its tides, only with the difference that the rise and fall of the water succeeded each other in much shorter intervals. The phenomenon began at noon and lasted until 2 p.m. Boys who were playing on the shore were so suddenly surprised by the rising lake that they were up to their knees in water before they had time to escape. In the evenings there was a violent thunderstorm, which also visited Berne at the same time.

From *Nature* **19**, 13 March, 446; 1879.

INTERESTING correspondence has appeared in the British Guiana *Royal Gazette*, we learn from the *Colonies and*

India, relative to the qualities assigned to the fruit of the papau-tree. It has been recently asserted, in an article in the *Pharmaceutical Journal*, "that the most interesting property attributed to it is the power of its juice to render bad flesh tender." Mr. Monro, of Georgetown, furnishes certain facts which he says are commonly known to the natives of British Guiana relative to this fruit. A horse tied near one of these trees rapidly loses health, and a stud horse becomes useless. Any pressure on the body of the animal leaves an inelastic indentation. The sap of the tree will soften steel, and before the process of tempering was known in the Colony, the blacksmiths used to drive their brittle chisels and plane vices into the wood, leaving them there for a day or two; and tough meat wrapped in the leaf for only a few minutes becomes tender.

From *Nature* **19**, 13 March, 447; 1879.

Unified field theory: dream becoming reality?

from F. E. Close

EINSTEIN spent the latter part of his life attempting to find one formula that would explain both the gravitational and the electromagnetic field. Pauli believed this to be impossible stating "what God hath put asunder, no man shall ever join".

Einstein failed in his quest but recent theoretical developments in high energy physics, combined with sophisticated experiments, suggest that we may be at the threshold of a profound unification of electromagnetism with the weak force of radioactivity and also the strong nuclear force. This will leave gravity as the final link in a grand synthesis.

Thus the centenary of Einstein's birth is an appropriate moment to outline why the high energy physics community is so optimistic that such a grand scheme may be at hand, even though gravity itself is not yet included.

Electromagnetic and weak interactions

The electromagnetic force has been well understood for 30 years. It is mathematically described by quantum electrodynamics, QED, the theory of the interaction of charged particles with photons (the quanta of the electromagnetic field).

As long ago as 1934 Fermi had recognised some similarities between the weak β -decay of the neutron and electromagnetic phenomena. This led him to propose a model for the weak interactions. This model was perfectly adequate as a description of laboratory phenomena but was found to contain internal inconsistencies if applied to extremely high energies. In 1957 Schwinger showed that if there existed electrically charged W bosons then the deficiencies of the Fermi model could be made less serious.

These W bosons of the weak interactions have a role analogous to the photon in QED. They are predicted to be about 70 GeV in mass (the proton is but 1 GeV mass) and it will be another couple of years before machines will be developed that can produce them.

In the 1960s Glashow, Salam and Ward and also Weinberg were instrumental in using this idea to create a model which unified QED and the weak interaction. If the W bosons had been massless, like the photon, then 'weak' interactions and electromagnetic interactions would have had essentially the same strength and been manifestly recognised as being 'the same'. The fact that $W^\pm$ are so massive is the

reason why interactions with W bosons are so much weaker than those with photons and makes electromagnetism and the weak interaction seem to be different.

In 1970 't Hooft showed that this model was completely self consistent, violated no sacred principles and was thereby a candidate theory unifying the weak and electromagnetic interactions. Accumulating data during the past 5 years, and in particular in 1978 (see *News and Views* 264, 505; 273, 99; 274, 11; 275, 267) now give solid evidence showing that this theory is very near the truth, and could even be completely correct.

This theory is known as quantum flavour-dynamics, QFD, and contains QED within it. The unified electromagnetic and weak force is often referred to as the 'electroweak' force.

QCD: a theory of strong interactions

Particles like the proton which feel the strong nuclear force appear to be complicated systems of more fundamental particles called quarks. Quarks have electrical charge and flavour and so partake in interactions with photons and W bosons, that is, the electroweak interaction. They also appear to carry a further kind of charge known as a colour charge. It is hypothesised that there exist coloured gluons, analogous to the photon of QED, which couple to this colour charge and hence generate the strong interaction. The resulting theory is called quantum chromodynamics, QCD, and has many similarities to QED. This theory is still very young and its richness is only gradually being understood. Quantitative definitive tests are slowly being found and the first data that are being accumulated appear to be in excellent accord with the predictions (see *News and Views* 277, 349; 1979).

Grand unification

QED involves interaction of electrical charge with photons; QFD involves interaction of flavour with W bosons and QCD involves interaction of colour charge with gluons.

This qualitative similarity between the three theories is manifested mathematically, the three differing only in internal symmetry properties which are described by group theory. QED is a $U(1)$ field theory containing one 'gauge boson'—the photon. An $SU(N)$ 'non-Abelian' field theory has N^2-1 gauge bosons; hence three ($W^+W^-W^0$) for the $SU(2)$ QFD and eight gluons for the $SU(3)$ QCD (see for example *News and Views* 277, 349).

The idea behind grand unification is to find a super group G which contains $U(1) \times SU(2) \times SU(3)$ (QED, QFD and QCD). The simplest example for G is the group $SU(5)$. The above (N^2-1) rule shows that there will be 24 gauge bosons. Twelve of them will be the photon, three W bosons and eight gluons. A further dozen are thereby predicted.

The $SU(5)$ supergroup accommodates a pair of leptons (for example e^- and ν) partnered by a pair of quarks (u and d). The quarks necessarily have colour while leptons do not. These features are all in accord with experience. This pairing of a lepton doublet with a quark doublet has been recognised for some time and the set is often referred to as the 'first generation of elementary particles' (see *News and Views* 271, 406; 1978). Nature appears to have made xerox copies: a second generation ($\mu^- \nu'$ and cs) and possibly a third generation ($\tau^- \nu''$ and $t(?)b$). This is acceptable in $SU(5)$, if somewhat unnatural.

The twelve new gauge bosons of $SU(5)$ can cause quarks to transmute into leptons. This will make the proton unstable (see below) and may provide a test of this idea. Indeed this prediction of proton instability is not unique to the $SU(5)$ idea and occurs in other candidate schemes where G is the group $SO(10)$ or the exceptional group E_6 . These different groups make different predictions for the menu of quark and lepton flavours that await discovery at the new accelerators.

Asymptotic freedom and grand unification mass

The possibility that we may be able to invent a sensible grand unification scheme has come from the discovery of the electroweak unification and also the possible discovery of the laws governing the strong interaction (QCD). Of particular significance in the latter case has been the discovery of the property of 'asymptotic freedom'. This appears pivotal to the grand unification idea.

The strength of the strong interaction at low energies is much greater than the electroweak interaction. The quark-gluon coupling decreases in strength as energy or mass increases until asymptotically it tends to zero (this is 'asymptotic freedom'). There will therefore exist an energy scale where the 'strong' interactions are no longer strong, but have comparable strength to the electroweak. At such an energy 'unification' can have a well defined meaning.

This energy scale can be calculated, and turns out to be about 10^{19} GeV, and so in a realm where gravity may also begin to be important. In the original heat of the big bang such energies may have been common and a true unity may have existed. As the Universe cooled so the different interaction strengths froze out giving the strong, electromagnetic and weak interactions of the present day.

The original symmetry of the hot Universe has gone. The heat generated in high energy accelerators has managed to give a glimpse of nature soon after the big bang and reveal hints of that long lost unity. As energies are increased so one reaches nearer to that original symmetry. Therein is the aesthetic beauty of particle physics and the grand unification ideal.

Progress so far

At present, high energy physicists are concentrating on deepening their understanding of QCD and of trying to find the correct 'super group' that will enable it to be unified with the electroweak interactions.

The simplest candidate for this unifying group is SU(5), proposed by Georgi and Glashow (*Phys. Rev. Lett.* **32**, 438; 1975) and investigated in some detail by Ellis and collaborators at CERN, Geneva. A natural consequence of such a grand unification is that quarks (which experience QCD) and leptons (which only experience the electroweak force) are related. For example, if Nature contained a charged lepton of mass 10^{15} GeV (the 'grand unification mass' where all interactions have the same strength) then it is predicted that there would also exist a quark of charge $-\frac{1}{3}$ at that same mass. In practice the Universe is very cold, and particles have masses of order 1 GeV rather than 10^{15} GeV. Ellis and co-workers showed that as the lepton or quark mass comes down from 10^{15} GeV to 1 GeV, so the quark to lepton mass ratio increases. As an example consider the following.

The ratio of strange quark to muon mass is known to be about five (0.5 and 0.1 GeV respectively). After the discovery of the τ lepton in 1975 one necessarily predicted the existence of a 'bottom' quark in the SU(5) framework. The τ is about 2 GeV in mass, hence 'nearer' to 10^{15} GeV than the 0.1 GeV muon, with the consequence that the ratio of bottom quark to τ mass will be nearer to unity than was the ratio of strange quark to muon. Ellis *et al.* calculated this and predicted a mass of about 5 GeV for the bottom quark (about twice that of the τ lepton). The lightest meson built from a bottom quark (b) and its antiquark ($\bar{b}$) would therefore be expected at about

10 GeV. The discovery of Y at 9.46 GeV, made from $b\bar{b}$ (see *News and Views* **275**, 267; 1978) is in remarkable accord with this prediction.

Another interesting feature of grand unified theories is that the intimate relationship between quarks and leptons implies that quarks can transmute into leptons, albeit very rarely. A consequence of this is that the proton, which is made from three quarks, will not be stable; there is a probability that it will decay into three leptons. Estimates for the lifetime of the proton vary but it could be as 'short' as 10^{33} years.

As the Universe itself is only of order 10^{10} years old, this prediction may seem rather esoteric. However, Avogadro's number enables one to study vast numbers of protons and experimentally a lower limit of about 10^{30} years has been obtained for the proton's lifetime (Reines *et al.* *Phys. Rev. Lett.* **34**, 493; 1974). Improvement of this is feasible and so it is possible that tests of some of these ideas may be forthcoming. Several authors have recently pointed out that proton instability could be an important ingredient in quantitatively understanding the observed excess of matter over antimatter in the Universe.

Some byproducts of grand unification ideas may soon be clarified at the new high energy electron-positron collider PETRA at Hamburg. In the SU(5) and SO(10) super group schemes it is predicted that a 'top' quark must exist, charge $\frac{2}{3}$ of the proton charge, to complete the symmetry of leptons and quarks. In the scheme based on the exceptional group E_6 , however, it is predicted that you cannot have such a quark but that instead a quark with charge $-\frac{1}{3}$ will exist. Hence a search for this (sixth flavour) quark promises significant consequences for theory.

Whichever particular scheme for superunification you buy, you find the prediction of a sixth flavour of quark. Mass estimates vary somewhat but about 20 GeV seems popular. This is tantalisingly close to the maximum energy that PETRA and its Californian counterpart PEP can attain.

In addition, the quantitative successes of grand unification (such as the prediction of the b quark mass) seem to require that no more than six or eight flavours of quark and of leptons can exist. Therefore it is possible that the next generation of accelerators will reveal the end of lepton-quark spectroscopy.

If QCD can be confirmed as the correct theory of the strong interaction (see *News and Views* **277**, 349; 1979) then it seems that we will indeed have all the ingredients to hand to build a successful grand unified theory.

The menu of quark and lepton flavours and the profound similarity (and subtle differences) between quarks and leptons and also their relative masses seem already becoming more clearly understood. It seems possible that a successful grand unification might also explain the various dimensionless parameters that pervade our Universe, like α the fine structure constant, the magnitude of the Cabibbo mixing angle and the weak-electromagnetic (Weinberg) mixing angle. The possibility that the proton is not stable is also an amusing consequence.

At the centenary of Einstein's birth, this seems to be the direction in which high energy theory is moving. However, the role of gravity and its incorporation in ultimate unification, Einstein's great unsolved problem, is still unclear. Now that superunification energies approaching the Planck mass are being discussed, it seems probable that we are approaching a point where ideas in gravity and high energy physics will have to fuse if we wish to approach a full understanding of how the present cool unsymmetrical Universe froze out from the big bang. $\square$

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High level nuclear waste isolation: borosilicate glass versus crystals

from G. de Marsily

IN a paper in this issue of *Nature* (page 219) Ringwood *et al.* advocate the use of an artificial crystalline material in which to immobilise the solid waste arising from the reprocessing of nuclear reactor fuel rods. He proposes to incorporate solid wastes in an artificial rock ('SYNROC') composed essentially of three minerals: hollandite, perovskite and zirconolite. According to Ringwood, up to 10% of waste could be incorporated into SYNROC; this material would be produced by hot-pressing the waste and ingredients at 1,200–1,300 °C, thus obtaining crystallisation, at a reasonable cost. This solution is clearly a serious potential competitor to the more usual glassification concept, which has been studied for more than 10 years, mainly in the UK and France, and is at present in operation in France at Marcoule.

As a geologist, I am concerned with

the leachability and stability of the waste in a geological formation, which will form the final barrier between the waste and our environment. From this viewpoint what are the differences between SYNROC and glass.

In their original form, the wastes produced by the reprocessing plants are liquid. Early projects suggested that this liquid be injected directly into the ground (either into a deep permeable layer, with immobile fluids, or into a cavity which would subsequently melt, due to the heat produced by the waste). Such projects have been disregarded, the radioactive elements being too readily available for transport by the ever present groundwater.

Incorporation of the calcined waste into a solid matrix then began to be considered. Many materials have been proposed, including glass, ceramic, cermets, metals, mica, feldspar, synthetic uraninite or thoriumite, and now SYNROC. So far glass has probably received most attention and given the best results. The leachability of glass depends on at least three distinct mechanisms: (1) dissolution of the borosilicate glass matrix in the water (in a salt environment, the solubility of silica increases, so that the thermally-driven fluid inclusions in the salt might have an important effect on the integrity of the glass); (2) molecular diffusion of the elements through the amorphous matrix, towards the surrounding solution, and (3) thermal diffusion (soret effect) of the elements in the glass, under the strong thermal gradients induced in the matrix by the heat produced by the waste. These three factors are also temperature dependent.

As we have discussed previously (Marsily *et al.* *Science* 197, 519; 1977), it becomes more and more obvious that the glassified waste will have to be stored in a cooling facility for 50 years or so after the extraction of the fuel from the reactor, before final disposal in a geological formation can be considered. This interim storage can perhaps take place in the final repository, but with a cooling mechanism (ventilation for instance). This will keep the temperature in the geological formation under 100 °C at 500 m depth, with a reasonable density of waste in the glass (of the order of 10 to 20%), and of glass in the geological formation (producing a heat load of the order 50 kW ha⁻¹ or less).

After 50 yr, the waste will produce 5 kW of heat per m³ of glass, at a 20% waste load. The rate of heat production 'decays' with a 'half life' of approximately 30 yr. It is of course

possible to reduce this rate by decreasing the waste load in the glass, but then the quantity of glass to be disposed of increases, and so does the area of the repository, the costs and so on. The suggested temperature of less than 100 °C will remain more or less steady for 1,000 yr due to both dissipation of heat in the rock and heat production decay. Accepting a temperature higher than 100 °C makes the safety evaluation of the repository much more uncertain (as a result of higher leach rates and mechanical instability of the rock and so on).

With such a temperature, and if a flow of water can reach the glass (that is, assuming the geological formation is not totally impervious and the container itself is not a good barrier), conservative estimates of the time needed to remove all the radioelements from the glass are in the range of a few thousand years. Much better results can be obtained if very little water flows through the repository, because the dissolution of the silica from the glass and the diffusion by the concentration gradients are then reduced; the leachability is also reduced if the silica content is increased, and the waste load reduced (for example to 10%, as in SYNROC). Note that other artificial barriers can also be added around the waste.

Now, what about the stability of the glass? The leach rates which I have considered above already assume that the glass matrix has suffered from thermal fracture as the canisters are decontaminated and cooled after fabrication. A factor of 20 is generally accepted for the increase in area per unit volume with respect to an undisturbed glass block of 150 l. Can other mechanisms increase this area? Many experiments, including resistance to radiation, with increased quantities of α -emitters (²⁴¹Am, ²⁴⁴Cm) to simulate the passage of 1,000 yr in 2 yr have, so far, given very good results. (If ²³⁹Pu is used instead of ²⁴¹Am, however, the mechanical resistance of the glass might be drastically reduced.) Only the possibility of crystallisation is considered dangerous, and this is one of the reasons why temperature should be kept as low as possible in the repository. Even then, crystallisation of the glass does not mean disaggregation. Phase separation (creation of a soluble molybdate phase) has been reported for some types of glass, but only during processing, and has not been suggested as a potential cause of instability.

However, it is well-known that artificial and natural glasses (obsidian, for example) brought to a high temperature and pressure can be metamorphosed into a clay-type material. That is what Ringwood has done: he brought samples of glass and SYN-

ROC to 350–400 °C and 1,000 bar, and found that the glass disintegrated (whereas SYNROC resisted). I have already discussed temperature, now what about pressure? The repository will lie at a depth of 500–1,000 m. After emplacement, it is generally agreed that it will be sealed with a buffer material (such as sand and bentonite, as proposed by the Swedes) and that grouting will be made to minimise the pore space of this buffer material. At the most, this grouting will be made at the lithostatic pressure, that is, of the order of 250 bar at 1,000 m. Now it is questionable whether the waste will be subjected to this pressure, or only to the hydrostatic pressure (for example, 100 bar). In any case, the pressure and temperature used by Ringwood seem inappropriate. Twenty-four hours at high temperature and pressure will never be equivalent to several thousand years in less severe conditions: the results obtained by Ringwood are as yet insufficient to prove that glass should be eliminated as a possible confining matrix.

On the other hand, the use of a crystalline structure instead of an amorphous matrix has always been attractive to geologists: elements trapped in a crystalline network are much less mobile, provided that the crystalline mineral has a very low solubility. This became evident in the study of the Oklo natural reactor which 'burnt' uranium approximately 2 billion years ago, in Gabon. Most of the radioactive 'waste' produced during the fission reaction has been found 'trapped' inside the crystalline structure of the uraninite mineral which formed the ore body. On the other hand, the elements which could not fit into the crystalline lattice of uraninite (because of a too small or too large ionic diameter) diffused and were apparently leached away.

One may then ask the following questions. Can the three minerals constituting SYNROC host all the radioelements present in the waste, and retain them with the same strength? Apparently only Cs, Sr and U have been actually tested on SYNROC B. Can a reasonable amount of waste (10% for example) be integrated in SYNROC, the present test having been done only with 1% of Cs, Sr and U? Is the stability and low leachability of SYNROC proved under more realistic conditions (water with low pH and more complex chemical composition than just NaCl, as may be found in a granitic environment)? Is radiation damage still insignificant for a 10% load of waste? The results obtained at the Battelle and Pennsylvania State University on a similar type of material ('supercaline') give approximately the same leach-

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ability as glass. And one must remember that stability of SYNROC at high temperatures and pressures does not automatically prove stability at other thermodynamic condition (consider for instance the weathering of granite). Is the industrial production of SYNROC as easy as Ringwood suggests? I confess to being slightly worried, for instance, over the control of the development of the crystalline phase inside the canister. This seems necessary in view of one result obtained by Ringwood, where insufficient mixing of the ingredients caused the appearance of an unknown 'accessory phase' rich in caesium, which was subsequently 'removed during leaching.' Also, the actual inclusion of ruthenium in hollandite is highly questionable in the process, given the high volatility of this element. One might also ask whether SYNROC can accept variations in composition of the waste (segregation of the liquid in the tanks) and the dissolution residues.

Nevertheless, the development of a confining matrix which would be stable for several million years in any environment is most desirable. If this could be achieved, the burden of proof of the safety of a repository would be transferred from the geologist to the manufacturer of SYNROC.

As a geologist, I cannot but support research in such a direction; as long as it is not used as an argument to delay further the search for a suitable geological formation which could contain the waste securely for a very long period of time. □

Cellulose microfibrils— an end in sight?

CELLULOSE is the most abundant macromolecule in the world; the figure usually quoted for its annual production is 10^{11} tonnes. Despite the obvious economic importance that such a figure implies, research into cellulose formation has so far failed satisfactorily to explain the way in which the functional unit of cellulose—the microfibril—is made. Viewed at its simplest, the microfibril is a crystalline association of long chains of β 1-4 linked glucose units, surrounded by a region of more amorphously associated chains. The whole structure is 6-20 nm wide, depending on its source, and intact microfibrils are of indeterminate length, certainly several microns long. The higher plant cell wall consists of a network of microfibrils associated with a matrix of other polysaccharides and proteins.

The formation of the microfibril can

be regarded as a two-stage process. The first stage is the biochemical formation of the polyglucan chains. Sugar units are added to the growing polymer as nucleotide complexes: the details of this process, and in particular the sites at which it occurs within cells are as yet unclear. The second process is the physical formation of the crystalline microfibril from the polyglucan. Here there are two rather distinct hypotheses to choose from.

The first supposes that the biochemical and physical processes occur more or less simultaneously, in other words that sugar units are added on the end of a pre-existing microfibril. Evidence for this is ultrastructural—the visualisation of ordered arrays of particles at the plasma membrane surface of algae having highly ordered cellulose walls. The particles are supposed to represent enzyme complexes engaged in the synthesis of the polyglucan chains. The evidence is persuasive, particularly since it can be further supposed that ordering of wall microfibrils might be mediated by movement of such terminating enzyme complexes within the surface of the fluid membrane. Attempts to show similar particulate complexes in higher plants however have for the most part been unsuccessful, although recent work using freeze-fracture techniques has been claimed to show terminal globuli associated with microfibril ends (Willison & Grout *Planta* 140, 53; 1978).

The alternative possibility is that the polyglucan first produced biochemically is in a soluble form, and that this intermediate associates to form the microfibril in a process not requiring direct structural linkage with the plasma membrane. This hypothesis has been recently examined by Colvin and his group, working with cellulose-producing bacteria (Colvin & Leppard *Can. J. Microbiol.* 23, 701, 1977; Colvin *et al. Can. J. Biochem.* 55, 1057; 1977; Sowden & Colvin *Can. J. Microbiol.* 24, 772; 1978). Colvin presents two lines of evidence in support of the idea that the biochemical and physical events of microfibril formation may not occur simultaneously. In structural terms, the authors describe a 'nascent' fibril, which is believed to represent an early stage in the formation of the classical microfibril. The nascent fibril has a diameter of up to 10 times that of the classical microfibril, and it consists of a dense crystalline core surrounded by an amorphous sheath which comprises most of the fibril's diameter. The nascent fibril is converted into the classical microfibril by dehydration. Thus in order to visualise the nascent fibril, only those techniques can be used which do not require the specimen to be dried before it is examined. Colvin

uses rapid freezing of 'never-dried' pellicles of bacterial cellulose, followed by deep etching of the specimen *in vacuo* and replication of the resulting etched surface. If the pellicles are first air dried, nascent fibrils are not seen, and the classical microfibril is formed.

These observations imply a change in thinking about the process of microfibril formation. The presence of nascent fibrils suggests that the acceptor for the sugar units may not be the end of a classical microfibril, but rather an unconsolidated polymer, or an association of such polymers. Direct structural involvement of membranes would not be required in this scheme.

Clearly for such a hypothesis to be viable it is necessary to demonstrate the reality of the postulated soluble intermediate polyglucan. This line of attack is the subject of the second of Colvin's three papers. The properties are described of a polysaccharide found in the medium in which the bacterial cells grow. This polysaccharide is prepared by precipitation from 60% ethanol, and further fractionated using ammonium sulphate precipitation. It appears to consist of chains of β 1-4 linked glucose units with on average a single glucose side chain on every third residue of the backbone. These branches would be expected to confer water solubility without affecting the basic linear shape of the molecule. As yet there is no direct evidence that this disperse family of polyglucans does in fact act as an intermediate in cellulose microfibril formation. Indeed, the branched structure of the polymer introduces a new complication in the form of an additional enzymic step to remove the single glucose side chains before crystallisation could take place. However, this work and its implications are at the very least sufficiently interesting to make a critical re-examination of the more established hypothesis worthwhile.

The key may after all lie with higher plant cells. Cellulose synthesis has been well studied biochemically in higher plants, but microfibril formation is naturally masked by the presence of a pre-existing cell wall. Now that the conditions for the formation of cell walls by isolated protoplasts are better understood, the time may be right to exploit this system in a more detailed way to examine *de novo* microfibril formation in higher plants. And of course, should Colvin's model prove to be applicable to cellulose formation in general, radical new thinking will be required to explain the differences in wall development which accompany cell differentiation in the higher organisms. □

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Eddington on Einstein's new theory

In 1916 Arthur Eddington, at the age of 34, was Plumian Professor of Astronomy at Cambridge, director of the university observatory and Secretary of the Royal Astronomical Society. This piece, contributed for the then equivalent of News and Views was one of the earliest simplified expositions of the General Theory in English. The author's name was inscribed over the first paragraph by the editor, Sir Norman Lockyer.

GRAVITATION AND THE PRINCIPLE OF RELATIVITY.

ACCORDING to the principle of relativity in its most extended sense, the space and time of physics are merely mental scaffolding in which for our own convenience we locate the observable phenomena of Nature. Phenomena are conditioned by other phenomena according to certain laws, but not by the space-time scaffolding, which does not exist outside our brains. As usually expressed, the laws of motion and of electrodynamics presuppose some particular measurement of space and time; but, if the principle is true, the real laws connecting phenomena must be independent of our framework of reference—the same for all systems of co-ordinates. Of course, it may be that phenomena are conditioned by something outside observation—a substantial æther which plays the part of an absolute frame of reference. But the following considerations may show that the ideal of relativity is not unreasonable. Every observation consists of a determination of coincidence in space or time. This is sufficiently obvious in laboratory experiments; and even the crudest visual observation resolves itself into the coincidence of a light-wave with an element of the human retina. If, then, we trace the path of adventure of a material particle, it intersects in succession the paths of other particles or light-waves, and these intersections or coincidences constitute the observable phenomena. We can represent the course of Nature by drawing the paths of the different particles—on a sheet of paper in a two-dimensional case. The essential part of the diagram is the order of the intersections; the paths between the intersections are outside observation altogether, and are merely interpolated. The sequence of phenomena will not be altered if the paper is made elastic and deformed in any way, because the serial order of the intersections is preserved. This deformation of the paper corresponds to a mathematical transformation of the space in which for convenience we have located the phenomena.

Until recently the application of the principle of relativity was limited to one particular transformation, namely, a uniform translation of the axes. In this case there is a wide range of experimental evidence in support of the principle. In 1915 Prof. A. Einstein¹ finally succeeded in developing the complete theory by which the postulate of relativity can be satisfied for all transformations of the co-ordinates. Gravitation plays a part of great importance in the new theory, and therein lies much of the practical interest of Einstein's work.

¹ Einstein, "Die Grundlage der allgemeinen Relativitätstheorie." (Leipzig: J. A. Barth, 1916). A detailed account appears in *Monthly Notices*, 1916, No. 9, by Prof. W. de Sitter, giving the astronomical applications. See also an article by de Sitter in the *Observatory*, October, 1916.

No attempt is made to explain the cause of gravitation—as a kink in space or anything of that nature. But the extended law of gravitation is determined, to which Newton's law is an approximation under ordinary conditions. It has long been suspected that there must be some modification of the law when the bodies concerned are in rapid relative motion; moreover, the "mass" of a moving body no longer has a unique meaning, so that a further definition, if not extension, of Newton's law is clearly needed. Now, although we do not seek a cause of gravitation in the properties of space, it may well happen that the law of gravitation is determined by these properties. The inverse-square law represents the natural weakening of an effect through spreading out in three dimensions; we may say that it is determined by the properties of Euclidean space. There is, therefore, nothing unreasonable in proceeding, as Einstein does, to examine whether a more extended law is suggested by the properties of generalised space—that is, by geometry.

The way in which gravitation enters into the discussion may be seen from the following example. Suppose an observer is in a closed lift; let the supports break and the lift fall freely. To the observer everything in the lift will now appear to be without weight; gravity has been suddenly annihilated. The acceleration of his frame of reference (the lift) is equivalent to an alteration of the gravitational field. Now an acceleration of the axes is one of the transformations contemplated by the general principle of relativity, and it is therefore necessary to allow that the gravitational field depends on the choice of co-ordinates. There is a "local" gravity, just as there is a "local" time or magnetic field depending on the co-ordinates selected.

We can now take a brief survey of Einstein's procedure. Suppose that space and time are measured by a system of co-ordinates x_1, x_2, x_3, x_4 ; x_4 is the time, but there is no need to discriminate between it and the others. If there is a gravitational field at any point, it can be abolished (as in the example of the lift) by choosing new co-ordinates x'_1, x'_2, x'_3, x'_4 accelerated with respect to the old. The necessary transformation could be specified in various ways; the way chosen involves the element of length ds measured by coincidences with a standard scale, and therefore independent of the choice of co-ordinates. Since in the x' co-ordinates there is no gravitation to complicate matters, we can safely use the usual formula,

$$ds^2 = dx_1^2 + dx_2^2 + \dots$$

and this when transformed to the old co-ordinates takes the most general form,

$$ds^2 = g_{11}dx_1^2 + g_{22}dx_2^2 + \dots + 2g_{12}dx_1dx_2 + 2g_{13}dx_1dx_3 + \dots$$

The ten g 's depend on the transformation, and can

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be used to specify it. But they do more than that; they define the original gravitational field, since they specify how it can be got rid of. They usually vary from point to point, because a different transformation is needed to eliminate gravity at different points. In the new theory the g 's are regarded as ten gravitational potentials specifying the field; and, in fact, one of them, g_{44} , is approximately the same as the Newtonian potential ϕ , except for a factor.

The inverse-square law can be expressed by the well-known differential equation $\nabla^2\phi=0$. Evidently the new law of gravitation must be a generalisation of this—an equation, or set of equations, involving the ten potentials instead of one. Also, to conform to relativity, the new equations must be unaltered by a change of co-ordinates. If new co-ordinates are used the g 's will be different, but the relations between the new g 's and new co-ordinates must be the same as those between the old g 's and old co-ordinates. The possible sets of relations which satisfy this are very limited in number. This subject, known as the theory of tensors, has been worked out very fully by Riemann, Christoffel, and others, and the possible sets of equations can be classified and enumerated. From this limited choice we have further to pick out a set of equations which will reduce to $\nabla^2g_{44}=0$ as a first approximation, and that is found to leave only one possibility. There is just one set of ten differential equations (of which, however, only six are independent) which satisfy both conditions. Einstein takes these as expressing his generalised law of gravitation. It is important to notice exactly how much of this is geometry. Geometry shows that if the equations hold for a particular set of co-ordinates, they hold for every set. We abolish the "if," and so assert a new law of Nature.

It is further necessary to consider what must be the generalised equivalent of Poisson's equation $\nabla^2\phi = -4\pi\rho$, which supplants Laplace's equation when matter is present. The extension is not difficult, since it is found that the ten equations above mentioned are the expression of a generalised principle of least action as applied to gravitational energy. Now mass is considered to be simply electromagnetic energy, and since there is no reason to believe that electromagnetic energy will behave differently from gravitational energy in regard to least action, we have only to include both forms of energy together in the equations, treating them as equivalent. It is not possible to write down here these final equations, since a very elaborate notation is needed for their expression. Though highly complicated in form, they can be applied without excessive labour to the more simple problems.

One or two of the more elementary consequences of this theory were given by Einstein some years ago. A ray of light must be bent in passing

through an intense field of gravitation; thus a star seen close to the limb of the sun during an eclipse should appear displaced $1.7''$ from its usual position. The vibrations of an atom must be slower in an intense field, so that the lines of the solar spectrum should be displaced slightly to the red as compared with terrestrial spectra. It has not yet been possible to put these predictions to a satisfactory test, and it has been left to the completed theory to furnish the first opportunity of an appeal to observation. In this the new theory has scored a most signal success, for it has cleared up the most celebrated case of discordance in gravitational astronomy.

From his generalised law of gravitation Einstein has deduced that the elliptic orbit of a planet will rotate in the direction of motion at the rate of

$$\frac{24\pi^3}{V^2} \cdot \frac{a^2}{T^2(1-e^2)} \text{ radians per revolution of the planet.}$$

Here V is the velocity of light, and a , T , e are the semiaxis, period, and eccentricity of the orbit. If v is the velocity of the planet, the amount is practically $6\pi v^2/V^2$ radians per revolution. For Mercury this works out at $43''$ per century—just the amount of the outstanding discordance between observation and theory. The theory also gives rotations for Venus and the earth, but their orbits are so nearly circular that the effect is imperceptible to observation. For Mars, with its strongly elliptic orbit, the correction is more important, and sensibly improves the accordance between theory and observation.³

It is rather difficult to grasp the fact that the same laws of Nature may hold when some bizarre system of co-ordinates is chosen. Suppose an observer A uses rectangular co-ordinates, and B, through some kink in his mind, uses polar co-ordinates without realising that he is doing anything unusual. For A a ray of light can travel along the straight line $x=\text{constant}$; but evidently it cannot travel along the circle $r=\text{constant}$, which is B's idea of a straight line. The answer is that B through his peculiar system of measurement will suppose that he is in an intense gravitational field; he will calculate the curvature in the ray of light produced by this field; and, making allowance for it, he will find that the light actually travels along its theoretical curve (i.e. curve for B, but straight line for A). Thus the same general laws of Nature are satisfied for B as well as for A; and it might be difficult to decide which of them had got hold of the absolute rectangular co-ordinates.

A. S. EDDINGTON.

³ The discordance of the perihelion of Mercury (now removed) was nearly 40 times its probable error. Of the sixteen secular variations of the four inner planets, all are now accordant, except the node of Venus, which deviates by $4\frac{1}{2}$ times its probable error. Among sixteen residuals we should expect to find one of three times the probable error, so that the evidence for the remaining discordance is not very strong. There are besides unexplained variations of the longitudes of the moon and planets, but these are in a different category.

Albert Einstein: 14 March, 1879—18 April, 1955

A guide for the perplexed

Kenneth Brecher

This brief collection of direct and indirect quotations by or about Albert Einstein is offered as a tribute in honour of the centenary of his birth. The selection makes no attempt to be complete but does try to be representative of his attitude towards science and mathematics. As this selection is addressed to a primarily scientific audience, his views concerning his two most important creations, quantum theory and relativity, are particularly stressed toward the end.

The most beautiful experience we can have is the mysterious. It is the fundamental emotion which stands at the cradle of true art and true science. Whoever does not know it and can no longer wonder, no longer marvel, is as good as dead, and his eyes are dimmed.

from *The World as I See It*,
by A. Einstein (Philosophical
Library: New York, 1934)

I believe with Schopenhauer that one of the strongest motives that lead persons to art and science is flight from the everyday life, with its painful harshness and wretched dreariness, and from the fetters of one's own shifting desires. One who is more finely tempered is driven to escape from personal existence and to the world of objective observing and understanding. This motive can be compared with the longing that irresistibly pulls the town dweller away from his noisy, cramped quarters and toward the silent, high mountains, where the eye ranges freely through the still, pure air and traces the calm contours that seem to be made for eternity.

With this negative motive there goes a positive one. Man seeks to form for himself in whatever manner is suitable for him, a simplified and lucid image of the world, and so to overcome the world of experience by striving to replace it to some extent by this image. This is what the painter does, and the poet, the speculative philosopher, the natural scientist, each in his own way. Into this image and its formation, he places the centre of gravity of his emotional life, in order to attain the peace and serenity that he cannot find within the narrow confines of swirling, personal experience.

from *The Scientific Imagination
Case Studies* (page 231), by
G. Holton (Cambridge
University Press: Cambridge, 1978)

The most incomprehensible thing about the universe is that it is comprehensible.

in *Albert Einstein: Creator and
Rebel* (page 18), by Banesh
Hoffman (Viking: New
York, 1972; Hart-Davis,
MacGibbon: London, 1973;
Paladin: London, 1977)

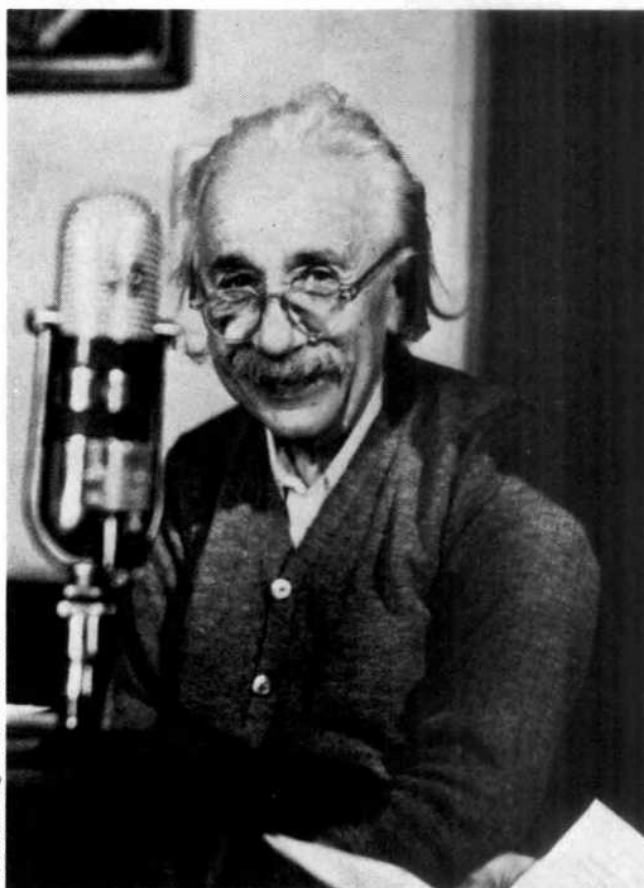
Out yonder there was this huge world, which exists independently of us human beings and which stands before us like a great, eternal riddle, at least partially accessible to our inspection.

from "Autobiographical
Notes", in *Albert Einstein:
Philosopher-Scientist*, edited by
E. A. Schilpp (Harper and
Row: New York, 1959)

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The essence of a man like me lies just in what he thinks and how he thinks—not in what he does or suffers.

from "Autobiographical
Notes", in *Albert Einstein:
Philosopher-Scientist*, edited by
E. A. Schilpp (Harper and
Row: New York, 1959)



Broadcasting from Princeton

Popperfoto

To the sphere of religion belongs the faith that the regulations valid for the world of existence are rational, that it is comprehensible to reason. I cannot conceive of a genuine scientist without that profound faith. The situation may be expressed by an image: science without religion is lame, religion without science is blind.

in *Einstein: His Life and
Times*, by P. Frank
(Knopf: New York, 1947)

Of all the communities available to us, there is not one I would want to devote myself to, except for the society of true searchers, which has very few living members at any time.

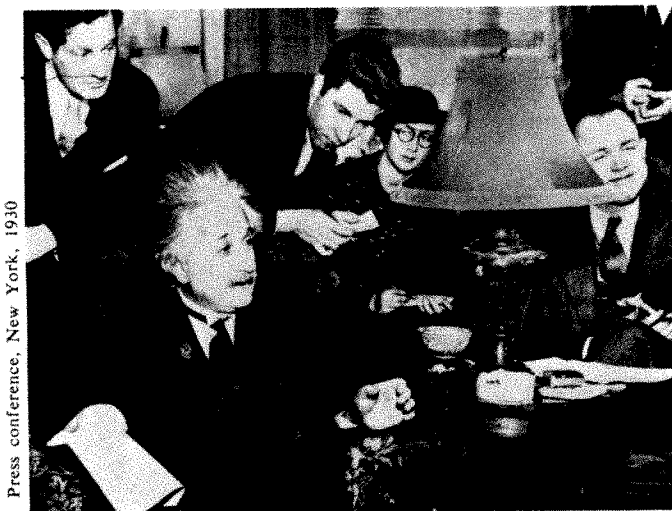
in letter to Max Born, April
2nd, 1924, from *The Born-
Einstein Letters*, by M. Born
(Walker: New York, 1971)

In every true searcher of Nature there is a kind of religious reverence; for he finds it impossible to imagine that he is the first to have thought out the exceedingly delicate threads that connect his perceptions. The aspect of knowledge which has not yet been laid bare gives the investigator a feeling akin to that experienced by a child who seeks to grasp the masterly way in which elders manipulate things.

in *Conversations with Einstein*,
by A. Moszkowski (Horizon
New York, 1970)

What I'm really interested in is whether God could have made the world in a different way; that is, whether the necessity of logical simplicity leaves any freedom at all.

to Ernst Straus, quoted in
*The Scientific Imagination Case
Studies*, by G. Holton
(Cambridge University Press:
Cambridge, 1978)



Press conference, New York, 1930

Popperfoto

I want to know how God created this world. I am not interested in this or that phenomenon, in the spectrum of this or that element. I want to know His thoughts, the rest are details.

in *Einstein: The Life and
Times*, by R. W. Clark (World
Publishing: New York, 1971;
Hodder and Stoughton:
London, 1979)

God is cunning but He is not malicious. (*Raffiniert ist der Herr Gott aber boshaft ist Er nicht.*)

inscribed at Princeton

Physics is essentially an intuitive and concrete science. Mathematics is only a means for expressing the laws that govern phenomena.

from *Lettres à Maurice
Solovine*, by A. Einstein
(Gauthier-Villars: Paris, 1956)

God does not care about our mathematical difficulties. He integrates empirically.

in *Quest* (page 279), by L.
Infeld (Gollancz: London, 1942)

How can it be that mathematics, being after all a product of human thought which is independent of experience, is so admirably appropriate to the objects of reality? Is human reason, then, without experience, merely by taking thought, able to fathom the properties of real things? In my opinion the answer to this question is, briefly, this: as far as the propositions of mathematics refer to reality,

they are not certain; and as far as they are certain, they do not refer to reality.

from "Geometry and
Experience", in *Ideas and
Opinions* (page 233), by
A. Einstein (Crown:
New York, 1954)

In the matter of physics, the first lessons should contain nothing but what is experimental and interesting to see. A pretty experiment is in itself often more valuable than twenty formulae extracted from our minds; it is particularly important that a young mind that has yet to find its way about in the world of phenomena should be spared from formulae altogether. In his physics they play exactly the same weird and fearful part as the figures of dates in Universal History.

from *Conversations with
Einstein*, by A. Moszkowski
(Horizon: New York, 1970)

What applies to jokes, I suppose, also applies to pictures and to plays. I think they should not smell of a logical scheme, but of a delicious fragment of life, scintillating with various colours according to the position of the beholder. If one wants to get away from this vagueness one must take up mathematics. And even then one reaches one's aim only by becoming completely insubstantial under the dissecting knife of clarity. Living matter and clarity are opposites—they run away from one another. We are now experiencing this rather tragically in physics.

in letter to Max Born, January
15, 1927, from *The Born-
Einstein Letters*, by M. Born
(Walker: New York, 1971)

It is in fact, nothing short of a miracle that the modern methods of instruction have not yet entirely strangled the holy curiosity of inquiry; for this delicate plant, aside from stimulation, stands mainly in need of freedom; without this it goes to wrack and ruin without fail.

from "Autobiographical
Notes", in *Albert Einstein:
Philosopher-Scientist*, edited by
E. A. Schilpp (Harper and
Row: New York, 1959)

The only rational way of education is to be an example—if one can't help it, a warning example.

To punish me for my contempt for authority, Fate made me an authority myself.

in *Albert Einstein: Creator and
Rebel* (page 24), by Banesh
Hoffman (Viking:
New York, 1972; Hart-Davis,
MacGibbon: London, 1973;
Paladin: London, 1977)

Great spirits have always encountered violent opposition from mediocre minds.

I remember a beautiful remark of his when he criticized a well-known American physicist. Einstein said he "couldn't really understand how anybody could know so much and understand so little"! Einstein always emphasized that you could know too many facts and get lost among them.

E. H. Hutten, in *Einstein:
The Man and His Achieve-
ment*, edited by G. J. Whitrow
(Dover: New York, 1973)

About ten years ago I spoke with Einstein about the astonishing fact that so many ministers of various denominations are strongly interested in the theory of

relativity. Einstein said that according to his estimation there are more clergymen interested in relativity than physicists. A little puzzled I asked him how he would explain this strange fact. He answered, a little smiling, "Because clergymen are interested in the general laws of nature and physicists, very often, are not." Another day we spoke about a certain physicist who had very little success in his research work. Mostly he attacked problems which offered tremendous difficulties.

By most of his colleagues he was not rated very highly. Einstein, however, said about him, 'I admire this type of man. I have little patience with scientists who take a board of wood, look for its thinnest part and drill a great number of holes where drilling is easy'.

Philipp Frank, "Einstein's Philosophy of Science", in *Reviews of Modern Physics*, 21, 349 (1949)

I might begin with a true story that some of you may know concerning a conversation between Einstein and the French poet Valery. When Einstein first came to Paris, a well known hostess achieved the triumph of having both Einstein and Valery in her drawing-room, and arranged for a conversation between the two that everyone else could listen to. As you know, Valery's mania was to say that he was more interested in the process of creation than in the actual opus which came out of this creative process . . . and he started asking questions of Einstein about how he worked.

"How do you work, and could you tell us something of this?" Einstein was very vague about it.

He said, "Well, I don't know . . . I go out in the morning and take a walk."

"Oh", said Valery, "Interesting, and of course you have a notebook and whenever you have an idea you write it out in your notebook."

"Oh", said Einstein, "No I don't".

"Indeed, you don't?"

"Well you know, an idea is so rare."

That is really as much as I think can be said as to really great creativity.

J. Monod, in *The Creative Process in Science and Medicine*, edited by H. A. Krebs and J. H. Shelley (Elsevier: New York, 1975)

He was asked whether he thought that everything could ultimately be expressed in scientific terms. Einstein replied: "Yes, that is conceivable, but it would make no sense. It would be as if one were to reproduce Beethoven's Ninth Symphony in the form of an air pressure curve."

G. Born, in *The Creative Process in Science and Medicine*, edited by H. A. Krebs and J. H. Shelley (Elsevier: New York, 1975)

During his Zurich stay the woman doctor, Paulette Brubacher, asked the whereabouts of his Laboratory. With a smile he took a fountain pen out of his breast pocket and said: "Here".

from *Albert Einstein: A Documentary Biography*, by C. Seelig (Staples: London, 1956)

The most important tool of the theoretical physicist is his wastebasket.

told by P. Morrison

Just as it is the pride of many people never to have any time, so it has been Einstein's always to have time. I

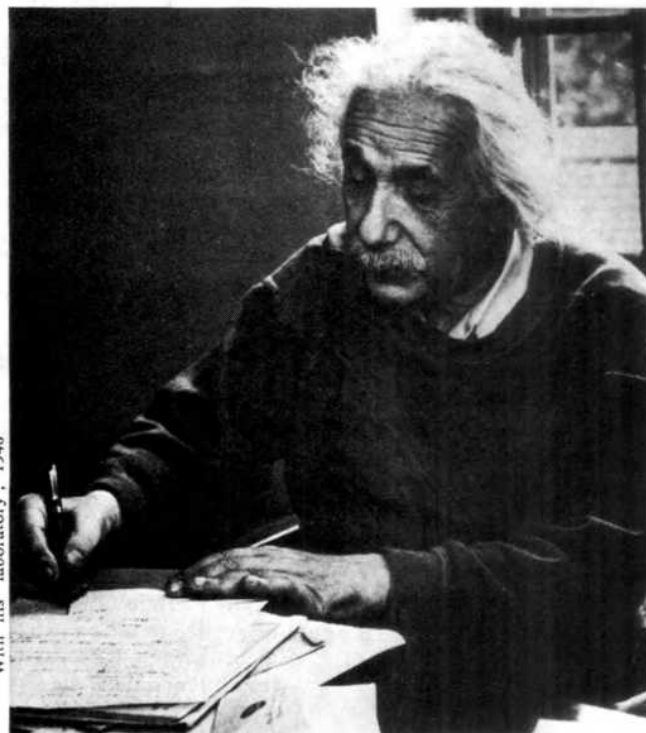
recall a visit I once paid him on which we decided to visit the astrophysical observatory at Potsdam together. We agreed to meet on a certain bridge in Potsdam, but since I was a good deal of a stranger in Berlin, I said I could not promise to be there at the appointed time.

"Oh," said Einstein, "that makes no difference; then I will wait on the bridge."

I suggested that that might waste too much of his time.

"Oh no," was the rejoinder, "the kind of work I do can be done anywhere. Why should I be less capable of reflecting about my problems on the Potsdam bridge than at home?"

in *Einstein: His Life and Times* (page 118), by P. Frank (Knopf: New York, 1947)



With his 'laboratory', 1948

Camera Press

The friendship of my thesis professor, Rudolf Ladenburg, and Einstein was a long one. They had been together in Berlin before coming to Princeton. Ladenburg liked to tell of their first meeting, about 1908, when he called upon Einstein in the Swiss Patent Office. Einstein told him that he was the first physicist that he had seen in five years. During these years Einstein had done much of his important work. He pulled out one drawer of his desk and said that it was his theoretical physics office. His duty of reading patents took little time, and he worked upon physics whenever he was free.

Y. Beers, *Am. J. Phys.*, 46, 506 (1978)

I never think of the future, it comes soon enough.

Who would have thought around 1900 that in fifty years time we would know so much more and understand so much less . . . Nowadays, one can be happy if one is not trampled down by the stampede of the buffalos.

in *Albert Einstein and the Cosmic World Order*, by C. Lanczos (Wiley: New York, 1965)

A theory is the more impressive the greater the simplicity of its premise is, the more different kinds of things it relates, and the more extended is its area of applicability . . . It [thermodynamics] is the only physical theory of universal content concerning which I am convinced that,

within the framework of applicability of its basic concepts, it will never be overthrown.

from "Autobiographical Notes", in *Albert Einstein: Philosopher-Scientist*, edited by E. A. Schilpp (Harper and Row: New York, 1959)

It was therefore quite a shock when he said, "But why should anybody be interested in getting exact solutions of such an ephemeral set of equations?". I remember very well this word "ephemeral". It meant that he did not consider his gravitational equations as the last word.

by C. Lanczos, in *Einstein: The Man and His Achievement* (page 49), edited by G. J. Whitrow (Dover: New York, 1973)



Press conference, New York, 1930

Popperfoto

But it [general relativity] is similar to a building, one wing of which is made of fine marble (left part of the equation), but the other wing of which is built of low grade wood (right side of the equation). The phenomenological representation of matter is, in fact, only a crude substitute for a representation which would do justice to all known properties of matter.

from "Physics and Reality" (1936), reprinted in *Ideas and Opinions* (page 311), by Albert Einstein (Crown: New York, 1954)

I can, if the worst comes to the worst, still realise that the Good Lord may have created a world in which there are no natural laws. In short, chaos. But that, there should be statistical laws with definite solutions, i.e., laws which compel the Good Lord to throw the dice in each individual case, I find highly disagreeable.

in *Albert Einstein: A Documentary Biography*, by C. Seelig (Staples: London, 1956)

You believe in a dice-playing God and I in perfect laws in the world of things existing as real objects, which I try to grasp in a wildly speculative way.

from letter to Max Born, quoted in *Albert Einstein: Philosopher-Scientist* (page 176), edited by E. A. Schilpp (Harper and Row: New York, 1959)

The present theory of relativity is based on a division of physical reality into a metric field (gravitation) on the one hand, and into an electromagnetic field and matter on the other hand. In reality space will probably be of a uniform character and the present theory be valid only as a limiting case. For large densities of field and of matter, the field equations and even the field variables which enter

into them will have no real significance. One may not therefore assume the validity of the equations for very high density of field and of matter, and one may not conclude that the "beginning of the expansion" must mean a singularity in the mathematical sense. All we have to realise is that the equations may not be continued over such regions.

in *The Meaning of Relativity* (page 129), fifth edition, by Albert Einstein (Princeton University Press: New Jersey, 1956)

Quantum mechanics is certainly imposing. But an inner voice tells me that it is not yet the real thing. The theory says a lot, but does not really bring us any closer to the secret of the 'old one'. I, at any rate, am convinced that He is not playing at dice.

from a letter to Max Born, December 4, 1926, from *The Born-Einstein Letters*, by M. Born (Walker: New York, 1971)

The Heisenberg-Bohr tranquilizing philosophy—or religion?—is so delicately contrived that, for the time being, it provides a gentle pillow for the true believer from which he cannot very easily be aroused. So let him lie there.

in a letter to E. Schrödinger, May 31, 1928, from *Letters on Wave Mechanics* (page 31), edited by K. Przibram (Philosophical Library: New York, 1967)

Is it conceivable that a field theory permits one to understand the atomistic and quantum structure of reality? Almost everybody will answer this question with "no". But I believe that at the present time nobody knows anything reliable about it. This is so because we cannot judge in what manner and how strongly the exclusion of singularities reduces the manifold of solutions...

One can give good reasons why reality cannot at all be represented by a continuous field. From the quantum phenomena it appears to follow with certainty that a finite system of finite energy can be completely described by a finite set of numbers (quantum numbers). This does not seem to be in accordance with a continuum theory, and must lead to an attempt to find a purely algebraic theory for description of reality. But nobody knows how to obtain the basis of such a theory.

in *The Meaning of Relativity* (page 165), fifth edition, by Albert Einstein (Princeton University Press: New Jersey, 1956)

You imagine that I look back on my life's work with calm satisfaction. But from nearby it looks quite different. There is not a single concept of which I am convinced that it will stand firm, and I feel uncertain whether I am in general on the right track... I don't want to be right... I only want to know whether I am right.

from *Lettres à Maurice Solovine*, by Albert Einstein (Gauthier-Villars: Paris, 1956)

One thing I have learned in a long life: that all our science, measured against reality, is primitive and childlike—and yet it is the most precious thing we have.

frontispiece to *Albert Einstein: Creator and Rebel*, by Banesh Hoffman (Viking: New York, 1972; Hart-Davis, MacGibbon: London, 1973; Paladin: London, 1977)

articles

Immobilisation of high level nuclear reactor wastes in SYNROC

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The elements occurring in high-level nuclear reactor wastes can be safely immobilised by incorporating them within the crystal lattices of the constituent minerals of a synthetic rock (SYNROC). The preferred form of SYNROC can accept up to 20% of high level waste calcine to form dilute solid solutions. The constituent minerals, or close structural analogues, have survived in a wide range of geochemical environments for periods of 20–2,000 Myr whilst immobilising the same elements present in nuclear wastes. SYNROC is unaffected by leaching for 24 h in pure water or 10 wt % NaCl solution at high temperatures and pressure whereas borosilicate glasses completely decompose in a few hours in much less severe hydrothermal conditions. The combination of these leaching results with the geological evidence of long-term stability indicates that SYNROC would be vastly superior to glass in its capacity to safely immobilise nuclear wastes, when buried in a suitable geological repository. A dense, compact, mechanically strong form of SYNROC suitable for geological disposal can be produced by a process as economical as that which incorporates radwaste in borosilicate glasses.

SEVERAL countries propose to solidify the high level nuclear wastes arising from reprocessing of nuclear reactor fuel rods, and to bury the solids in suitable geological repositories. Because of the intensely radioactive nature of the waste (Table 1) this material must be prevented from reaching the biosphere for a period which may be as long as 1 Myr. This requires, among other things, that the waste be solidified in a form possessing extreme stability and resistance to leaching and alteration in appropriate geological environments.

The most popular procedure advocated by the nuclear power establishment during the past 25 yr has been to incorporate the radwaste into a borosilicate glass. The product typically contains ~25% of radwaste. There are, however, some serious disadvantages of this procedure which have become recognised only comparatively recently. Borosilicate glasses readily devitrify when subjected to the action of water and steam at elevated pressures and temperatures, for example at 300–400 °C and 300–1,000 bar^{2,3}. Pressure–temperature conditions in the lower end of this range may well be encountered by glass cylinders after burial in geological repositories. Moreover, it would be extremely difficult to design a repository in which groundwater can be prevented from gaining access to radwaste. (This includes

repositories in salt formations⁴). There is thus a high probability that glass cylinders will devitrify within relatively short intervals after burial. Devitrification in these conditions can cause a drastic increase in effective surface area and in leachability of certain highly dangerous species such as caesium and the actinides³.

It is unlikely that the problem can be avoided by reducing the proportion of waste and ageing the glass for 30 yr to achieve lower temperatures⁵. Detailed studies³ show that the mechanism of alteration is complex. One of the mechanisms involves diffusion of water into the glass structure. This precedes the actual devitrification and causes the interior of the glass to lose its strength. Another mechanism involves selective leaching of components from regions where devitrification is occurring, thereby producing a disintegrated material with a high degree of heterogeneity. Moreover, the rate of alteration is non-linear with time. Because of the complexity of the reaction mechanisms, it is not possible to predict the stabilities of glasses with respect to devitrification in geological environments at low temperatures in the vicinity of 100–150 °C.

Table 1 Principal components of a typical calcine (radwaste)¹

	Mol %
Rare earths (REE elements)	26.4
Zr	13.2
Mo	12.2
Ru	7.6
Cs	7.0
Pd	4.1
Sr	3.5
Ba	3.5
Rb	1.3
U + Th	1.4
Am + Cm + Pu + Np	0.2
Fe	6.4
(PO ₄)	3.2
Na	1.0
Others (mainly Tc, Rh, Te, I and processing contaminants (including Ni, Cr)	9.0

Incorporation of radwaste in crystalline materials

McCarthy^{1,6} and Roy⁷ have previously recognised some of the uncertain aspects of immobilisation in glasses and have proposed that radwaste be incorporated in ceramic materials composed of crystalline phases. A phase assemblage of this

nature would be much closer to thermodynamic equilibrium than glass, so that the risks arising from devitrification of glasses would be avoided. These authors also described the basic crystal chemical principles underlying the incorporation of radwaste elements in crystalline phases.

There is, of course, an enormous range of crystalline phases, phase assemblages and methodologies which can be conceived for the purposes of storing radwaste elements. The particular strategy chosen by McCarthy led to the formulation of a series of materials designated by the general name of 'Supercalcine'. These materials are produced by adding ~50% of the oxides of Si, Ca, Al and Sr to the high level waste before calcination (Table 2). The above components are added in carefully-defined proportions so that during calcination, they will react with the components of radwaste to form a specific assemblage of desired crystalline phases. The preferred assemblage for waste derived from light-water reactors contains phases possessing apatite, fluorite, scheelite, pollucite and spinel-type structures.

McCarthy and Roy deserve credit for recognising at an early stage the disadvantages of glass as an immobilisation medium, and for perceiving the advantages of incorporating radwaste within crystalline phases. However, it is now clear that their Supercalcine strategy does not achieve many of the potential advantages of immobilising radwaste within crystalline phases. A detailed discussion of weaknesses of the Supercalcine strategy in this respect is given in refs 3 and 4.

An alternative method of radwaste immobilisation in crystalline materials was described by Ringwood⁴. This was based on incorporation of radwaste elements as dilute solid solutions in the constituent minerals of a synthetic rock—SYNROC. The preferred method of producing this SYNROC was to melt a mixture of TiO₂, BaO, SiO₂, ZrO₂, Al₂O₃, CaO and K₂O (Table 2, column I) at 1,300 °C and cool slowly to produce a synthetic rock composed of the minerals 'hollandite' BaAl₂Ti₆O₁₆, perovskite CaTiO₃, zirconolite CaZrTi₂O₇, Ba-felspar BaAl₂Si₂O₈, kalsilite KAlSiO₄ and leucite KAlSi₂O₆. Alternatively, this mineral assemblage could be produced by hot-pressing the above composition in subsolidus conditions. About 10% of radwaste calcine would be intimately mixed with the above SYNROC components before melting and crystallisation. Ringwood showed that nearly all the elements present in high-level waste (Table 1) were incorporated as dilute solid solutions within the individual minerals of SYNROC, and could therefore be immobilised for long periods when SYNROC was buried in a suitable geological environment.

The actual composition and mineral assemblage of SYNROC (Table 2) differs drastically from that of Supercalcine. There is also an important difference in concept. Because of the large proportion of radwaste (>50%) in Supercalcine, the radwaste

elements largely control the nature of the crystalline phase assemblage. This greatly restricts flexibility and yields a phase assemblage which possesses some undesirable characteristics^{3,4}. However, SYNROC contains only ~10% of radwaste. Thus, the 90% of added components controls the nature of the phase assemblage and the radwaste atomic species simply substitute in low concentrations within the crystal lattices determined by the major added components, as in nature. This provides a great deal of flexibility in selecting a crystalline phase assemblage with the most desirable immobilisation characteristics.

An important characteristic of SYNROC, therefore, is that its particular mineral assemblage (Table 2) remains essentially the same, whether the radwaste component amounts to 0%, 5%, 10% or even 20%. The advantages of this procedure are discussed more fully in refs 3 and 4.

We have since modified and simplified the original composition (termed SYNROC A in Table 2) by eliminating the silicate minerals Ba-felspar + kalsilite ± leucite. The modified version of SYNROC B is composed essentially of the minerals 'hollandite' + perovskite + zirconolite. Because of the absence of silicates, the titanium content is much higher and there is a superficial resemblance to the high-titanium ceramics developed by the Sandia Corporation⁷. However, Table 2 shows that the detailed chemical and mineralogical composition of SYNROC in fact differs fundamentally from that of the Sandia material.

Our main reason for developing SYNROC B was to incorporate radwaste caesium in the hollandite phase, which has proved to be exceptionally resistant to leaching. This was found to require the removal of kalsilite, leucite and Ba-felspar from the assemblage. Because of the absence of silicates, the melting temperature of SYNROC B is much higher than that of SYNROC A. Accordingly, we prefer to produce SYNROC B by hot-pressing and recrystallisation in the solid state at ~1,300 °C, as discussed subsequently.

Mineralogy of SYNROC B

When the SYNROC B bulk composition (Table 2, column 2) is hot-pressed at 1,200–1,400 °C, the minerals are found to possess hollandite, perovskite and zirconolite structures. Typical compositions of these minerals are given in Table 3.

'Hollandite': natural hollandite has the formula BaMn₈O₁₆, where manganese can occur in 2+, 3+, and 4+ valence states. There are, however, a large number of structural isotypes, including BaAl₂Ti₆O₁₆, in which the Mn²⁺ and Mn⁴⁺ ions of natural hollandite are replaced by Al³⁺ and Ti⁴⁺. The most abundant mineral in SYNROC A and B is BaAl₂Ti₆O₁₆. In subsequent discussions, this phase will be referred to as 'hollandite'.

Table 2 Comparison of SYNROC with alternative ceramic waste forms

	I SYNROC A	II SYNROC B	III Typical Supercalcine*	IV Sandia ceramic†
Radwaste (wt %)	10	10	50	~25
Inert additives (wt %)	90	90	50	~75
		Composition of inert additives (wt %)		
SiO ₂	13	—	68	Minor
TiO ₂	33	57	—	~90
ZrO ₂	10	11	—	—
Al ₂ O ₃	16	10	11	Minor
CaO	6	13	19	—
BaO	17	9	—	—
SrO	—	—	2	—
Na ₂ O	—	—	—	Minor
K ₂ O	5	—	—	—
Mineral structures	Hollandite, perovskite, zirconolite, Ba-felspar, kalsilite, leucite	Hollandite, perovskite, zirconolite	Scheelite, cubic zirconia, spinel, apatite, corundum, pollucite	Rutile, metal, cubic zirconia, pollucite, Gd ₂ Ti ₂ O ₇ , amorphous silica

* From ref. 1. † From ref. 7.

Table 3 Compositions of coexisting phases in SYNROC B, doped with ~1% each of Ca_2O , UO_2 and SrO

	'Hollandite'	Perovskite	Zirconolite
TiO_2	72.0	56.5	48.4
ZrO_2	0.3	0.8	30.6
UO_2	<0.1	0.8	3.8
Al_2O_3	13.1	0.4	2.6
NiO	0.9	<0.1	<0.1
BaO	12.0	<0.2	<0.2
SrO	<0.1	2.5*	1.3
CaO	<0.2	39.2	13.8
Ca_2O	1.5	<0.1	<0.1
Total	99.7	100.2	100.5

* In other experiments, up to 7.5% SrO has been found in perovskite, with smaller amounts in zirconolite.

The hollandite structure is closely related to that of rutile, but differs in having tunnels parallel to the vertical axis in which very large atoms such as K, Rb, Cs, Ba, Sr and Pb may be stably bound. Moreover, many elements including Mg, Co, Ni, Cu, Cr, Fe, Mn, Mo, Rh, Ru, Sn and Zr are capable of replacing Al and Ti in the octahedral lattice sites. Because of the great flexibility and range of permitted ionic substitutions, and its resistance to leaching, 'hollandite' is a key component of SYNROC. We have also synthesised pure hollandite forms of $\text{Ca}_2\text{Al}_2\text{Ti}_6\text{O}_{16}$, $\text{K}_2\text{Al}_2\text{Ti}_6\text{O}_{16}$, and $\text{SrAl}_2\text{Ti}_6\text{O}_{16}$ and demonstrated the existence of extensive solid solutions with $\text{BaAl}_2\text{Ti}_6\text{O}_{16}$. Perovskite, CaTiO_3 : this mineral is capable of taking an extremely wide range of other elements into stable solid solution. The elements which are capable of replacing calcium in its lattice site include Sr^{2+} , Ba^{2+} , Na^{+} , all rare earths (REE^{3+}), Y^{3+} , Cd^{2+} , Mn^{2+} , Am^{3+} , Pu^{3+} whilst the elements capable of entering the titanium lattice site include Nb^{5+} , Zr^{4+} , Mo^{4+} , Pu^{4+} , Rh^{4+} , U^{4+} , Sn^{4+} , Ru^{4+} , Fe^{3+} , Cr^{3+} , Al^{3+} . Many more substitutions are known, but only those relevant to radwaste immobilisation have been mentioned. Zirconolite, $\text{CaZrTi}_2\text{O}_7$: this mineral possesses a distorted pyrochlore structure. A large number of pyrochlore isotypes are known and a wide range of atomic substitutions are possible. Thus, the eight-fold sites can be occupied by Na, K, Ca, Sr, Ba, Pb, Y, REE, Bi, Zr, U, Th, whilst Ta, Nb, Ti, Fe and Mn can substitute in the six-fold sites.

Incorporation of radwaste elements in SYNROC minerals

The relative proportions of different elements present in high-level wastes are given in Table 1. We have doped samples of SYNROC A and SYNROC B with 1–3% of non-radioactive isotopes of most of these elements. These compositions were then crystallised by appropriate heat-treatments at 1,150–1,350 °C and distributions of the tracers between coexisting phases were measured by electronprobe microanalyses. These experiments showed that nearly all the radwaste elements in Table 1 readily enter and form homogeneous solid solutions with the SYNROC minerals, as discussed elsewhere^{3,4}. The results are summarised in Table 4, showing which phase is the host for a particular element. On general crystal chemical grounds, we can be confident that the remaining rare earth elements will behave similarly to La, Gd and Y whilst the actinide elements (including plutonium, americium and curium) will behave similarly to uranium and/or the rare earths, entering zirconolite, and, to a lesser extent, perovskite. In SYNROC B, rubidium will doubtless behave similarly to potassium and caesium, thereby entering the hollandite phase. Phosphorus is found to combine with Ba and Ca to form minor amounts of an orthophosphate phase $(\text{Ba}, \text{Ca})_3(\text{PO}_4)_2$, whilst palladium and tellurium are reduced to the metallic state (Te as NiTe).

It is remarkable that almost the entire range of radwaste elements can be incorporated in only three crystalline phases, which are also thermodynamically compatible. This is a consequence of the structures and crystal chemical properties of these phases which permit an extremely wide range of ionic

substitutions. In principle, it would be possible to omit the perovskite phase, as the radwaste elements occurring in perovskite can also enter 'hollandite' and zirconolite. However, we retain perovskite in the SYNROC B assemblage because it preferentially accommodates ^{90}Sr , one of the most dangerous of radwaste isotopes. With this particular mineralogy, each of the three SYNROC B minerals thus accommodates preferentially a particularly hazardous radwaste element or class of elements—caesium enters 'hollandite', strontium enters perovskite, whilst actinide elements mainly enter zirconolite.

Accelerated leaching tests on SYNROC and glasses

We have investigated the relative stabilities of different waste forms by subjecting them to the action of water and aqueous solutions at elevated pressures and temperatures. The samples used were in the form of short cylinders with diameters of 2–4 mm. These were sealed in silver tubes, together with 12 times their volume of water or 10 wt % NaCl solution. After sealing, the tubes were placed in hydrothermal apparatus and subjected to the desired pressure-temperature conditions.

Borosilicate glasses possessing compositions proposed for radwaste immobilisation were completely devitrified and often disintegrated when subjected to the effects of pure water and 10 wt % NaCl solutions at temperatures of 350–400 °C and at 1,000 bar for 24 h. Extensive losses of caesium (50–95%), uranium (30–90%) and other cations were also observed.

In contrast, samples of SYNROC A (doped with 1% of Cs) remained unaltered when subjected to the effects of pure water at 1,000 bar, 24 h, and at temperatures up to 600 °C, demonstrating superiority to glass in this respect. However, when subjected to the action of 10% NaCl solution in the same conditions, kalsilite and leucite phases showed partial and extensive decomposition at 400 and 500 °C respectively, accompanied by loss of caesium. At 600 °C, these phases were completely decomposed and replaced by sodalite, with total loss of caesium. A similar series of experiments conducted on a sample of SYNROC A containing accessory pollucite ($\text{CaAlSi}_2\text{O}_6$) which is the host for caesium in Supercalcine and in the Sandia ceramic (Table 2, Columns III and IV) showed that this mineral was similarly unstable when subjected to the action of NaCl solutions in the above conditions. It seems then, that framework-type silicates such as pollucite, leucite and kalsilite may not be ideal for immobilising radwaste caesium in the presence of groundwaters containing dissolved sodium.

Samples of SYNROC B containing 1% each of Cs, Sr and U were next treated with pure water and 10% NaCl solutions in similar conditions to those used on glass and SYNROC A, but at 400, 500, 600, 700 and 800 °C. There was no significant alteration. The individual mineral phases remained stable and there was no measurable loss of Cs from 'hollandite', U from zirconolite or of Sr from perovskite. Moreover, there was no indication of entry of Na from the NaCl solution into the 'hollandite'. Similar leaching experiments were also carried out at 900 and 1,000 °C and at 5,000 bar for 24 h. In the 900 °C

Table 4 Summary of experimental data showing host phases for principal high-level waste elements introduced into SYNROC B

'Hollandite' $\text{BaAl}_2\text{Ti}_6\text{O}_{16}$		Zirconolite $\text{CaZrTi}_2\text{O}_7$	Perovskite CaTiO_3
Cs^{+}	Mo^{4+}	U^{4+}	Sr^{2+}
K^{+}	Ru^{4+}	Zr^{4+}	(U^{4+})
(Na^{+})	Rh^{3+}	Y^{3+}	(Y^{3+})
Ba^{2+}	Fe^{3+}	Gd^{3+}	(Gd^{3+})
	Cr^{3+}	La^{3+}	(La^{3+})
	Ni^{2+}		
	Fe^{2+}		

Actinide elements and rare earths preferentially enter zirconolite when this phase is present. However, they can also be accommodated by perovskite.

experiments, the 'hollandite' in the peripheral regions of the sample altered to rutile and caesium was lost into solution. However, the interior was unchanged. Zirconolite and perovskite throughout the entire sample were unaltered and retained their uranium and strontium quantitatively. At 1,000 °C, the samples were altered more extensively, but again, the central region was not affected.

The major minerals of SYNROC B exhibit a truly remarkable degree of resistance to hydrothermal alteration as compared to borosilicate glass, Supercalcine and SYNROC A. The extreme stability of these minerals justifies the conclusion that SYNROC B would constitute an excellent medium for radwaste immobilisation.

The remarkable ability of 'hollandite' to retain caesium and to resist exchange with sodium is probably a consequence of its crystal structure. Exchange can occur only through uniaxial diffusion of alkali elements through the narrow channels in the lattice parallel to the C axis. These are effectively blocked by Ba^{2+} ions which are tightly bonded because of their double charges and the caesium ions are thus locked-in. In contrast, open framework structures such as kalsilite, leucite and pollucite offer much easier three-dimensional access, thereby permitting exchange with sodium ions in solution and ultimate dissolution.

One problem encountered with the first batch of runs on SYNROC B arose from the loss of caesium from an unknown accessory phase (other than 'hollandite') during leaching. Broad-beam microprobe analyses of starting materials indicated the presence of 30–40% more caesium than could be accounted for by the 'hollandite'. This caesium was presumably present in another accessory phase which was removed during leaching. The formation of this phase was interpreted to result from incomplete equilibration during hot-pressing, due to imperfect mixing of components in the initial starting materials. This interpretation was supported by a subsequent series of four runs on modified SYNROC B compositions containing excess Al_2O_3 (13%) and, separately, excess TiO_2 (67%). The samples were mixed more thoroughly and hot-pressed at 1,350 °C, resulting in much coarser crystals and presumably, a closer approach to equilibrium. Samples of these runs were leached in 10% NaCl solutions for 24 h. No detectable losses of caesium from the bulk specimens were observed.

Radiation damage

Concern has been expressed that radiation damage to crystals of ceramic immobilisation media might enhance the solubility of radwaste elements in groundwater. Radiation damage, principally from actinide elements which emit α particles, causes disorder of the host lattice leading to the metamict state, sometimes accompanied by extensive volume expansion. However, experimental studies reviewed in ref. 4 show that, providing a radioactive element is substituting in a stable lattice site (such as U^{4+} substituting for Zr^{4+} in zircon), its solubility in aqueous solutions is not markedly increased by radiation damage. Aqueous solutions at elevated pressures and temperatures simply cause recrystallisation to the original state without loss of crystal-chemically compatible radioactive elements.

Most of the actinides in SYNROC would be incorporated in zirconolite. In nature, this mineral usually contains up to 1% of UO_2 and ThO_2 . Moreover, its structure is quite similar to that of pyrochlore, which commonly contains several per cent of UO_2 + ThO_2 . Natural zirconolite and pyrochlore minerals are usually metamict because of the intense accumulated radiation damage from α particles. Nevertheless, they seem to have survived in their host rocks for periods exceeding 2,000 Myr without losing appreciable amounts of uranium and thorium. The temperatures, pressures and leaching conditions encountered by the natural minerals were probably far more severe than those of any proposed radwaste repository. The stability of these minerals in natural conditions and their demonstrable capacity to retain uranium and thorium provide grounds for confidence that synthetic zirconolite in SYNROC will be capable of immobilising the actinide elements for the required period of

~1 Myr, when buried in a carefully-chosen geological-geochemical environment.

In view of the the proved stability of 'hollandite' and perovskite in SYNROC B as demonstrated by their extreme resistance to leaching in experimental conditions, we can be confident that these minerals will be able to immobilise caesium and strontium for the required decay period of ~1,000 yr when SYNROC is buried in a suitable geological repository.

Operation of the SYNROC process

Our preferred process for immobilising radwaste is illustrated in Fig. 1. It is envisaged that high level waste (HLW) solution would continue to be flash-heated to produce 'calcine' as at present. SYNROC would be produced by a similar process from an aqueous solution-suspension containing the components in their correct proportions. Oxide mixtures produced by this procedure possess a high degree of reactivity and homogeneity.

About 90% of SYNROC would then be intimately mixed with ~10% of HLW calcine. The mixture would be introduced into a thick-walled cylindrical nickel container and uniaxially cold-pressed to ~70% of its theoretical density. A thick nickel lid would then be placed on the cylinder, separated by a thin copper seal.

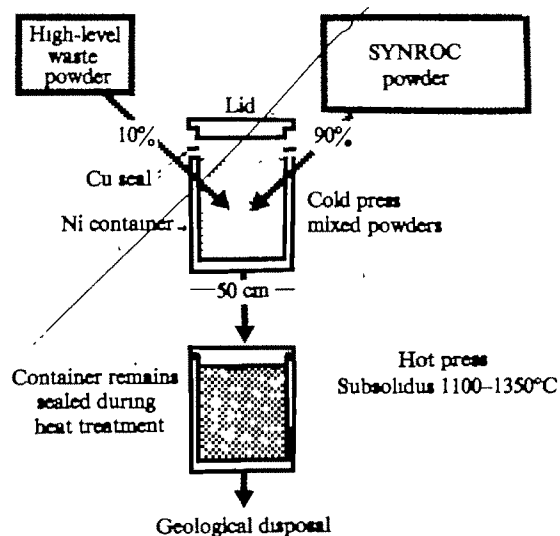


Fig. 1 Flowchart for the immobilisation of radwaste in SYNROC and sealing of the resultant material in thick-walled nickel canisters.

The entire assembly would then be hot-pressed, either uniaxially or isostatically, at a temperature of 1,200–1,300 °C. In these conditions, rapid and complete subsolidus equilibration and recrystallisation of HLW calcine and SYNROC components occur, leading to the formation of a well-crystallised SYNROC mineral assemblage containing the HLW calcine components in solid solution. The mineral assemblage is mechanically strong and close to theoretical density.

During hot-pressing, the nickel container is mobile and flows inwards as the volume of the SYNROC contracts. The lid is sealed on to the container simultaneously by the copper, which melts at a lower temperature and alloys with nickel on either side. Two important advantages accrue from the above procedure. First, the formation of crystalline SYNROC in its terminal state and final encapsulation and sealing of the SYNROC in a metal capsule are performed in a single operation. Second, volatile components such as caesium and ruthenium are quantitatively retained by SYNROC minerals during the hot-pressing operation, and become permanently incorporated in the lattices of SYNROC minerals as described earlier. Radioactive contamination of the production plant and of the environment is thereby minimised. In contrast, during the production of borosilicate glasses, substantial amounts of caesium, ruthenium and other volatile elements are lost by

volatilisation. The recovery of these elements for recycling is an expensive operation.

Conclusion

If our society is to take advantage of the potential benefits of nuclear power, there can be no excuse for any policy which does not use the most advanced technology to safeguard future generations from the attendant dangers of high level nuclear wastes. It can hardly be claimed that immobilisation of radwaste in borosilicate glasses, currently favoured by the nuclear power establishment, satisfies this requirement.

We have demonstrated that a form of synthetic rock, SYNROC, can be produced which is vastly superior to glass in its ability to immobilise radwaste for long periods in appropriate geological-geochemical environments. Because of the simplicity of the production technique, and the absence of a need to

recycle escaping volatiles such as Cs and Ru, the cost of producing SYNROC may be comparable to, or even less than, glass.

SYNROC is immune from devitrification and is composed of mutually compatible phases, which possess crystal structures identical to those of minerals which are known to have survived in geological environments at elevated pressures and temperatures for periods of 20–2,000 Myr and to have retained radioactive elements for these periods. This fact provides grounds for confidence that the far shorter immobilisation period required for radwaste can be attained in the much less extreme pressure-temperature conditions present in a suitable geological repository. This confidence is reinforced by direct accelerated leaching tests at elevated pressures and temperatures.

Note added in proof: Pudovkina *et al.*⁹ have since observed that the mineral zirkelite which occurs in the gem gravels of Sri Lanka and contains up to 20% (UO₂+ThO₂), possesses the zirconolite structure.

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Evidence for late Precambrian plate tectonics in West Africa

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In the Gourma and Iforas regions (Mali) rifting occurred around 800–850 Myr ago along the eastern margin of the West African craton with a triple point in Mali, the Gourma being interpreted as an aulacogen. Oceanic closure around 600 Myr led to a collision between the passive continental margin of the West African craton and an active continental margin to the east displaying island arc and marginal trough volcano-clastic assemblages bordering a deformed continental mass intruded by a high-level batholith. The suture is marked by a string of positive gravity anomalies corresponding to the emplacement of ultrabasic and basic rocks including perhaps ophiolites. East-West shortening was accompanied by the translation onto the West African craton foreland of nappes including internal nappes displaying high pressure-low temperature metamorphism assemblages.

A CONTROVERSIAL subject in orogenesis^{1,2} is whether plate tectonics had an important role in the Proterozoic^{3,4} or whether *in situ* ensialic models fit the facts^{5,6} in the best way. To seek an answer, attention has been focused on the Pan-African, the youngest and best preserved of Precambrian orogenic belts. In the past few years a detailed geological and geophysical investigation has been carried out on the critical Iforas-Gourma region in Mali^{7–10} (Fig. 1), an area of about 300,000 km², crossing the West African craton and the Pan-African mobile belt to the East. The results show convincing evidence of a Wilson cycle ending with collision between a passive continental margin and an active continental margin.

Pre-Pan-African rifting and the Gourma aulacogen

The West African craton, stable since 1,700 Myr, is partly covered by thin flat-lying cratonic sediments younger than 1,000 Myr (refs 11, 12), but along its eastern margin the Gourma basin is characterised by deep subsidence with an accumulation of over 8,000 m of sediments⁹ (Fig. 2). The observed sedimentary sequence with an early terrigenous clastic phase at the base (formations I and II), followed by differentiated carbonate deposits indicating lateral passage platform-slope-trough (formation III) and ending with prograde continental clastic sediments (formations IV and V) is typical of a passive margin¹³. Furthermore analysis of palaeocurrents in the overlying Bandiagara Sandstone Group shows a converging drainage pattern centred on the Gourma with transport from the south-west and west. The shape of the basin, as defined by the distribution of slope sedimentary facies (breccias, turbidites) in the carbonate sequence and by the gravity pattern, is that of a trough orientated WSW-ENE representing a gulf of subsidence within the West African craton, which here forms an embayment. It presents all the characteristics of a typical aulacogen¹⁴. A detailed sedimentological study will be given elsewhere. Gravimetrically it is marked by positive anomalies which can be traced westwards across the West African craton and this suggests a deep crustal heavy source¹⁵. We conclude that a major change in sedimentation occurred around 850–800 Myr ago with rifting along the eastern margin of the West African craton with a triple point in Mali: the Gourma is thought to be a failed arm which has evolved as an aulacogen, a comparable situation with that of the Benue trough with respect to the Gulf of Guinea in the Cretaceous⁴.

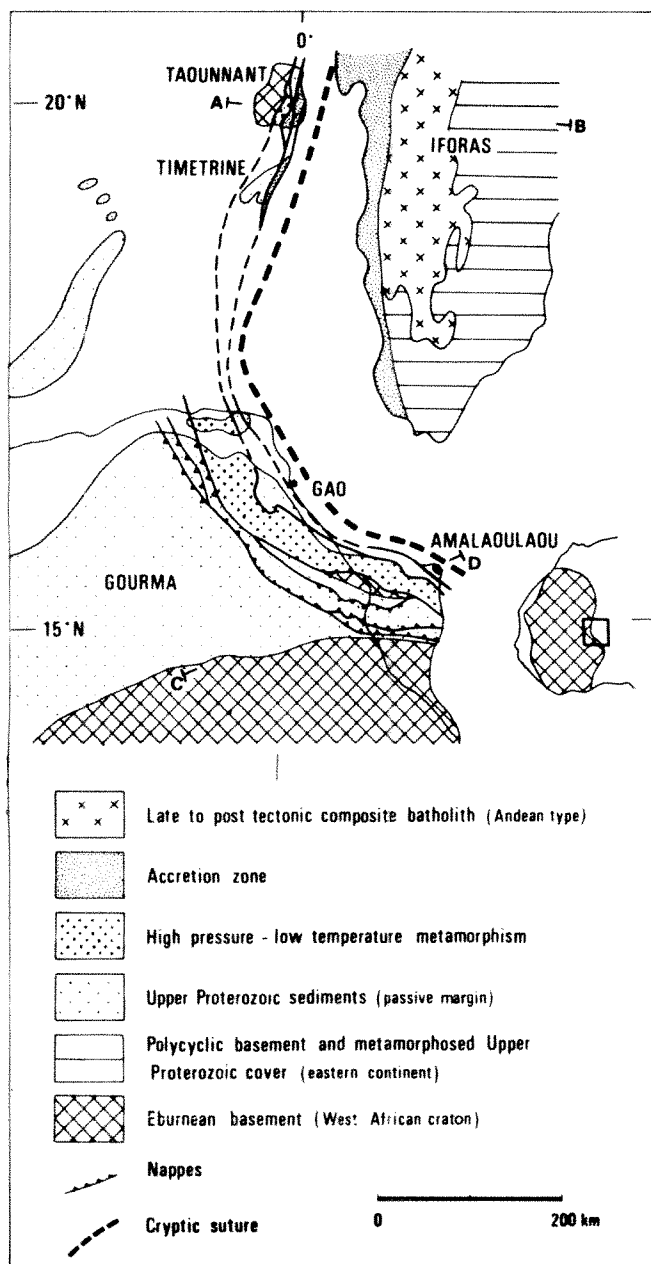


Fig. 1 Major structural units of the Iforas-Gourma region (Mali).

The associated magmatism would be represented to the North in northwestern Hoggar by the intrusion at around 800 Myr of gabbros and ultrabasic rocks into Upper Proterozoic quartzites and dolomites of shelf type^{12,16}, and by the presence of deformed north-south dyke swarms of alkaline and peralkaline metarhyolites, undersaturated soda-trachytes and metaphonolites superimposed on basic dyke swarms which may represent the roots of a pre-Pan-African palaeorift¹⁶. In northeastern

Gourma at Amalaoulaou gabbros and ultrabasic rocks are believed to have intruded the base of the crust at ~850 Myr before high pressure-high temperature granulite conditions and to have been tectonically emplaced around 600 Myr at a high level in the suture zone (Lancelot and de la Boisse, personal communication).

The suture

Oceanic closure 200 Myr later during the Pan-African is marked by a suture outlined by a string of positive gravity anomalies with amplitudes of over 30 mgls locally attaining 80 mgls which may be followed over a distance of 2,000 km and which correspond in the Iforas-Gourma region to the position of basic and ultrabasic complexes (mainly layered metagabbros and quartz gabbros) and may include ophiolites (Fig. 3). Interpretation of profiles across the anomalies¹⁵ using the inverse approach¹⁸ and linear programming¹⁹ shows that the structures, considered as homogeneous bodies with densities $>2.8 \text{ g cm}^{-3}$ and generally $>2.9 \text{ g cm}^{-3}$ continue to depths varying from 6 to 20 km. Geometry shows that bodies with a density of 3 g cm^{-3} must be unrooted. In shape they generally show an easterly dip which is in accord with the general movement pattern with thrusting towards the West African craton and with direct field observations made along the banks of the Niger.

South-west and west of the suture

In the Gourma (Figs 1, 4) nappes outcrop over a zone 300 km by 50–80 km wide²⁰. The internal nappes (mainly micaschists and quartzites) characterised by high pressure-low temperature metamorphism display flat-lying foliation. Foliation planes bearing phengite cut earlier sharp folds and the grade of metamorphism increases to the south-west where it attains eclogite conditions with a very pure jadeitic pyroxene (de la Boisse, personal communication). Stretch lineation association with NE-SW minor folds perpendicular to the general strike, are well developed within the nappes and along the lower grade mylonitic quartzite soles of the nappes. In northern Gourma the internal nappes come into direct contact with the para-autochthonous folded greenschist facies Gourma formations by underthrusting towards the south-west; to the south they are faulted against the Bourré Massif. The Bourré Massif²¹ is a sub-vertical horst of Eburnean granite dated at $2,080 \pm 20 \text{ Myr}$ ²² intrusive into subsequently retrograded amphibolite facies Birrimian sediments, overlain in unconformity by upper Proterozoic sericite quartzites and basal polygenic conglomerate. The horst was extruded after the passage of the external nappes, a situation comparable to that of the Mont Blanc in the western Alps. The external nappes are sub-horizontal and probably pellicular. They consist essentially of schistose formations belonging to the passive margin displaying greenschist facies metamorphism devoid of high pressure conditions. Over large areas one observes an upright succession and flat schistosity and a-type lineations within the nappes. The Gourma formations I–V described above¹³ as forming a pre-Pan-African aulacogen have been strongly deformed to form the para-autochthonous foreland. Major folds are overturned to the south-west and are superimposed on early pre-nappe east-

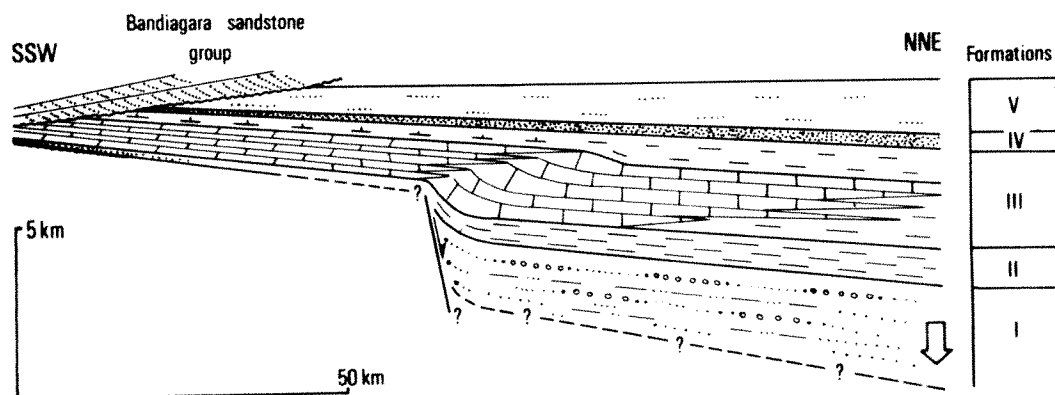


Fig. 2 Schematic SSW-NNE section showing variations in facies and thickness of the Gourma formations I–V.

west folds⁹. Between the front of the nappes and Hombori (100 km) several tens of kilometres of shortening implies a 'décollement' with respect to the underlying basement. The non-metamorphic Bandiagara Sandstone Group overlies in unconformity the Gourma Formations I-V and has been affected by a later phase of folding.

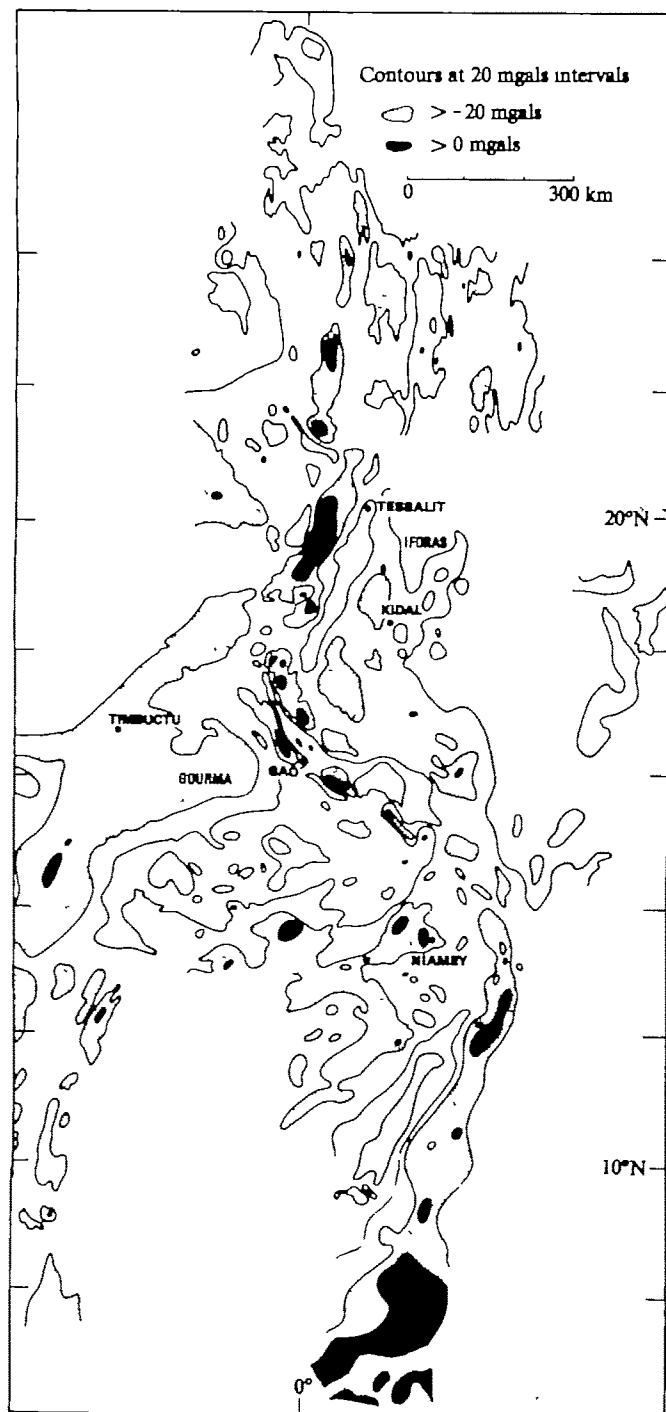


Fig. 3 Simplified Bouguer anomaly map of the eastern margin of the West African craton.

Four-hundred kilometres further north, in the Taounant region two flat-lying superimposed nappes have been mapped directly overlying eroded Eburnean (2,000 Myr old) basement. The lower one consists of a mylonitised unit of quartzites-dolomites presumably belonging to the passive continental margin whereas the overlying one is composed of strongly epidotised metabasalts and diabbases containing a blue amphi-

bole, which are thought to come from an oceanic domain to the east. Similarly to the South in the Timetrine, the quartzites, meta-arkoses, phyllites, large serpentine bodies and metabasalts with blue amphibole, are also interpreted as nappes, but here the relationships with the Eburnean basement are obscured by the Cretaceous.

A characteristic feature west of the suture is the total absence of autochthonous Pan-African magmatism. To the east of the suture a strongly deformed zone ~100 km wide is composed of a late Upper Proterozoic volcano-clastic assemblage. It consists of flysch-like metagreywackes with turbidites, conglomerates composed exclusively of volcanic and plutonic pebbles, dacitic breccias and meta-basalts and later meta-andesites volcanic greywackes and a terrigenous flysch unit, suggesting island arc and marginal sea environments, still active during the earlier deformation of the more internal continental domain of the Iforas, a situation similar to that of the upper Cretaceous to Mid-Tertiary flyschs of Western Alps. This assemblage is comparable to that of the 'Série verte' described 300 km further north in North-west Hoggar¹⁶ where geochemical studies have shown the immature nature of the greywackes²³ and the probable derivation of andesites in liaison with a subduction zone by partial melting of the mantle followed by low pressure fractionation²⁴. This zone is injected by a considerable volume of predominant pre-tectonic ultrabasic rocks, gabbros and diorites the volume of more acid terms, tonalites and adamellites increasing eastwards. This accretion zone, apparently devoid of an ancient sialic substratum, follows the sheared western margin of the Iforas. It is significant that greywacke formations with associated calc-alkaline magmatism which are widely developed in western Hoggar and the Iforas are totally absent west of the suture. Such an absence would be explained by the former existence of an ocean separating two continents.

The Iforas is a highly deformed sheared continental domain of 2,000 Myr old granulite basement with remnants of supracrustal platform deposits (quartzites and dolomites) overlain by late Upper Proterozoic marginal sea volcano-clastic sediments including a possible tillite intruded by abundant pre-, syn- and post-tectonic granitoids. A zone of late high temperature-low pressure metamorphism with the local occurrence of granulite facies rocks, charnockites and norites (Aguelhoc, Iforas, Egalis, North-west Hoggar) coincides with the westernmost known area of sialic rock and its Upper Proterozoic cover²⁰. A vast composite late to post-tectonic calc-alkaline batholith capped by flat-lying rhyolitic lavas (Nigritian)¹⁰ and cut by sub-alkaline and alkaline ring-complexes occupies the external zone of the continental domain. It coincides with a regional positive gravity anomaly. Emplacement occurred in a zone subjected to pronounced vertical uplift leading to unroofing during the Pan-African. Existing U-Pb data on Zircons (Lancelot, personal communication) suggest this late batholith to have been emplaced in a relatively short interval between 615 and 590 Myr, the dates 616 ± 11 Myr and 613 ± 3 Myr corresponding respectively to a diorite and quartz monzonite, and 586 ± 13 Myr to a hedenbergite-hastingsite-perthite granite belonging to the Kidal ring-complex. Magmatic activity in this zone, however, must have taken place over a long period of time, as the late batholith cuts foliated diorites corresponding to a deeply eroded pre-tectonic palaeobatholith about 700 Myr old. A striking feature of the batholith is the presence of spectacular dyke swarms. Early east-west striking swarms consist essentially of subordinate basic dykes, quartz-microdiorites micro-adamellites and felsites which locally were truncated by high-level adamellite and perthite granite. They are cut by important north-south swarms composed of subordinate basic dykes and several generations of quartz microsyenite, granophyres and rhyolites which can be followed over a distance of over 250 km in the axis of the batholith and which coincide with the Nigritian rhyolite fields and the alignment of ring-complexes. In a different context the batholith displays features reminiscent of an Andean-type batholith²⁵. The general disposition of calc-alkaline magmatism east of the suture suggests an easterly

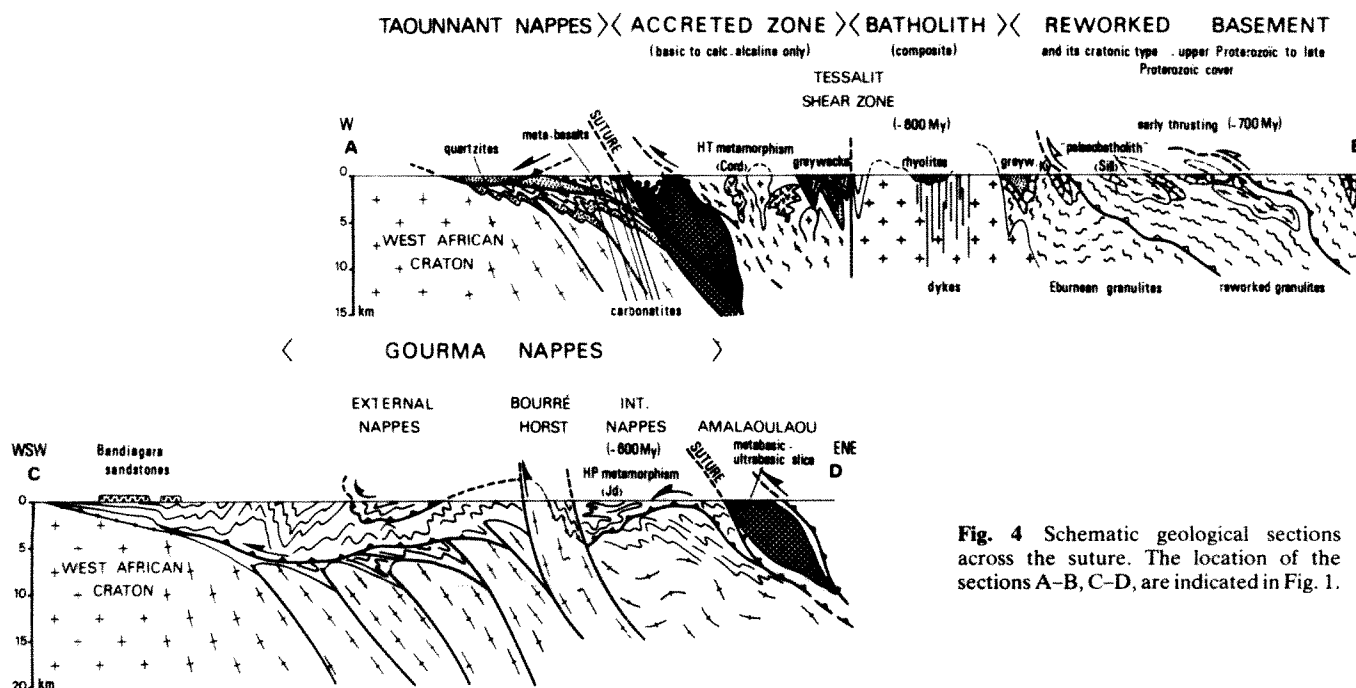


Fig. 4 Schematic geological sections across the suture. The location of the sections A-B, C-D, are indicated in Fig. 1.

dipping palaeo-subduction zone and the high pressure–low temperature metamorphism of the internal nappes of the Gourma would fit with this picture. The late alkaline rocks in the ring-complexes may be related to verticalisation of the Benioff plane accompanied by a diapiric rise of mantle material east of the suture²⁶.

General movement pattern

The Eburnean basement and supracrustal Upper Proterozoic formations of the Iforas have a complex structural and metamorphic history. Early Pan-African intracontinental deformation dated by the earliest syntectonic granites at 693 ± 0.3 Myr (Renaud Andreopoulos, personal communication) and which took place in barrowian metamorphic conditions generated large northwesterly moving crystalline nappes which were subsequently refolded, leading to considerable tectonic thickening and locally pronounced NW–SE metamorphic gradients²⁷. This early phase of deformation produced ENE–WSW structures which have also been observed in the accretion zone and suggest an early collision still not well defined. Late Pan-African deformation directly related to collision with the West African craton resulted in considerable east–west shortening in western Iforas and in the translation of nappes onto the foreland west and south-west of the suture. A characteristic feature east of the suture is the spectacular development of shear belts with a complex history. There is evidence for early sinistral movement along N° 20 trending shear belts and in the closing stages of collision for dextral movement on north–south belts leading to the northwards displacement of western and central Iforas with respect to eastern Iforas. Molassic sediments similar to the ‘Série Pourprée’ of western Hoggar dated at around 530 Myr (ref. 7) occur in grabens and fill a north–south palaeo-rift on the West-African craton between the suture and Timetrine-Taounnant where it is associated with under-saturated syenites and carbonatites. Late movements as young as the Upper Cambrian produced open north–south folds in the molassic sediments and a system of conjugated NNW sinistral and ENE dextral wrench faults, a system largely developed throughout the Pan-African mobile zone of West Africa²⁸.

Conclusions

All the evidence points to a modern-type orogenic belt involving a collision during the closing stages of the Pan-African, between the passive continental margin of the West African craton and

the active continental margin of an eastern continent. This conclusion fits the recognition of obducted ophiolites at Bou Azzer (Morocco) along the northern margin of the West African craton²⁹ and supports the collision hypothesis advanced for the Togo–Benin segment³. Looking at the Touareg shield (Hoggar, Air, Iforas) as a whole, the general pattern of shear belts and late Pan-African deformation seen at an eroded level, shows striking analogies with the tectonic pattern of Asia produced by the collision of India³⁰. Palaeomagnetic evidence suggests important horizontal displacement during the Pan-African³¹, and proposed reconstructions for 800 Myr and 675 Myr indicate open situations between North America and Gondwanaland and oceanic closure at 600 Myr (refs 32, 33). As no measurements exist for the West African craton, these results are not incompatible with the former presence of a pre-Pan-African ocean situated east of the West African craton.

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Structure of the human fetal globin gene locus

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We have derived a 'map' of restriction enzyme sites in and around the human γ -globin genes. This has enabled us to show that there are two γ -globin genes per haploid set, that the genes contain 'introns' within the same regions of DNA as the human β and δ -globin genes, and that the genes are 3,500 base pairs apart. We conclude that the correct gene organisation of the human β -like globin locus is $\gamma^A\gamma^B$.

DURING human fetal life, the major haemoglobin is HbF, consisting of two α -globin and two γ -globin chains ($\alpha_2\gamma_2$). In the last stages of fetal development, a switch occurs from γ -globin synthesis to adult β -globin synthesis; by a few weeks after birth, the major haemoglobin is HbA ($\alpha_2\beta_2$) accompanied by a much lower level of HbA₂ ($\alpha_2\delta_2$)¹. In some populations (such as Saudi Arabians), in most persons at times of erythropoietic stress (as in severe anaemia, β -thalassaemia, or sickle cell disease) and in pregnancy, the proportion of HbF in adult peripheral blood rises. In addition, in the rare condition known as hereditary persistence of fetal haemoglobin (HPFH) there is no HbA or HbA₂ and the person, who is clinically asymptomatic, survives solely on HbF (refs 2, 3).

It is known that there are at least two non-allelic γ -globin genes, one of which codes for a γ -globin chain with glycine and the other with alanine at amino acid position 136 of the 146 amino acid γ -chain (γ^G and γ^A respectively)⁴. In people with a mutation at some other position, either the γ^G or the γ^A -globin chain shows the mutation, but never both. It can be concluded that there must be at least two γ -globin genes per haploid genome, one each for the γ^G and γ^A -globin chains. Molecular hybridisation to cellular DNA of γ -globin complementary DNA supports this conclusion⁵⁻⁷.

However, the chain synthesis ratios of γ^A and γ^G are not those predicted if protein synthesis reflects gene number, and therefore it has been suggested that there are three or four γ -globin genes per haploid genome⁸. It has also been suggested that a third γ -globin gene may occur, so-called γ^T , coding for HbF_{Sanchez}, with threonine rather than isoleucine in position 75 (ref. 9). γ^T expression varies greatly among individuals and may represent either a third non-allelic γ -globin gene, or a commonly occurring allele of one of the existing genes. It is not yet possible to distinguish genetically between these alternatives.

We have recently reported the physical map of the human δ and β -globin gene loci¹⁰ as have others¹¹. Here we describe similar experiments which allow the mapping of the human γ -globin genes within their surrounding DNA sequences.

The development of techniques for the mapping of single-copy genes¹²⁻¹⁵ makes it possible to construct a physical map of restriction enzyme sites in and around the γ -globin genes. Normal human placental DNA is digested with restriction

enzymes and denatured. The fragments are separated by electrophoresis on a 1.2% agarose gel, transferred from the gel to a nitrocellulose filter using the method of Southern¹² and hybridised with ³²P-labelled pH γ G1 DNA followed by autoradiography to reveal the position of the fragment containing γ -globin gene sequences. pH γ G1 is a pCR1-derived recombinant that contains 500 base pairs of double-strand complementary DNA prepared from human γ -globin mRNA¹⁶. The limitations of this technique have been extensively discussed^{10,12,14,15}.

Our approach to mapping the γ -gene locus has been to identify a restriction endonuclease which gives only a single fragment containing all the γ -globin gene sequences hybridising to the specific γ -globin recombinant probe, to locate the gene sequences within this fragment by making use of restriction enzymes which are known to cleave within the globin coding sequence, and to distinguish between the several (in this case, two) gene sequences by identifying a restriction site present in one and absent in the other.

Table 1 Summary of sizes of γ -globin gene fragments generated by a variety of restriction enzymes in single and double digests

<i>Bgl</i> III	<i>Bam</i> HI	<i>Eco</i> RI	<i>Pst</i> I	<i>Xba</i> I	<i>Bgl</i> III + <i>Bam</i> HI	<i>Bgl</i> III + <i>Eco</i> RI
13	15.0	6.5	10.0	7.5	6.0	3.0
	5.0	2.5	5.0	5.0	5.0	2.5
	2.6	1.65	4.0	3.7	2.1	1.65
		0.65	0.9			0.65
<i>Bgl</i> III + <i>Pst</i> I	<i>Bgl</i> III + <i>Xba</i> I	<i>Bam</i> HI + <i>Eco</i> RI	<i>Bam</i> HI + <i>Xba</i> I	<i>Eco</i> RI + <i>Pst</i> I	<i>Pst</i> I + <i>Xba</i> I	
5.0	6.0	2.6	7.5	4.0	5.0	
2.2	5.0	1.65	5.0	1.8	3.7	
0.9	2.25	1.05	2.6	1.6	0.9	
		0.65		0.8		

Sizes are in kilobase pairs.

High molecular weight DNA was isolated from placentae of three normal infants of English, Welsh and Dutch ancestry. The DNA was cleaved with the restriction enzyme BglII, and after electrophoresis, transfer and hybridisation to ³²P-labelled pH γ G1 DNA only a single fragment of about 13 kilobases is seen. This fragment contains all the γ -globin gene sequences in human DNA. (All γ -globin gene fragment sizes are presented in Table 1.)

Several restriction endonucleases are known to cleave the γ -globin gene sequence from direct analysis of plasmid recombinants constructed with cDNA prepared from γ -globin mRNA¹⁶⁻¹⁸. Table 2 lists these enzymes, together with the corresponding amino acid positions coded for at the cleavage site. BamHI and EcoRI cleave both the γ^A and the γ^G genes, in

Table 2 Restriction enzyme sites within the A_γ or G_γ coding sequence

	A_γ	G_γ
<i>EcoRI</i>	121–122	121–122
<i>BamHI</i>	99–100	99–100
<i>PstI</i>	135–136–137	None
<i>XbaI</i>	None	None
<i>BglII</i>	None	None

Sites given are for the amino acids coded for by the restriction site in the appropriate γ -globin gene. Numbering is from the N(5') terminus of the protein chain. The positions of the *EcoRI* and *BamHI* sites are from refs 16 and 17; the *PstI* site from ref. 18, and the absence of *XbaI* and *BglII* sites from our unpublished data.

the same coding positions as for the human β and δ -globin genes, whereas *PstI* cleaves only the A_γ gene and not the G_γ (ref. 18). It is known that the coding sequence for A_γ contains a *PstI* site at the position coding for amino acids 135–136–137 (–CTGCAG–). This site is absent from the G_γ gene because of the presence of glycine rather than alanine at this position of the amino acid chain, giving a sequence altered to (–CTGGAG–) which is not cleaved.

When normal DNA is cleaved with *EcoRI*, four fragments hybridising to the γ -globin probe are obtained, of sizes 6.5, 2.5, 1.65 and 0.65 kilobases (Fig. 1). Assuming at this stage that there are two genes, this is consistent with the presence of at least one additional *EcoRI* site between the two genes. If this prediction is valid, these four fragments include two derived from the 3' end of the genes and two derived from the 5' end.

A double digest with *BglII* and *EcoRI* again gives four fragments (Fig. 1), three of which (at 2.5, 1.65 and 0.65 kilobases) are identical to those from an *EcoRI* digest and one of which, of 3.0 kilobases, is new and replaces the band of 6.5 kilobases, from which it must be derived. (It is too large to be

generated from any other *EcoRI* fragment). Therefore an *EcoRI* site internal to one of the γ -globin genes must be located 3.0 kilobases from one of the bounding *BglII* sites, and a second *EcoRI* site must lie 3.5 kilobases to the other side of this *BglII* site. The three smallest *EcoRI* fragments are not cleaved by *BglII* and therefore must lie entirely within the large 13-kilobase *BglII* single digest fragment.

BamHI single digests of human DNA give three hybridising fragments of 15, 5.0 and 2.6 kilobases (Fig. 1). As both genes contain a *BamHI* site, these three fragments could result from the *BamHI* cleavage of each gene, with no additional *BamHI* site in the DNA sequence between the two genes. The two *BamHI* sites are localised within the 13-kilobase *BglII* fragment, as shown using a double digest of *BamHI* plus *BglII*. The 15.0-kilobase *BamHI* fragment is cleaved by *BglII* to give a 6.0-kilobase double digest fragment; the 2.6-kilobase *BamHI* is cleaved by *BglII* to give a 2.1-kilobase double digest fragment (Fig. 1). The 5.0-kilobase *BamHI* fragment is not cleaved by *BglII*. Thus, *BamHI* sites are located 6.0 and 2.1 kilobases, respectively, from the bounding *BglII* sites of the 13-kilobase *BglII* fragment. Other *BamHI* sites are 15.0 and 2.6 kilobases, respectively, outside these internal *BamHI* sites, as defined by the *BamHI* single digest fragments.

From these data, the distance predicted between the two *BamHI* sites within the 13-kilobase *BglII* fragment is 4.9 kilobases. We conclude that these *BamHI* sites generate the 5.0-kilobase fragment seen in the *BamHI* single digest, and that this fragment is sandwiched between the 15.0 and the 2.6 kilobase *BamHI* fragments (Fig. 2).

The 5.0-kilobase *BamHI* fragment has at its termini the only two *BamHI* sites which are present within the 13-kilobase *BglII* fragment. These must be the *BamHI* sites which are located within the two γ -globin genes (Table 2). The 5.0-kilobase *BamHI* fragment contains two 'half-genes' therefore (one to the 3' side and one to the 5' side of the intragenic *BamHI* site), and each of the other *BamHI* fragments contains one 'half-gene'.

It is possible to orientate the *BamHI* and *EcoRI* 'maps' with respect to the *BglII* sites by use of a *BamHI/EcoRI* double digest. We have shown that both the 2.6-kilobase *BamHI* and the 6.5-kilobase *EcoRI* fragments contain a *BglII* site. There are two possibilities: either the *BglII* site is the same in both the *EcoRI* and the *BamHI* fragments (as shown in Fig. 2), which predicts that the 6.5-kilobase *EcoRI* fragment will be cut by *BamHI* to yield a 2.6-kilobase double digest fragment. Alternatively, the 6.5-kilobase *EcoRI* and the 2.6-kilobase *BamHI* fragments are located at opposite ends of the 13-kilobase *BglII* fragment. In this case there would be no *BamHI* cut in the 6.5-kilobase *EcoRI* fragment (Fig. 2). The fact that a double digest with *BamHI/EcoRI* gives fragments of 2.6, 1.65, 1.05 and 0.65 kilobases, eliminates this second possibility.

The existence of four *EcoRI* fragments is compatible with two γ -globin genes. The *BglII/EcoRI* digest above locates an *EcoRI* site within one of these. The *BamHI* plus *EcoRI* digest locates the *EcoRI* site in the other γ -globin gene, and establishes that there are two γ -globin genes. The strongly hybridising 2.5-kilobase *EcoRI* fragment is cleaved by *BamHI* to give a 1.65-kilobase double digest fragment (Fig. 1); alternative possibilities are inconsistent with a *PstI/EcoRI* double digest (Fig. 3a).

The orientation of the 2.5-kilobase *EcoRI* fragment with respect to the *BamHI* sites is established by experiments with 3'- and 5'-specific probes (data not presented; see Fig. 4 legend). The 15-kilobase *BamHI* fragment does not hybridise with 5'-specific probes, whereas the 5.0-kilobase *BamHI* fragment and the 2.5-kilobase *EcoRI* fragment do. Hence the orientation of the 2.5-kilobase *EcoRI* fragment can only be that shown in Fig. 2. According to the *BamHI-EcoRI* map derived from these data, the 1.65-kilobase *EcoRI* fragment would be uncut by *BamHI* and would co-migrate with the 1.65-kilobase *BamHI + EcoRI* double digest fragment.

Therefore, the intragenic *BamHI* and *EcoRI* sites in both coding sequences are 800–1,000 base pairs apart. From the direct sequence known from the cDNA plasmids, it is known

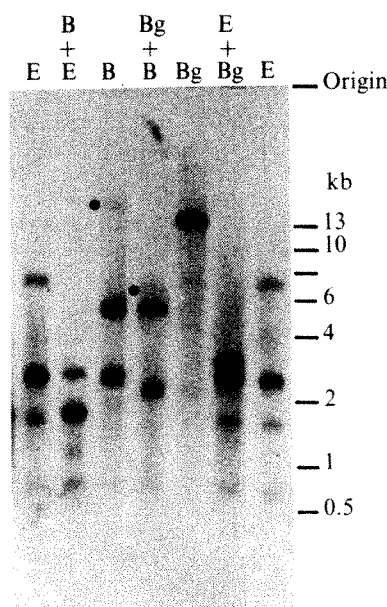


Fig. 1 γ -Globin gene containing fragments generated by *EcoRI* (E), *BamHI* (B), *BglII* (Bg) and combinations of these enzymes. The calibration scale is in kilobase pairs and faint bands are marked by ●. 30 μ g of placental DNA, prepared according to ref. 10, was digested with the appropriate restriction endonuclease, denatured with alkali and electrophoresed on a 1.2% Agarose gel. The denatured DNA was transferred to a cellulose nitrate filter sheet by blotting¹², the filter cut into strips and hybridised with 50 ng per ml of ³²P-labelled pH γ G1, at 65 °C in 3 $\times$ SSC 0.1% SDS, for 2 d (SSC is 0.15 M NaCl, 0.015 M Na citrate pH 7.0). After hybridisation, strips were washed with 3 $\times$ SSC 0.1% SDS at 65 °C and finally with 0.1 $\times$ SSC 0.1% SDS also at 65 °C. Labelled components were then detected by autoradiography. All details of hybridisation and washing procedures and of the size markers used are as described in refs 14 and 15.

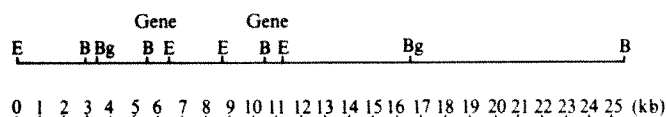


Fig. 2 Partial restriction site map of the γ -globin genes. Only approximate positions of the structural genes are shown. E, *EcoRI*; B, *BamHI*; Bg, *BglII*.

that the corresponding sites in the mRNAs are only 67 base pairs apart. This difference defines an intervening sequence (intron) between the *EcoRI* and the *BamHI* sites, at the same positions as those previously demonstrated in the rabbit and mouse β -globin genes and in the human δ and β -globin genes (refs 10, 11, 15, 19 and T. Maniatis, personal communication). The 1.05-kilobase *EcoRI*+*BamHI* double digest fragment is presumably generated from the intragenic *BamHI* and *EcoRI* sites flanking the large intron—the size of this fragment is in reasonable agreement with that predicted (0.85) from the map position of the *EcoRI* and *BamHI* sites described above. Figure 2 shows the map of the *BglII*, *BamHI* and *EcoRI* sites located by these experiments. (We will refer to the γ -globin genes as 'left' and 'right' as displayed in this figure.)

Cleavage of human DNA with *XbaI* gives three γ -globin gene fragments of 7.5, 5.0 and 3.7 kilobases (Fig. 3). A double digest with *BglII* yields fragments of 6.0, 5.0 and 2.25 kilobases. Thus, the 3.7-kilobase *XbaI* fragment is cleaved by *BglII*: this places an *XbaI* site 2.25 kilobases from the *BglII* site and a second *XbaI* site 3.7 kilobases away from this *XbaI* site. The 7.5-kilobase *XbaI* fragment is cleaved by *BglII* to give the 6.0-kilobase double digest fragment and this places an *XbaI* site 6.0 kilobases from the second *BglII* site. To orientate the *XbaI* sites with respect to the *BamHI* sites and within the *BglII* fragment, *XbaI* plus *BamHI* double digests were carried out. This yields the 7.5-kilobase *XbaI* fragment, the 5.0-kilobase fragment present in both *BamHI* and *XbaI* digests and the 2.6-kilobase *BamHI* fragment (Fig. 3). As the 2.6-kilobase *BamHI* fragment is generated from the 3.7-kilobase *XbaI* fragment, this allows the orientation of the *XbaI* sites with respect to the *BglII* sites. The only possible explanation for the existence of the 5.0-kilobase *BamHI* plus *XbaI* double digest fragment is that the two *XbaI* sites within the *BglII* fragment are located very close to the

position of the two *BamHI* sites mapped above. As *XbaI* does not cleave the γ -globin cDNA, and as the *BamHI* sites considered here are within the coding sequence of both γ -globin genes, the *XbaI* sites must be present in the intron, between the *BamHI* and *EcoRI* sites in both γ genes.

PstI generates γ -globin gene fragments of 5.0, 4.0 and 0.9 kilobases and a faint band of about 10 kilobases (Fig. 3). The *PstI* sites have been mapped in a double digest of *PstI* with *BglII*, *EcoRI* or *XbaI*. Cleavage of human DNA with *PstI* plus *BglII* gives γ -gene fragments of 5.0 and 0.9 kilobases (present in the *PstI* digest) and a double digest fragment of 2.2 kilobases (Fig. 3). This fragment must be derived from the 4.0-kilobase *PstI* fragment by *BglII* cleavage which places a *PstI* site 2.2 kilobases from a *BglII* site and also a *PstI* site 4.0 kilobases away from this *PstI* site. The *PstI* and *XbaI* double digest orientates the *PstI* sites within the *BglII* fragment: fragments of 5.0, 3.7 and 0.9 kilobases are obtained (Fig. 3) showing that the 4-kilobase *PstI* fragment contains the 3.7-kilobase *XbaI* fragment. A 5.0-kilobase fragment is present in both *PstI*, *XbaI* and *PstI*+*XbaI* digests which suggests that the *PstI* and *XbaI* sites which generate these 5-kilobase fragments must map near to each other. This is confirmed by the fact that one of the *PstI* sites, located above using *PstI* plus *BglII* double digests, maps very close to an *XbaI* site, independently located using a double digest with *BglII*, which defines one end of the 5.0-kilobase *XbaI* fragment. The 4.0 and 5.0 kilobase fragments are therefore contiguous on the DNA at a single *PstI* site. The position of the 0.9-kilobase *PstI* fragment will be discussed below.

Orientation of the physical map

To orientate the physical map with respect to the direction of transcription it is necessary to determine the orientation of the structural gene sequence. We have done this by hybridising restriction endonuclease digests to separate probes for the DNA regions to the 5' or 3' side of the intragenic *EcoRI* site. Plasmid pH γ G1 was cut with a mixture of *EcoRI* and *HindIII* restriction endonucleases: this gives two fragments of about 4 and 10 kilobases containing the γ -globin gene sequence to the 3' and 5' side of the *EcoRI* site, respectively. The two fragments were purified by gel electrophoresis, recovered from the gel²⁰ and labelled with ³²P by nick translation.

Filters carrying *EcoRI*-digested human DNA were hybridised with 3'- and 5'-specific probes. The 5' probe detected only the 6.5 and 2.5 kilobase fragments whereas the 3' probe detected the 1.6 and 0.65 kilobase fragments (Fig. 4).

Similar experiments (not presented) indicated that the 15-kilobase *BamHI* fragment contains only 3' information. This, combined with the fact that the 2.5-kilobase *EcoRI* fragment is only 5' containing, indicates that the orientation of the 2.5-kilobase *EcoRI* site with respect to the *BamHI* site in the right hand γ -gene can only be that shown in Fig. 2.

Localisation of the 3' *EcoRI* fragments

The mapping data described above do not allow us to locate the 1.65 and 0.65 kilobase *EcoRI* fragments. As both fragments contain DNA sequences to the 3' side of the *EcoRI* site in the γ -globin genes we anticipated that one fragment must be derived from the γ gene, and one from the α gene. To assign the *EcoRI* fragments to the γ genes we performed a *PstI* and *EcoRI* double digest. The α gene has a *PstI* site at position 136 and *PstI* should therefore cut the 3' *EcoRI* fragment of this gene whereas the γ gene has no such site. Strongly hybridising fragments of 4.0 and 1.8 kilobases, together with a fainter band at 1.6 kilobases were seen in this digest (Fig. 3). The 4.0 and 1.8 kilobase bands contain the 5' regions of the gene to the left and right in the map in Fig. 2, up to the *PstI* sites in each respective intron. The size of these fragments fits exactly with the map deduced in the preceding sections. The 1.6-kilobase band is the size expected for the 3' *EcoRI* fragment uncut by *PstI*. This fragment therefore must lie within the 5-kilobase *PstI* fragment and adjoin the 6.5-kilobase *EcoRI* fragment of the left hand

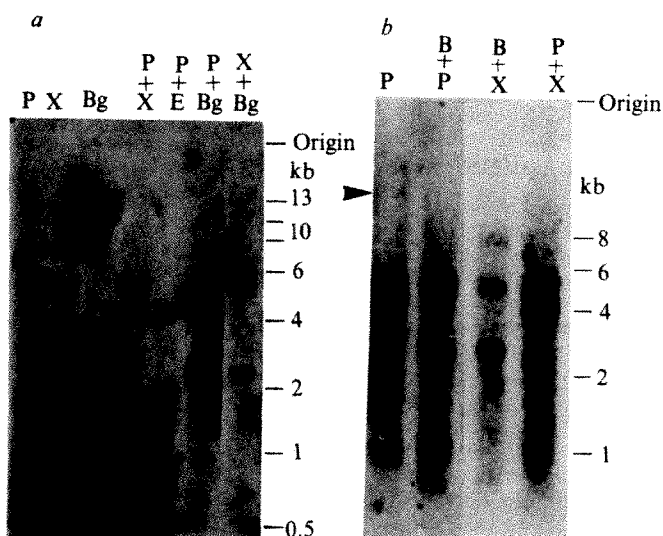


Fig. 3 a, γ -globin gene containing fragments generated by *PstI* (P), *XbaI* (X), *BglII* (Bg) and *EcoRI* (E) in single and double digests. Sizes are in kilobases. The position of certain faint bands is marked ●. b, γ -globin gene containing fragments generated by *PstI* (P), *XbaI* (X) and *BamHI* (B) in selected single and double digests. Sizes are in kilobases. The arrow marks the position of the very faint 10-kilobase *PstI* fragment seen in some digests. Experimental conditions for both experiments are as in Fig. 1.

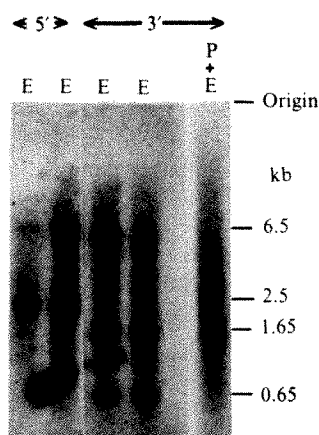


Fig. 4 The orientation of the γ -globin genes within the neighbouring DNA. 30 μ g of *EcoRI* digested DNA (E) or 30 μ g of *EcoRI*+*PstI* digested DNA (E+P) was alkali denatured and run on 1.2% Agarose gels and transferred to filters (as described in Fig. 1). Strips were then hybridised with ^{32} P-labelled 4-kilobase pH γ G1 *EcoRI*+*HindIII* fragment (3' specific) and the 10-kilobase *EcoRI*+*HindIII* fragment (5' specific), as described in Fig. 1. In each case a threefold excess of unlabelled fragment of the opposite specificity is included in the hybridisation to reduce cross hybridisation due to probe contamination¹⁴. The running position of each size fragment is indicated.

γ -gene. The 0.65-kilobase *EcoRI* fragment cannot be seen clearly in the digest. The *PstI*/*EcoRI* digest was hybridised with a specific probe for the regions of the γ -gene to the 3' side of the intragenic *EcoRI* site (as described above in Fig. 4) to confirm these assignments. The 1.6-kilobase fragment is visible (Fig. 4) whereas the 0.65-kilobase fragment cannot be seen. (Compare with the *EcoRI* digests in Fig. 4 where both the 1.6-kilobase fragment and the 0.65-kilobase *EcoRI* fragment are clearly visible.) The 0.65-kilobase *EcoRI* fragment is cut by *PstI*, (presumably) at the region coding for amino acids 135–136–137 of the γ -globin polypeptide chain (see Table 1) which should remove 45 base pairs of DNA homologous to our probe. The remaining 100 base pairs should however be detected by the γ -probe. We have shown that this is the 3' end of the γ -gene and the failure to detect this 100-base pair fragment may be the result of low homology between the 3' region of the γ -gene and the γ -cDNA probe in use here (data not presented). A similar problem arose in our study of the δ - β -locus¹⁰ where we could detect the 3' regions of the δ -globin gene with β -globin cDNA sequences only with difficulty.

Finally, the *PstI* cleavage of the 0.65-kilobase *EcoRI* fragment explains the 0.9-kilobase *PstI* fragment. This fragment is derived from the γ -gene and extends from the *PstI* site in the intervening sequence of the gene to the *PstI* site at the DNA coding position for 136 of the γ -globin chain. We predict that a fourth *PstI* fragment containing the regions to the 3' side of the DNA coding for amino acid 137, should also be visible. This is seen as a very faint 10-kilobase fragment in some *PstI* digests (see Fig. 3, for example). As discussed above, we believe that this fragment is difficult to see for three reasons: it does not cross react well with our γ -probe, it contains only 100 base pairs of DNA homologous to our probe and it is large.

The arguments presented here show that the leftward γ -gene in Fig. 2 is γ -gene and the rightward gene is γ -gene. We have not shown that the 3' *EcoRI* fragments are contiguous with their respective 5' fragment at the intragenic *EcoRI* sites of the γ -genes. This could most simply be achieved by use of γ -globin chain mutants which have lost the *EcoRI* site, but these mutants are extremely rare^{1,2,22}. For the γ -gene, non-contiguity of the 2.5 and the 0.65 kilobase *EcoRI* fragments implies that a second *PstI* site must occur exactly 0.9 kilobases from both the *PstI* site close to the *BamHI* site, and the intragenic *PstI* site. Similarly, the γ -gene cannot contain more than an additional 0.75 kilobases of DNA, as this is all the DNA that is not included in gene-containing

fragments between the two γ -globin genes. We believe, therefore, that it is reasonable to assume contiguity of fragment round the intragenic *EcoRI* sites.

All the restriction site information derived above can be combined into the single map in Fig. 5a. We believe that this is the simplest map compatible with our data; other more complicated maps are not excluded, however. For example, the difficulty of detecting small fragments containing only short regions of structural genes makes it hard to eliminate the possibility that additional *EcoRI* sites are present (within an additional intron?) in the 3' regions of the two genes. We believe that this is unlikely for the same reasons discussed for the contiguity of the *EcoRI* fragments around the intragenic *EcoRI* sites. Molecular cloning of this locus will ultimately resolve this problem.

The major features of the map, shown in Fig. 5b, are as follows. We identify the γ -gene by the presence of the *PstI* site to the 3' side of the intragenic *EcoRI* site. The γ -gene is proved to be to the 3' side of the γ -gene as predicted previously from genetic evidence^{3,22}. Both genes contain introns between the *BamHI* sites (at position coding for amino acids 99–100) and the *EcoRI* site (at positions 121–122). These introns are approximately 800–1,000 base pairs in length, similar to those found in the human δ - and β -globin genes. Similar sequences in the same position have previously been found in mouse¹⁹ and rabbit β -globin genes¹⁵. The two γ -globin genes are separated by 3.5 kilobases of DNA, as compared with approximately 7.0 kilobases of DNA separating the human δ - and β -globin genes¹⁰ and the map spans 25.5 kilobases of DNA.

Several more general conclusions can be drawn. Our data are consistent with the presence of only two γ -globin genes; for more to be present, a reduplication of an entire region of DNA greater than 25 kilobases in length and sufficiently conserved to retain identical restriction sites over this length would be

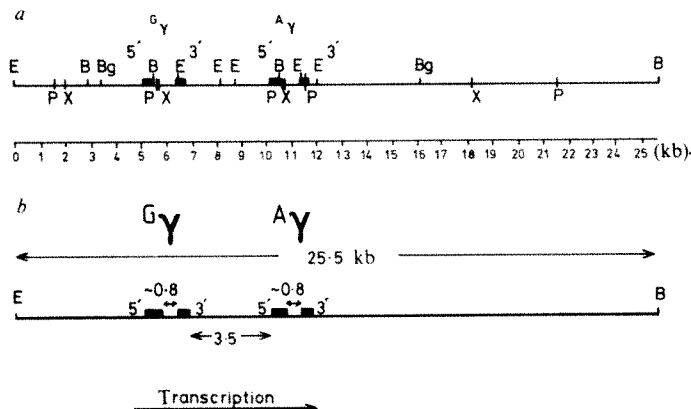


Fig. 5 a, Physical map of the linked γ - and γ -genes. The position of the coding sequences are indicated by filled boxes. It should be stressed that only those restriction sites that generate fragments containing a portion of coding sequence are detected in these experiments. The coding sequences are shown as two blocks. There is no evidence to exclude the possibility that these blocks might contain further short introns. E, *EcoRI*; B, *BamHI*; Bg, *BglI*; P, *PstI*; X, *XbaI*. b, The major features of the human fetal globin gene locus.

required. In the individuals examined (from persons of English, Dutch and Welsh parentage) there is no demonstrable third γ -gene; therefore it is likely that the gene for HbF_{Sardinia} is an allele of one of the two γ -globin genes or that there is a polymorphism of γ -gene number in humans. We did not determine whether HbF_{Sardinia} was expressed in cord blood in these individuals and therefore final determination of the γ -globin gene must await investigation of a person who definitely synthesises this chain. In the small number of DNA samples used, no polymorphisms in the restriction fragment patterns were seen.

The demonstration of close linkage of γ - and γ -genes enables us to exclude one of the two models for the organisation of the

β -related globin gene family locus in humans. The existence of the fusion globins, Hb Lepore, where the N-terminal protein sequence of the δ -globin chain is fused to the C-terminal sequence of the β -globin chain²³, and Hb Kenya²⁵ where the N-terminal protein sequence of γ is fused to the C-terminal sequence of β -globin, had shown that the gene order must be either $\alpha\gamma\beta$ or $\alpha\beta\gamma$. We have demonstrated that the former is correct.

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letters

Infrared observations of the X-ray quasars 0241+622 and MR2251-178

TWO new quasars have recently been found through X-ray observations (the only other one being 3C273, see refs 1; 2). Ricker *et al.*³ have reported observations confirming the identification of the X-ray source 2251-178 with a quasar of visual magnitude 14.1. Canizares, McClintock, and Ricker⁴ have reported visual spectrophotometric observations of this quasar, and find its visual spectrum is much like other low redshift quasars. Apparao *et al.*⁵ have reported the discovery of a low redshift quasar identified with the X-ray source 4U0241+61. Margon and Kwitter⁶ have reported detailed visual spectrophotometry of this quasar, 0241+622, and likewise find that its spectrum is typical of low redshift quasars. Because of the importance of establishing any morphologically unique characteristics of X-ray quasars, these quasars should be observed at all possible wavelengths. We report here IR observations of the two newly discovered X-ray quasars. These observations indicate that the X-ray quasars have IR properties typical of low redshift quasars, and, along with 3C273, are among the brightest known quasars at IR wavelengths.

All observations reported here of 4U0241+622 and MR2251-178 were obtained on the 5 m Hale telescope on Palomar Mountain. Broadband photometry from 1.25 to 20 μ m of 0241+622 was obtained on 26 March 1978 UT and again on 20 July 1978 UT. Photometry from 1.25 to 10 μ m was obtained of MR2251-178 on 17 July 1978 UT and 20 July 1978 UT. These data are plotted in Fig. 1 along with the flux observed from 3C273 (ref. 7).

Spectrophotometric observations of 0241+622 from 1.5 to 2.5 μ m with a resolution $\Delta\lambda/\lambda \sim 0.05$ using a cryogenically cooled circular variable filter wheel spectrometer were obtained on 26 March 1978 UT; these observations were repeated on 20 July 1978 UT with a circular variable filter wheel of resolution

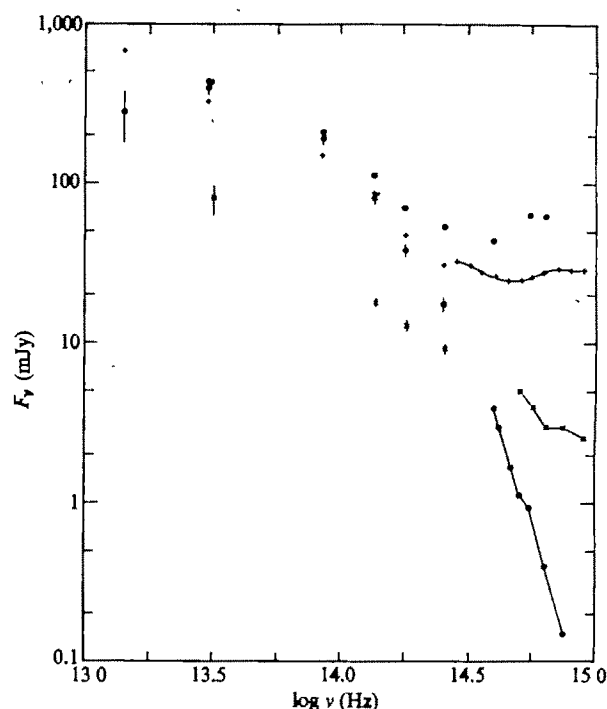


Fig. 1 The IR and visual energy distributions of the quasars 0241+622, MR2251-178, and 3C273 plotted against frequency. The data for 3C273 (+) is from ref. 7, the optical data for 0241+622 (●) is from ref. 6 and the optical data for MR2251-178 (x) is from ref. 4. ○ represent the de-reddened energy distribution of 0241+622, assuming $A_v = 4.6$ mag.

$\Delta\lambda/\lambda \sim 0.01$. Both sets of observations are shown in Fig. 2. The data were calibrated and corrected for atmospheric extinction using observations of the nearby B2Ia star 10 Persei. The data suggest that $P\alpha$ emission redshifted to $1.956 \mu\text{m}$ is present, but the atmospheric extinction limits the accuracy of the determination of the line flux of $P\alpha$.

Before the IR energy distributions of these quasars can be compared, the observed flux from 0241+622 must be corrected for significant galactic foreground extinction. Margon and Kwitter⁶ adopt a value of visual extinction $A_v \sim 4.6$ mag based on five separate estimates of the extinction. The present broad-band observations give an additional estimate of the extinction. For 10 quasars with redshifts $z \leq 0.2$, Neugebauer *et al.*⁷ find that the mean V ($2.2 \mu\text{m}$) = 3.1 mag with a spread of ± 0.4 mag. The observed V ($2.2 \mu\text{m}$) colour of 0241+622 is 6.7 mag if the flux did not vary between the times of the visual and IR observations. If the intrinsic V ($2.2 \mu\text{m}$) colour of 0241+622 is 3.1 ± 0.4 mag, the quasar thus suffers 3.6 ± 0.5 mag of differential extinction between $0.5 \mu\text{m}$ and $2.2 \mu\text{m}$. If a standard Van de Hulst number 15 reddening curve^{8,9} is applied, this implies $A_v = 4.0 \pm 0.6$ mag, consistent with the value adopted by Margon and Kwitter.

A further, though weak check on the extinction is obtained from consideration of the $P\alpha$ line flux. The flux in the $P\alpha$ line based on the data in Fig. 2, is $3 \times 10^{-16} \text{ W m}^{-2}$; the determination is uncertain because of the difficulty in determining the true continuum and in assessing the line flux shortwards of $1.96 \mu\text{m}$. The $H\beta$ flux as measured by Margon and Kwitter is $\sim 4.5 \times 10^{-17} \text{ W m}^{-2}$, giving a ratio $P\alpha/H\beta \sim 7$. The observed ratio $P\alpha/H\beta$ in 3C273 is 0.3 (refs 10, 11), in agreement with theoretical calculations if the region is optically thick to Lyman series photons¹². If 0.3 is the intrinsic value of $P\alpha/H\beta$ and if the normal reddening curve applies, a visual extinction $A_v = 3.2$ mag is implied. In view of the large uncertainty in the $P\alpha$ line flux and in the intrinsic $P\alpha/H\beta$ ratio, we consider the agreement with other determinations of A_v acceptable.

The de-reddened IR energy distribution of 0241+622, based on $A_v \sim 4.6$ mag, is shown in Fig. 1. For wavelengths $\leq 10 \mu\text{m}$ the energy distributions of all three quasars are remarkably similar and in this case cannot be distinguished from other low

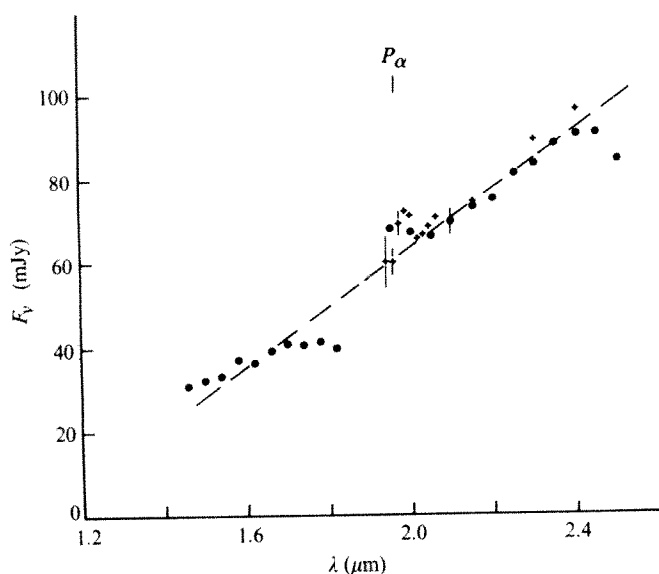


Fig. 2 The near-IR spectrum of 0241+622, plotted against wavelength. ● represent data obtained with a spectral resolution of $\Delta\lambda/\lambda \sim 0.05$, while + represent data obtained with a spectral resolution of $\Delta\lambda/\lambda \sim 0.01$.

Table 1 Emitted power per fractional bandwidth (νF_ν)

Wavelength	0241+622	2251-178	3C273
$2 \mu\text{m}$	12	4.7	120
$0.5 \mu\text{m}$	30	4.0	160
$2.2 \text{ \AA}$ (5.5 keV)	2	1-9	10-50

Values are in units of 10^{37} W . The visual and IR data for 3C273 are from ref. 7, while the X-ray data for all these quasars are from ref. 5; the visual data for 0241+622 are from ref. 6; the visual data for MR2251-178 are from ref. 4.

redshift quasars. For a sample of eight low redshift quasars ($z \leq 0.2$) studied by Neugebauer *et al.*⁷, the average $2-10 \mu\text{m}$ spectral index α ($f_\nu \propto \nu^\alpha$) is -1.1 with a total spread of ± 0.3 . The intrinsic $2-10 \mu\text{m}$ index of 0241+622 is 0.9, while that of 2251-178 is 1.0. Both of these are thus well within the range of indices of the sources studied by Neugebauer *et al.*

It is of interest to compare the observed IR, visual, and X-ray luminosities of 0241+622, 2251-178, and 3C273. Table 1 shows the power per fractional bandwidth, νF_ν , where ν is the rest frequency and F_ν is the emitted power density, for the X-ray, visible and IR wavelengths; the Hubble constant has been taken as $50 \text{ km s}^{-1} \text{ mpc}^{-1}$. These observations do not show a strong correlation between the IR, visual, and X-ray luminosities of these quasars, although to within factors of three the ratios of IR, visual, and X-ray luminosities are similar for all three quasars. Note, however, that the X-ray flux for 3C273 has varied by a factor of 5 over a 2-yr period¹³ while the visible and IR fluxes have remained essentially constant (see ref. 7); this suggests that there is no direct link between the X-ray and visual/IR emission mechanisms.

The main distinguishing feature of these quasars at IR wavelengths is their brightness. The three currently known X-ray emitting quasars are comparable in visual brightness to the brightest quasars in the compilation of Burbidge, Crowne, and Smith¹⁴ and are the three brightest observed quasars at $2.2 \mu\text{m}$ (see ref. 7). The fact that the three quasars which are known to be X-ray sources are among the brightest quasars in the optical wavelengths supports the suggestion of Ward *et al.*¹⁵ and Canizares, McClintock, and Ricker⁴, that all quasars are relatively bright X-ray emitters, and sensitive X-ray surveys should provide a powerful tool for the discovery of new quasars.

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Infrared observations of the black-hole candidate V861 Sco

V861 Sco was found to be an eclipsing binary by Cousins and Lagerwey¹ and its radial velocity curve has been determined by Walker². The primary is a B01 star, and taking suitable values for its mass the radial velocity curve suggests the unidentified secondary has a mass in the range 5–12 $M_{\odot}$. Recently Polidan *et al.*^{3,4} showed that the system may be an X-ray source; it is therefore a black-hole candidate. We therefore thought that observations at other wavelengths would be of interest and we report here IR observations from 1.2 to 3.6 μm .

V861 Sco was observed from 25 July to 17 August 1978 at the Cabezon observatory, Tenerife, with the 1.5 m IR flux collector. The observations were made with the Leicester University and Royal Edinburgh Observatory IR photometers, both of which are of conventional design, use InSb detectors, and have identical interference filters. Each observation is the average of a number of 20 s integrations. The error bars for our data shown in Fig. 1 are the standard error of the mean found from these integrations. V861 Sco is very low on the southern horizon from Tenerife, and can therefore only be observed once each night.

The standard used was SAO227504 which is less than 0.5° from V861 Sco. This standard was in turn calibrated against α Cyg ($J = 1.00$, $K = 0.89$, $L = 0.78$, Johnson⁵), which was just rising at the time of observation of V861 Sco, and subsequent observations of α Cyg allows a correction for atmospheric extinction to be made. This procedure gives rise to a light curve of very reliable shape but whose absolute calibration has errors of ± 0.1 magnitudes.

The ephemeris used is calculated from the data of Cousins and Lagerwey¹ and gives the primary minimum a phase of zero.

$$\phi = (\text{JD} - 2440493.236) \times 0.127418 - 0.123$$

We have not used the ephemeris quoted by Cousins and Lagerwey¹ as this uses an epoch of JD = 2430000 which exaggerates any errors in the period. As can be seen from our data and the error in the period, this ephemeris is quite adequate at present and does not need clarifying as suggested by Polidan *et al.*⁴. Furthermore, this ephemeris places Polidan's possible X-ray eclipse at about primary minimum, when the secondary is in front of the B01a primary, which is not very satisfactory.

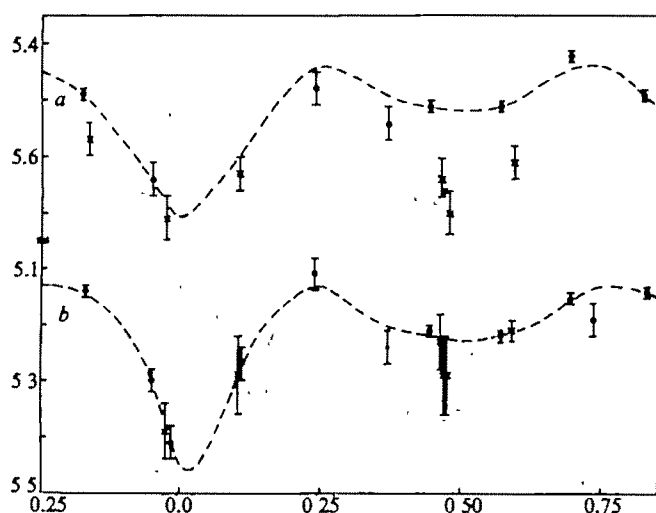


Fig. 1 Light curves of V861 Sco: a, 1.2 μm ; b, 2.2 μm . ●, Our magnitudes, ×, magnitudes from Tanzi *et al.*⁶.

Table 1 IR eclipse depths and optical data of the secondary minimum

	U	B	V	J	K
Δm_1	0.31	0.27	0.27	0.27	0.33
Δm_2	0.19	0.19	0.19	0.08	0.10
B_2/B_1	0.65	0.73	0.73	0.32	0.34
A_2/A_1	0.30	0.26	0.26	0.24	0.29

Figure 1 shows our 1.2 and 2.2- μm light curves and also includes the IR data of Tanzi *et al.*⁶. Their data agrees well with ours at 2.2 μm but is about 0.1 mag fainter at 1.2 μm ; this is probably due to our error in the absolute calibration due to the large air-masses. Neither our data nor that of Tanzi *et al.*⁶ is of sufficient quality to draw a useful 3.6- μm light curve.

The main interest of these observations is the shallow IR secondary minimum. Table 1 shows our best estimate of the IR eclipse depths together with the optical data taken from Cousins and Lagerwey¹. The ratio of secondary to primary surface brightness, B_2/B_1 , is calculated from these depths. We have also calculated A_2/A_1 , the eclipsed area at either primary or secondary eclipse divided by the area of the primary, assuming the eclipse is total. If the eclipse is not total this column is a lower limit for A_2/A_1 .

The rapid decrease of the surface brightness of the secondary from the optical to the IR clearly shows that this object is non-stellar. For example, if we take -0.79 for the $V-K$ colour of the B01a primary, we can show the $V-K$ colour of the secondary is -1.71 which doesn't correspond to any known star.

The non-stellar secondary is probably a compact object surrounded by an accretion disk. In the IR the cool outer regions of the disk are optically thick to free-free radiation and a low surface brightness is observed. In the visible region of the spectrum, however, the outer regions of the disk would be optically thin, allowing the hotter central region to be observed.

The presence of an optically thin accretion disk is also supported by the emission lines seen by Walker² and Polidan *et al.*³.

It may be argued that we have taken the eclipse depths at face value and not made any allowance for gravity darkening, ellipticity or heating effects on the near side of the primary. These effects can be removed by 'rectifying' the light curve and this has been done by Walker⁷ for the optical data. If the main distortion to the light curve is the ellipticity of the primary this effect should be the same in both the visible and near IR. Thus we might apply Walker's rectification to the IR light curves but, as this has an amplitude of 0.15 mag, it would more than remove the secondary minimum. Walker's rectification may therefore be excessive.

Any rectification applied to the IR light curves will only make the ratio B_2/B_1 smaller still, so our conclusion that the secondary is non-stellar remains. The shallow IR secondary eclipse also means that the area of the primary seen at phase 0.25 cannot be more than 10% greater than the area seen at phase 0.5. This small ellipticity of the primary implies that it does not fill its Roche lobe. If the primary were to fill its Roche lobe a difference of 17% would be needed; this requirement is almost independent of the assumed mass ratio. Thus the photosphere does not apparently fill its Roche lobe; however, the likely extended atmosphere of the primary could give a substantial mass transfer through the inner lagrangian point. Alternatively, if we regard the mass transfer as being due to a stellar wind then the secondary must contain a compact object, which would have to be a black hole. This conclusion is independent of any assumption about the possible X rays.

It should also be noted that if, following Walker², we take $A_v = 1.47$ the whole system has a K excess of 0.4 mag. This cannot, of course, be due to the eclipsed radiation from the secondary. If the eclipses are partial, and the secondary is effectively larger at IR wavelengths this would give the IR excess. Alternatively the whole system may be surrounded by free-free emitting material.

We conclude that these observations support the idea that the secondary of V861 Sco contains a black hole. However, a more convincing identification of the X-ray source with V861 Sco is needed. Even if V861 Sco does not turn out to be an X-ray star it would still seem to be an interesting system. Either way the IR data should assist with any future models of the system.

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HEAO 1 observations of high-energy X rays from 3C273

THE first detection of high-energy (13–120 keV) X rays from the vicinity of the quasar 3C273 is reported here. This object is one of three quasars associated with X-ray sources^{1–3}. Low energy (2–10 keV) X rays^{3–5} and γ rays (50–500 MeV)⁶ have been previously observed.

The present observations were made with the 13–180 keV slat collimated detectors of the high energy X-ray and low energy γ -ray (A4) experiment on the HEAO 1 satellite⁷. These detectors each have areas of $\sim 100 \text{ cm}^2$ and fields of view of $\sim 1^\circ \times 20^\circ$ (FWHM). The narrow slats intersect on the +Y axis and are inclined with respect to the Y-Z plane of the satellite at angles of -30° (left slat) and $+30^\circ$ (right slat).

The A4 detectors were pointed at 3C273 for ~ 6 h on 30 June, ~ 8 h on 3 July, and ~ 10 h on 5 July 1978, resulting in a positive

detection. In addition, the source was observed for ~ 20 d during December 1977 and January 1978 in the scanning mode (satellite spinning). During each rotation of the satellite the source appears a maximum of ~ 10 s in the field of view; however, there was no detection of this source.

During the pointed observations the detectors were alternately directed at 3C273 and at a reference position $\sim 4^\circ$ from the quasar to obtain background data. Background data during the scanning observations were obtained from data adjacent to source crossings. Individual photon events were pulse height analysed in 64 channels from ~ 13 to ~ 180 keV and binned in five coarse energy bands from 13–25, 25–45, 45–85, 85–120, and >120 keV. Count rates in these bands were computed by averaging the background-subtracted data corrected for detector response, assuming a source at the position of 3C273 and using the Goddard Space Flight Center (GSFC) attitude solution. No data were used when the Earth or other known X-ray sources were in the field of view. A comparison of the GSFC attitude solution with the more accurate solution of the scanning modulation collimator (A3) experiment for several orbits indicated no significant (>5 arc min) differences. The hard X-ray source is localised to a region of $\sim 4.5 \text{ deg}^2$ which includes 3C273.

The results of the observations are summarised in Table 1. The June/July 1978 data show statistically significant fluxes independently in each detector of $\sim 8\sigma$, $\sim 6\sigma$, $\sim 4\sigma$, and $\sim 3\sigma$ in the four energy bands from 13 to 120 keV. The December 1977/January 1978 scanning data yield only an upper limit (our detection was only at 2σ level of confidence) combining all data from ~ 13 to ~ 120 keV. The ratio of the count rate in December 1977/January 1978 to the rate in June/July 1978 is 0.8 ± 0.5 . We note that the X-ray flux of 3C273 is variable on a time scale of months^{8–9} and that during our June/July 1978 observations 3C273 was in a high state⁹.

The count rate spectrum for the June/July 1978 observations is shown in Fig. 1a. Figure 1b shows the inferred photon spectrum, derived from detector efficiency curves determined by multiple scans over the Crab Nebula. Previous measurements at lower X-ray energies² and at γ -ray energies⁶ are also included for comparison. The combined Ariel V and HEAO A4 data (in the region of 15 keV) indicate variability. The HEAO A4 data taken alone are consistent with a single power law photon spectrum with spectral index $\gamma = 1.67 \pm 0.14$ (1σ error). If we include the COS B data in the fit the spectral index is 2.05 ± 0.03 (1σ error).

The HEAO 1 cosmic X-ray (A2) experiment and the COS B experiment also observed 3C273 during June and July 1978 (not shown in Fig. 1). The COS B data (50–500 MeV) are consistent with their measurements in June 1976 but an intensity decrease

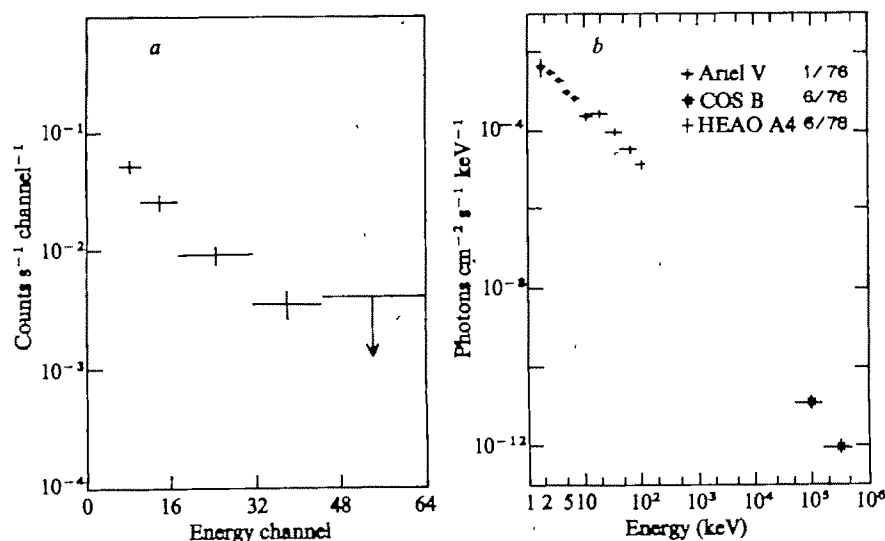


Fig. 1a HEAO A4 count rate spectrum for the June/July 78 observations. Only left slat detector data are shown. Errors are $\pm 1\sigma$ and include estimates of systematic errors due to background variations. Upper limits are at a 2σ confidence level. **b**, Photon spectrum from 1 keV to 500 MeV (see text for refs). HEAO A4 spectral points are obtained by combining left slat and right slat data. Errors are $\pm 1\sigma$ and include estimates for the uncertainty in converting from counts to photons.

Table 1 3C273 observational parameters

December 1977/January 1978

Left slit

only: keV
PHA count rate* 13–120 $(1.1 \pm 0.6) \times 10^{-2}$ ct s⁻¹ per channel

June/July 1978

Left slit:

PHA count rate* 13–25 $(5.1 \pm 0.6) \times 10^{-2}$ ct s⁻¹ per channel
25–45 $(2.5 \pm 0.4) \times 10^{-2}$
45–85 $(0.9 \pm 0.2) \times 10^{-2}$
85–120 $(0.3 \pm 0.1) \times 10^{-2}$
>120 $<0.4 \times 10^{-2}$
13–120 $(1.4 \pm 0.2) \times 10^{-2}$

Right slit:

PHA count rate* 13–25 $(4.2 \pm 0.7) \times 10^{-2}$ ct s⁻¹ per channel
25–45 $(2.2 \pm 0.3) \times 10^{-2}$
45–85 $(0.8 \pm 0.2) \times 10^{-2}$
85–120 $(0.3 \pm 0.1) \times 10^{-2}$
>120 $<0.3 \times 10^{-2}$
13–120 $(1.3 \pm 0.2) \times 10^{-2}$

Photon flux†

13–25 $(2.8 \pm 0.4) \times 10^{-4}$ photons cm⁻² s⁻¹ keV⁻¹
25–45 $(1.0 \pm 0.2) \times 10^{-4}$
45–85 $(3.6 \pm 0.6) \times 10^{-5}$
85–120 $(1.5 \pm 0.4) \times 10^{-5}$ *Errors ($\pm 1\sigma$) include an estimate of systematics due to background variations.†Combined data from the right and left slit. Errors ($\pm 1\sigma$) include an estimate of systematics due to the conversion from counts to photons.

by as much as a factor of 2 cannot be excluded (Swanenburg, personal communication). The A2 data (2–60 keV) agree well with the A4 observations at 20 keV but indicate a flatter spectrum at lower energies. No single power law spectrum in the range 2–60 keV yields a satisfactory fit to the A2 data. However, if a fit to a single power law is forced, the fit yields a spectral index of ~ 1.4 (ref. 10).

A comparison of our data with those at lower X-ray energies and higher γ -ray energies indicates that there is an overall trend for spectral steepening from low to high energies with perhaps a break near ~ 20 keV. It is possible that the high energy γ -rays do not originate from the same region as the X rays¹¹. This should be considered in any comparison of spectral data in the X-ray and γ -ray regions.

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Energy sources for X-ray cluster emission

SOME galactic clusters are strong X-ray emitters¹, and we consider here the problem of establishing certain models for the source of this radiation. In the first model we suppose that the X rays are thermal bremsstrahlung from a hot quasi-isothermal inter-cluster gas. Typical parameters for the temperature, particle density and total mass of the gas are² $T \sim 3 \times 10^8$ K, $n \sim 10^{-3}$, $M \sim 3 \times 10^{44}$ kg. If we assume³ relative nuclei solar abundances, then the thermal energy contained in the gas is $E_T \sim 2 \times 10^{64}$ erg. This is $\sim 10\%$ of the total rest mass energy of a $10^{11} M_\odot$ galaxy and clearly poses a problem for potential energy sources.

To check the necessity of having a 'current' energy source (light times away) one must consider the cooling rate of the gas and compare it with its estimated age. The essential kinetic energy loss is from free-free bremsstrahlung transitions and particle-ion inelastic collision which excite ion-electron levels. However, the above kinetic energy is so high, $1.5 kT \sim 40$ keV that few ions exist with many electrons. For example, Fe XXVI, the hydrogenic iron ion, is bound by only 9.2 keV. Taken together with the small number of heavy nuclei present at all, the latter cooling mechanism is completely negligible. For a constant density plasma then, the cooling rate is⁴

$$\frac{3}{2} nK \frac{dT}{dt} = 1.42 \times 10^{-27} n_e \sum_i n_i Z_i^2 \left(\frac{T}{K} \right)^{1/2} \text{ erg cm}^{-3} \text{ s}^{-1} \quad (1)$$

where n is the total number of free particles per cm³ and n_i is the density of nuclei of charge Z_i . We consider only electron-nucleus collisions as in the non relativistic approximation ($T \ll 6 \times 10^9$ K) the radiation from electron-electron collisions is much weaker⁵. Taking $n = 4.6 \times 10^{-3}$ cm⁻³ as a typical value with $n_{He} = 0.1 n_H$ the temperature falls according to

$$T(t) = \{T_0^{1/2} - 157.5t\}^2 \text{ K} \quad (2)$$

where t is in 10^9 yr and T_0 is the initial temperature in K. If we take $T_0 = 9 \times 10^8$ K then after 10^{10} yr the gas cools by only 10%. We conclude then that the hot gas may be very old, heated from its past with no current source necessary. We seek, therefore, energy sources within the cluster that were operating in the remote past and had short astronomical lifetimes with efficient energy production. The quasars are natural candidates. Due to their high intrinsic luminosities $\sim 10^{45}$ – 10^{48} erg s⁻¹, their lifetimes are necessarily short $\sim 10^8$ yr. As there are few quasars of small redshift (assumed cosmological) the quasar period of galactic evolution must be associated with young galaxies. One advantage of the quasar model is the following. If the heat input was too large, then the gas would no longer be gravitationally confined in the cluster. Instead it would envelop them. Abell clusters 399 and 401 may be examples of the overheating case⁶. Conversely, if the heat input was too small then the cluster

would not be an X-ray emitter at all. This would explain why some clusters have little or no X-ray emission. If we assume that the gas is at least as old as the galactic cluster we can estimate the age of the cluster in this model. Schmidt⁷ estimates that the space distribution of quasars is $10^{-6}(1+z)^6$ quasars Mpc^{-3} , where z is the redshift. This is valid up to $z \approx 2.5$. Assuming⁸ that the space density of galaxies is 0.03 galaxies Mpc^{-3} requires that the number of quasars relative to the number of galaxies at redshift z is $dN_{\text{rel}}/dz \approx 3 \times 10^{-5}(1+z)^6$. Thus we expect $4.3 \times 10^{-6}(1+z_0)^7$ relative number of quasars to have existed between $z = z_0$ and $z = 0$. If the energy per quasar which goes into heating the surrounding gas is R we deduce that the age of the cluster z_c in units of z is

$$z_c = (5.84) \left(\frac{E_T}{RS} \right)^{1/7} - 1 \quad (3)$$

where S is the number of galaxies in the cluster. Values of z greater than or less than z_c of equation (3) give a gas too hot or too cold than is observed. z_c depends weakly on the unknown number R . One may estimate this as follows. The only way that a completely ionised dilute intercluster gas can be effectively heated to such high temperatures is from collisional heating. Charged particles, cosmic rays, streaming out of quasars are the primary heating mechanisms in this model. According to Ipavach's analysis⁹ the maximum cosmic ray luminosity for a giant $10^{12} M_\odot$ galaxy is $10^{45} \text{ erg s}^{-1}$ for 10^8 yr yielding $3 \times 10^{62} \text{ erg}$. Taking into account that only a small percentage of the cosmic ray flux deposit their energy within the intercluster gas we estimate that a reasonable $R \sim 10^{61} \text{ erg}$.

We have applied the above analysis to Cluster Abell 2218. The parameters of this source have been given by Birkinshaw¹⁰. We take $T \sim 3 \times 10^8 \text{ K}$; $M_{\text{gas}} \sim 3 \times 10^{44} \text{ kg}$; $n \sim 4.6 \times 10^3 \text{ cm}^{-3}$; $S \sim 6.2 \times 10^4$. Then from equation (3)

$$z_c (\text{Abell 2218}) \approx 2.24 \quad (4)$$

This corresponds ($H = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$) to an age of $16.2 \times 10^9 \text{ yr}$.

The second model that we consider is a specific realisation of Katz's suggestion¹¹ that the X-ray emissions are from many distinct 'galactic type' sources rather than from an all pervading hot intergalactic gas. According to Rees¹², the 2.7° microwave background is not attributed to the cooling of big bang primordial radiation but to a later $t \sim 10^6$ – 10^7 yr epoch of intense stellar nucleosynthesis and the observed ratio $N_\gamma/N_b \sim 10^8$ is naturally derived. An essential prediction of the Rees' cosmology is the appearance of dead remnants of massive stars strewn throughout the Universe, being the relics of the first few millennia. It seems reasonable that such objects could form large dead star clusters. In particular, one could imagine an extensive system composed of a black hole cluster with attendant matter in the form of captured gas and galaxies. This would then form the missing virial mass. A small percentage of the black holes would be in binary systems with a normal star, recently condensed out of the cluster medium. Taking $10^{37} \text{ erg s}^{-1}$ as the average X-ray luminosity of a black hole binary we would require $\sim 10^6$ – 10^8 binary systems within a cluster. If the black hole cluster is the virial mass defect then $10^{15 \pm 1} M_\odot$ per cluster is in the Rees' remnants: if each has an average mass of $100 M_\odot$ then only 10^{-8} – 10^{-4} of the available remnants need be in X-ray binary systems. Alternatively, the remnants may be supermassive and accreting matter from a diffuse uniform density ρ environment. The accretion rate is $\rho \pi R^2 v$. For rates 10^{-10} – $10^{-8} M_\odot \text{ yr}^{-1}$ (giving typical black hole luminosities) we require 10^4 – 10^5 black hole remnants with mass $M \sim 10^{11}$ – $10^{12} M_\odot$ for $\rho \sim 10^{-3} \text{ protons cm}^{-3}$. The interpretation then is that the extensive X-ray emission from galactic clusters is a physical manifestation following from the Rees' cosmology: this has a dramatic and testable prediction. Because of the rotating black hole sink, the accretion disks have a strong density gradient of optically thin to optically thick material¹³. Consequently the spectrum is expected to consist of a thermal bremsstrahlung low energy part from

the optically thick region and a high energy inverse Compton part from the optically thin region. The spectrum therefore should drop with a black body slope to $\leq 50 \text{ keV}$ and then begin to increase to a broad (comptonised) hump out to a few hundred keV. It would be desirable to have good X-ray data between the region 10–500 keV if X-ray cluster emission has this two component form.

Finally the two models discussed here may be unified. If the mysterious quasar source has its origin in gravitational collapse¹⁴ then a smaller black hole cluster may be the remnant of early quasar activity.

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Auto-irradiation effects in DT pellets

FUSION reactors based on magnetic containment and operating in a quasi steady-state will need to be refuelled during their burn period. The ballistic injection of solid pellets of hydrogen isotopes, first suggested in 1954¹, still seems to be the most promising method, and two satisfactory techniques for fabricating solid hydrogen pellets have been developed. In one, a continuous extrusion of solid hydrogen is thermally chopped into cylindrical pellets². In the other, a jet of liquid hydrogen is acoustically broken into uniform droplets which are then frozen by reducing the pressure to below the triple point value³. Both techniques have worked well with deuterium, but fusion reactors will need DT pellets, and we consider here how the radioactivity of tritium will affect the situation. Two effects are apparent with characteristic times which may be short enough to be of practical significance: thermal stress arising from the deposition of decay heat throughout the pellet and electric stress caused by the charging of the pellet due to the escape of β particles from its surface. For a pellet of radius a , the thermal stress varies as a^2 and the electric stress as a^{-2} , which leads to both upper and lower size limits at radii where the effective stress approaches the yield stress of solid hydrogen. Fortunately the range of pellet radii envisaged in current reactor concepts (10^{-2} – 10^{-4} m) falls mid-way between the two limits.

The heat produced by the β decay of tritium in an equimolar DT mixture is 1.0 W mol^{-1} . In a thermally insulated sample this would cause a uniform temperature rise, but in pellets prepared by the techniques mentioned and injected into a cold vacuum chamber, there will be radial temperature gradients caused by

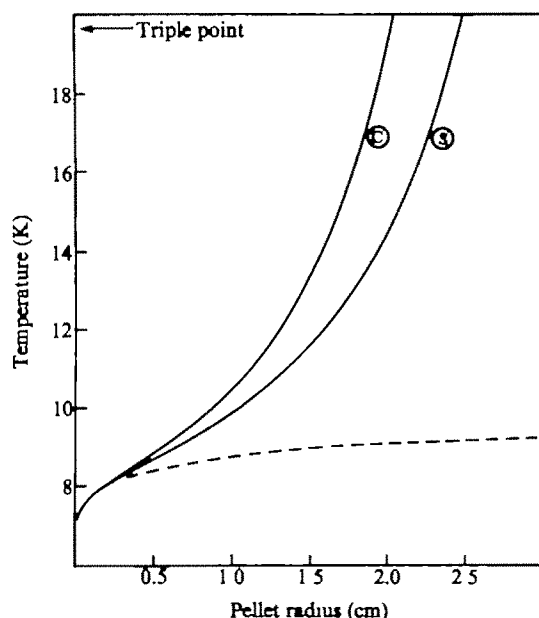


Fig. 1 Variation of equilibrium temperatures with pellet size. The solid curves are the central temperatures for cylindrical (C) and spherical (S) pellets. The dashed curve is the surface temperature. The triple point temperature of DT is 19.8 K.

outward heat flow. This can happen during extrusion by conduction through the nozzle; in isolated pellets evaporation establishes an equilibrium surface temperature which increases with pellet radius. By equating evaporative loss with decay heat we find this to be 7.0–8.7 K for the size range quoted above; radiative heat transfer is negligible in these conditions. To arrive at the equilibrium radial temperature distribution we assume the pellets to have constant surface temperature T_s and uniform heat generation A_0 , and a thermal conductivity K which varies with temperature. This leads to a parabolic distribution of the form:

$$\int_{T_s}^{T_c} K dT = \frac{A_0}{C}(a^2 - r^2)$$

where the numerical factor C is 4 for a cylinder and 6 for a sphere⁴.

The thermal conductivity of solid DT is unknown, but it has been suggested that when freshly frozen it may behave like 72% para H_2 (ref. 5). The variation of central temperature with pellet size shown in Fig. 1 is calculated on this assumption, and suggests that pellets smaller than about 2 cm radius are unlikely to have molten centres.

The thermal stress at the surface of a pellet whose temperature is a function only of radius is proportional to the mean temperature minus the surface temperature⁶, that is

$$(\sigma)_s = \frac{\alpha E}{(1-\nu)}\{\bar{T} - T_s\}$$

where α is the linear expansion coefficient, E is Young's modulus and ν is Poisson's ratio. By averaging the parabolic temperature distribution we find the equilibrium surface stress to be:

$$(\sigma)_s = \frac{\alpha E}{(1-\nu)} \cdot \frac{A_0 a^2}{DK}$$

where the numerical factor D is 8 for a cylinder and 15 for a sphere. The growth rate of this stress is a function of the dimensionless quantity $\kappa t/a^2$, where κ is the thermal diffusivity; the time t to reach 75% of the equilibrium stress is $0.25a^2/\kappa$ for a cylinder and $0.16a^2/\kappa$ for a sphere⁴.

There are no published experimental data on the mechanical or thermal properties of DT, so to estimate the surface stress and

Table 1 Data for thermal stress calculation

Quantity	Unit	$T = 5$ K	$T = 10$ K	Material	Ref.
B_s	$\text{mol m}^{-3} \text{K}^{-3}$	0.26	0.26	DT	5
H	W mol^{-1}	1.0	1.0	DT	
αA_0	$\text{W m}^{-3} \text{K}^{-1}$	6.6	26	DT	
K	$\text{W m}^{-1} \text{K}^{-1}$	0.2	0.8	nH_2	5
$(1-\nu)$		0.7	0.7	nD_2	7
E	MN m^{-2}	460	420	D_2	8
κ	$\text{cm}^2 \text{s}^{-1}$	0.14	0.083	nD_2	9
Stress					
cylinder	GN m^{-2}	$2.7a^2$	$2.4a^2$		
sphere	GN m^{-2}	$1.4a^2$	$1.3a^2$		
(t)_{75%}					
cylinder	10^4 s	$1.8a^2$	$3.0a^2$		
sphere	10^4 s	$1.2a^2$	$1.9a^2$		

To evaluate the stress we note that the solid density of hydrogen closely obeys the empirical relationship⁵ $(\rho_s)_T = (\rho_s)_0 - B_s T^3 \text{ mol m}^{-3}$, whence $\alpha = B_s T^2 / \rho_s$. As $A_0 = H\rho_s$, we have $\alpha A_0 = HB_s T^2$.

its growth rate we have used a judicious mixture of measured data for other solid combinations of the hydrogen isotopes together with some predictions for DT based on them. The values used to plot the graphs in Fig. 2 are given with their sources in Table 1.

Electrical stress on isolated pellets arises from charging due to the escape of β particles born sufficiently near the surface and having outwardly directed velocities. The electrostatic energy of a sphere with a surface charge Q is $\mathcal{E} = Q^2/8\pi\epsilon_0 a$ and hence the outward electrostatic pressure at the surface is

$$P = -\frac{d\mathcal{E}}{dv} = \frac{Q^2}{32\pi^2\epsilon_0 a^4}$$

If its radius a is small enough, a pellet will charge up to a value Q_{crit} beyond which disruption occurs; we suggest that the critical pressure at this point can be identified with the yield stress. It is

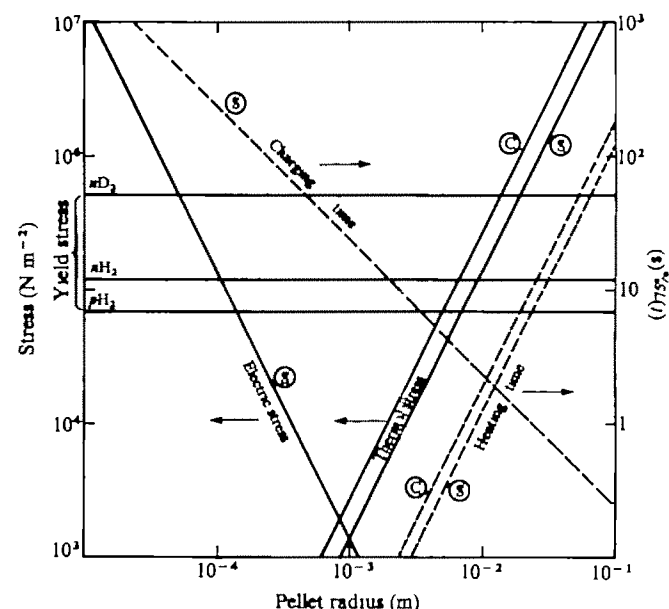


Fig. 2 Stress in DT pellets. The solid sloping lines show the steady state electrical and thermal stresses. The broken lines show the times to reach 75% of these stresses. (C) refers to cylindrical pellets, (S) to spherical. The pellet surface temperature is 5 K. The horizontal lines are measured yield stresses for polycrystalline hydrogen.

analogous to the Rayleigh stability criterion for charged liquid droplets¹⁰, which can be written:

$$\gamma \geq Q^2/64\pi^2\epsilon_0 a^3$$

where γ is the surface tension. If pellets behave like droplets, we may expect further charging to expel from the surface small fragments carrying sufficient charge to restore the stability criterion $Q \leq Q_{\text{crit}}$. For sufficiently large radii Q_{crit} will not be reached, as it would imply a potential greater than that corresponding to the maximum β energy ($V_{\text{max}} = 18$ keV); such pellets will be electrostatically stable.

The rate of charging is $dQ/dt = e(dn/dt)$, where dn/dt is the electron loss rate from the surface. It has the initial value:

$$\left(\frac{dn}{dt}\right)_0 = 4\pi a^2 RS$$

where R is the thickness of a hypothetical layer from which all β particles are thought to escape and S is the activity. As the potential of pellets of all radii rises towards V_{max} asymptotically, it is convenient to make quantitative assessments in terms of their potential V . Using the relationship $Q = 4\pi\epsilon_0 a V$, we have:

$$\left(\frac{dV}{dt}\right)_0 = eaRS/\epsilon_0$$

which we evaluate by taking R to be approximately the half-range for self absorption in tritium, quoted by Evans¹² as 0.039 mg cm^{-2} ; the value of S for an equimolar DT mixture is $2.13 \times 10^{17} \text{ decays kg}^{-1} \text{ s}^{-1}$. This gives:

$$\left(\frac{dV}{dt}\right)_0 = 1.5 \times 10^6 \text{ a V s}^{-1}$$

The time taken to charge to a given fraction of V_{max} will depend on the β energy spectrum, but we consider it sufficiently accurate for these estimates to assume it to be flat, so that:

$$V = V_{\text{max}} \left\{ 1 - \exp \left[- \left(\frac{dV}{dt} \right)_0 t / V_{\text{max}} \right] \right\}$$

Figure 2 shows the equilibrium electric stress,

$$P_{\text{max}} = \epsilon_0 V_{\text{max}}^2 / 2a^2 = 1.4 \times 10^{-3} / a^2 \text{ N m}^{-2}$$

and the charging time to 75% of P_{max} ,

$$(t)_{75\%} = 2.4 \times 10^{-2} / a \text{ s}$$

to facilitate direct comparison with the thermal stress.

In assessing our results the first point is that the total stress is a minimum for pellets of ~ 1 mm radius. This is a convenient size for fusion reactor concepts¹³; a total injection rate of 100 pellets per second would give a gross output of about 4,000 MW(th) at 10% burn-up per pass.

In the absence of data on the strength of solid DT, the measured yield stresses for polycrystalline H_2 and D_2 ⁸ are shown in Fig. 2. Using these figures as a guide, we see that pellets smaller than 10^{-2} m radius are unlikely to suffer early disruption due to thermal stress. Larger pellets may need to be fabricated and used within a few seconds, otherwise they may be prone to surface cracking and central melting, both of which could increase their ablation rate on entering a hot plasma. Electric stress seems to be of little consequence unless pellets are smaller than 10^{-4} m radius, where the slow charging rate will diminish its practical importance.

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Mercury(II) sulphide: a photo-stable semiconductor

THERE is much interest in photo-electrochemical cells which use a semiconductor-electrolyte junction to transduce light into electrical and chemical energy¹. The semiconductors investigated to date fall into two main groups, the first of which contains photostable material but which shows response in the UV rather than the visible region of the spectrum, for example, n-titanium dioxide², n-strontium titanate³, n-potassium tantalate⁴, n-tin oxide⁵ and n-barium titanate⁶. The second group comprises compounds which respond to the visible region of the spectrum but are photochemically unstable such as n-gallium phosphide⁷, n-indium phosphide⁸, n-cadmium sulphide⁹, and n-cadmium selenide¹⁰. Attempts have been made to extend the spectral response of the materials belonging to the first group, by doping¹¹ and chemically affixing dyes to the surface of the semiconductor¹². Some success has been attained in stabilising some of the members of the second group; for example, cadmium sulphide and selenide are stable in polychalcogenide solutions¹³. If cells containing a semiconductor-electrolyte junction are to be of any practical use in the harnessing of solar energy then the following criteria for the semiconductor should be met: (1) they should absorb visible radiation; and (2) be photostable and capable of use in powder form. Clearly, if the conduction and valence bands of the semiconductor have energies that facilitate proton reduction and oxidation of hydroxyl ions respectively this would be an added bonus as the cells could be used to either photo-assist or photo-electrolyse the water¹⁴. We report here on mercury(II) sulphide (cinnabar) band gap = 2.1 eV¹⁵ which fulfils most of the above criteria¹⁶.

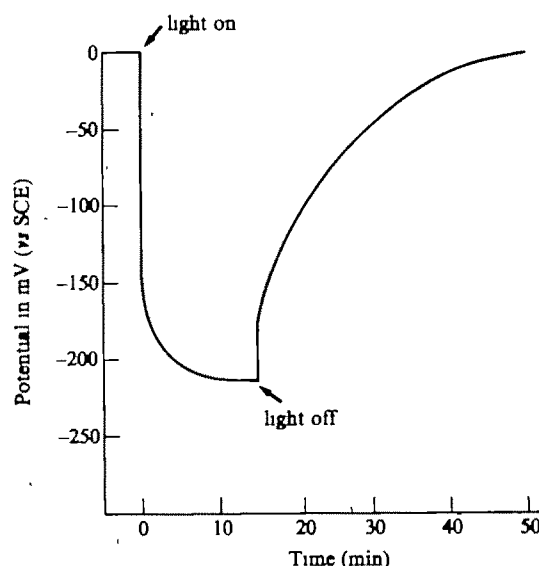


Fig. 1 Plot of potential versus time for red HgS in pH 7 (unbuffered) 0.1 M NaNO_3 , with respect to an SCE.

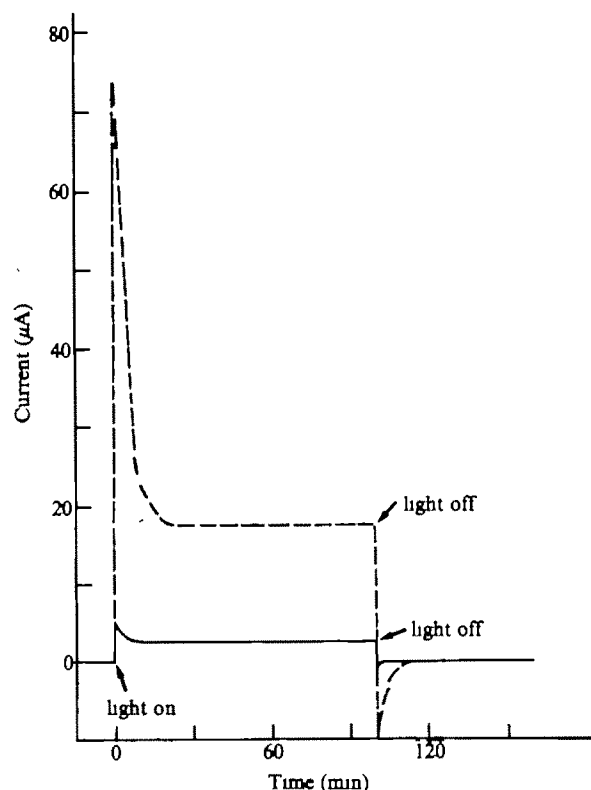
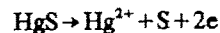


Fig. 2 Plot of current versus time for HgS (held at 0 V versus SCE) in pH 7 (unbuffered) 0.1 M NaNO₃. Solid line, untreated red HgS. Dashed line, red HgS, blackened by irradiation in potassium iodide. Effective area of electrode irradiated is 2.25 cm² and light intensity is $\sim 2.5 \times 10^{-2}$ W cm⁻².

The electrodes consist of a platinum mesh coated with cinnabar (optoacoustic spectra show that the samples absorb little radiation above 620 nm and presumably therefore, contain very little *meta*-cinnabar). The coating is carried out by dipping the mesh into a suspension of cinnabar produced by boiling a deoxygenated aqueous solution of the powder followed treatment with an ultra-sonic probe. The mesh is removed from the suspension and then dried by hot air. To obtain reproducible results the electrode is stored in an oxygen-free unbuffered 0.1 M sodium nitrate solution (pH 7) for at least 15 h. When such an electrode is placed in an electrolyte solution such as an unbuffered 0.1 M sodium nitrate solution (pH 7) and is connected to a voltmeter (impedance 10^7 – $10^8 \Omega$) with an SCE as reference, illumination of the electrode generates a photo-e.m.f. The light source is a 1.8 kW xenon lamp and the light is filtered by passing it through a 0.28 M copper(II) chloride solution maintained at 5°C. This filter solution has maximal transparency at 515 nm and has a half-band width of 90 nm. The direction of the e.m.f. and plot of the photo e.m.f. versus time (Fig. 1) are all typical of the behaviour of an n-type semiconductor. The photo-e.m.f. is somewhat dependent on the history of the sample but usually falls in the range of -150 to -300 mV. When the cinnabar electrode is connected to a platinum counter electrode and a potential of 0 V maintained between the two by means of a potentiostat and a SCE reference, illumination causes a current to flow (Fig. 2). As expected, the photocurrent increases as the anodic bias is increased. From a determination of the biasing voltage at which zero current flows, the flat band potential of these electrodes was determined as ~ -0.25 V versus SCE at pH 7. This value varies with pH according to the Nernst equation (that is, ~ -60 mV per pH unit). When inert electrolytes such as sodium nitrate, are used, irradiation of such electrodes or suspension of mercury(II) sulphide does not lead to any solubilisation of mercury (as detected by conventional atomic absorption spectroscopy where the lowest reliable

detection of mercury is $7.5 \mu\text{g ml}^{-1}$). We conclude therefore, that the photocurrents are photoelectrolytic currents.

Anodic oxidation could occur on illumination:



One mercury atom should be solubilised per two electrons passed anodically. Stability of HgS was confirmed by passage of enough positive photocurrent to yield a theoretical mercury concentration of $62 \mu\text{g ml}^{-1}$ in the electrolyte: atomic absorption analysis showed that within the detection limit, no mercury solubilisation by anodic oxidation occurs.

Solubilisation of mercury does, however, occur when the electrolyte contains easily oxidisable ions, such as iodide. With these ions, high currents are produced and the red cinnabar changes colour and becomes brownish-black. Preliminary studies have shown that mercury(II) sulphide undergoes a photochemical reaction with iodide and other reducing ions, but as yet the products have not been identified. However, when the darkened electrodes are removed from the electrolyte, washed well with distilled water and then used in an inert electrolyte, no further solubilisation of mercury occurs. Blackening of electrodes by this procedure enables one to prepare electrodes which give significantly higher currents at zero bias ($\sim +15 \mu\text{A}$) than the untreated cinnabar electrodes (see Fig. 2). A bonus is that the darkening extends the spectral response. The nature of the material responsible for the dark colour is uncertain, although X-ray powder photographs and optoacoustic spectra suggest that some of the colour is due to presence of *meta*-cinnabar.

Electrodes which deliver high currents at zero bias are also effective in photo-assisting the electrolysis of water. Thus at pH 12, using degassed sodium nitrate or chloride as electrolyte and a bias of $> +0.2$ V (versus SCE) leads to gas evolution at the platinum counter electrode and gas bubbles on the semiconductor surface can often also be seen.

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An auroral effect on the fair weather electric field

EVIDENCE is presented here for coupling between the upper and lower atmosphere by electrical means related to aurorae. Electric field data obtained with rocket-launched parachute-borne probes are shown to be consistent with the hypothesis that aurorally-produced radiation can short out vertical electric field 'cells' in the mesosphere, with corresponding effects on the lower atmosphere electric field.

Effects of solar sunspot-related activity on the lower atmosphere electric field occur too frequently to be explained by direct modulation of the 'classical' global electrical circuit¹, which requires large variations in galactic cosmic-ray flux or rare solar cosmic-ray events which penetrate to the lower stratosphere². Available data indicate responses of the lower atmosphere field to much smaller and more frequent solar and geomagnetic disturbances². Israëli¹ reported observations of the direct effects of aurorae on the surface field by early investigators, and more recent observations of such effects, including balloon measurements, are cited in refs 2 and 3.

One mechanism which may explain some such observations involves the existence in the mesosphere of a large vertical electric field, with a total potential of the order of 100 kV.

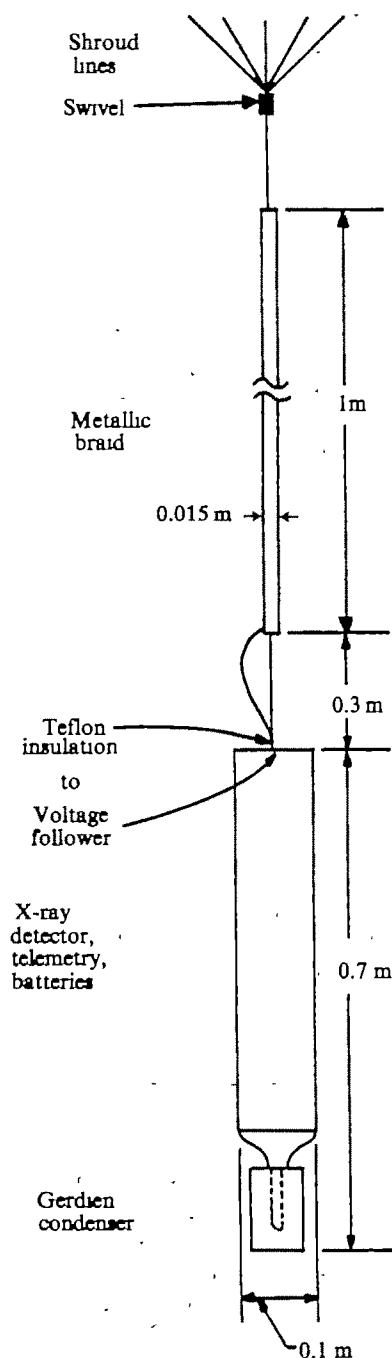


Fig. 1 Electric field antenna configuration.

Where this mesospheric field is shorted by ionisation produced by radiation, then this potential may be added to the lower atmosphere potential (~ 300 kV), producing an increase or decrease depending on the direction of the mesospheric field. Large mesospheric fields were first observed in the USSR⁴. Sceptical of this result, we concluded that such fields should have appeared as base-line shifts in previously obtained conductivity measurements using parachute-borne electrical conductivity probes. On checking old data we found evidence of such base-line shifts in several cases⁵, with the largest electric field effect

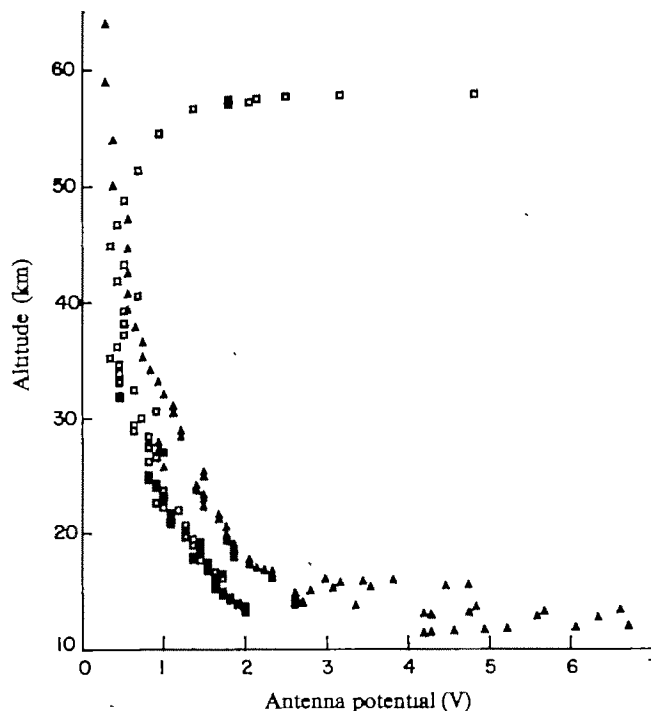


Fig. 2 The variation of antenna potential with altitude: $\square$, quiet, 22 March 1978 06.52 UT; $\triangle$, disturbed, 27 March 1978 10.26 UT.

appearing in a measurement made in Chilca, Peru, near the magnetic equator. (This may indicate that the fields are generated primarily at low latitudes.) In operation Aurorozone I, aimed at measuring the atmospheric effects of aurorae at Poker Flat Research Range near Fairbanks, Alaska, in September 1976, it was observed that a baseline shift indicating a large electric field was present only during quiet conditions, vanishing during disturbed periods of auroral activity (J. D. Mitchell, personal communication), presumably due to enhanced electrical conductivity. This led to the hypothesis that observed lower atmosphere effects were due to the shorting out of a permanent electrical field cell in the mesosphere, with the associated potential appearing in the lower atmosphere circuit⁶. The existence of such large mesospheric fields may be related to aerosol layers, which make the atmospheric ionisation relatively immobile⁷.

To investigate this phenomenon further, electric field measurements were conducted at Poker Flat during operation Aurorozone II in March 1978. The instruments used 1-m metallic antennas above parachute-suspended payloads, as shown in Fig. 1. Teflon insulation was used to connect the antenna to an electrometer follower (Intersil ICH 8500A). The payload section included telemetry, and also X-ray detectors and a Gerdsen condenser for charged particle measurements (provided by other experimenters). This inherently asymmetric system is capable of measuring relative electric fields at night, when photo-emission from surfaces is unimportant.

Figure 2 shows data obtained from two such launches on Super Arcas rockets. The data, in potential difference between

the antenna and payload (accurate to ± 0.3 V), are approximately the same as the downwards electric field in V m^{-1} , the effective length of the dipole being of the order of 1 m. The data show the field during a very quiet period (22 March, 06.25 UT) and during a disturbed condition (27 March, 10.26 UT) which occurred following the onset of a geomagnetically disturbed period when the Poker Flat riometer indicated over 5 dB of enhanced galactic noise absorption, with extensive visible aurora. There was no reported thunderstorm activity or precipitation (rain or snow) in Alaska during these periods (H. Cole, personal communication).

The quiet data indicate the normal fair weather pattern at lower altitudes, but show an enhancement at high altitudes. The rocket performed less well than expected, so that the peak implied by the highest altitude data points was not attained. The disturbed data indicate a small field at high altitudes, but a substantially higher field at lower altitudes, with some evidence of ragged structure. If the ionosphere is nearly equipotential at a higher altitude, say 100 km, then shorting the mesospheric part of the circuit would be expected to produce a correspondingly altered field in the lower atmosphere, as is observed.

This effect could penetrate to a lower latitude as the amount of radiation necessary to short the mesospheric field may be relatively small. Beyond this the effect would decrease towards lower latitudes as determined by mapping of the field. A more complete global description of the electrical response of the atmosphere to geomagnetic disturbances would require that future auroral zone 'campaigns' be supplemented by measurements at various latitudes. Because of the inferred importance of mesospheric fields to the complete global circuit this would require rocket measurements supplemented by balloon and surface measurements. The most reasonable way of acquiring mesospheric data would seem to be using low cost meteorological rockets as are widely used for wind and temperature measurements, utilising electric field and charged particle sensors deployed on parachutes.

Note that the coupling mechanism suggested above is not necessarily the only one of importance. Direct modulation of the lower atmosphere field has been demonstrated in connection with large solar cosmic ray events⁸. Magnetospheric and ionospheric fields have been shown to map to low altitudes⁹. Rapid responses of the surface field to auroral events^{3,10} may be induced by aurorally produced charge separation.

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Note added in proof: Combining Gerdien condenser conductivity data (J. D. Mitchell, personal communication) with the electric field measurements shows that vertical current density increases rapidly above 30 km in both the quiet and disturbed cases. This seems to indicate that currents with density much higher than the 'fair weather' current circulate in the upper stratosphere and mesosphere, which may explain the nature of the effect described above.

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Geomagnetic activity and the high latitude magnetic field of the Sun

SMITH *et al.*¹ using Pioneer 11 in 1976 did not observe any sector structure at the heliographic latitude of about $+16^\circ$. At this latitude the field polarity was consistently directed away from the Sun. Its direction was in accord with the direction of the high-latitude magnetic field near the north pole of the Sun. This result, as well as indirect data²⁻⁴, shows that the high latitude solar field extends far into interplanetary space. The evidence for this is shown by the analysis of the yearly *aa* indices^{5,6} between 1868 and 1976. We find intensification of geomagnetic disturbances during those 11 yr periods when the extended solar magnetic field polarity in the northern hemisphere is away from the Sun.

From measurements by the Mount Wilson^{7,8}, Cambridge⁹ and Crimea¹⁰ magnetographs one finds that the general magnetic field of the Sun varies with a period of ~ 22 yr. Then assuming the first year of the 11-yr cycle and the even numbered years as the beginning of each oscillation and taking the 22-yr Hall cycle period as the unit, one finds that the high latitude field of the Sun is maximum near the phase 0.5. During this interval the field vector is in the same direction as the vector of the angular velocity of the solar rotation, that is, the magnetic field polarity is away from the photosphere in the northern hemisphere. During the interval near phase 1.0, the absolute field value attains a maximum again but its polarity near the poles reverses. The high latitude field approaches zero and reverses near the 0.25 and 0.75 phases, in the neighbourhood of the 11-year cycle maxima.

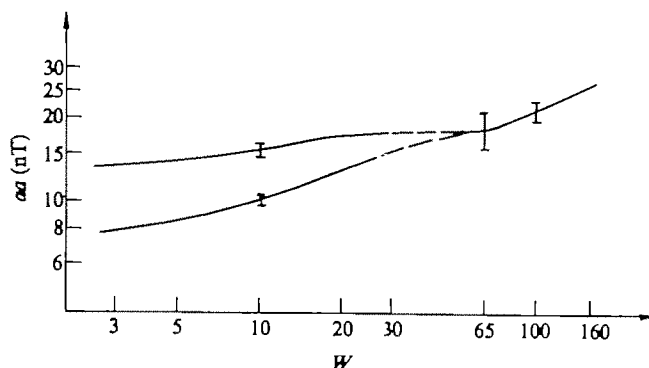


Fig. 1 The relationship of the yearly *aa* indices of geomagnetic activity with Wolf number (*W*). The lines for $W < 27$ are the approximations from the text, for $W > 60$ the *aa* values are calculated for intervals of *W*. The errors bars are shown.

The Earth migrates at 7°S – 7°N heliographic latitude throughout the year. So the Earth crosses the current sheet, which is almost parallel to the solar equatorial plane and separates the two hemispheres of opposed field polarity. Taking into account Earth's migration and the time variation of the inclination of the geomagnetic dipole, as derived in ref. 11, we find that the projection B_s of the interplanetary magnetic field (IMF) in the direction of the geomagnetic dipole is large enough to have some geomagnetic effect. Near phase 0.5 this component B_s is parallel to the geomagnetic dipole (antiparallel to the geomagnetic field at the magnetopause) and one may expect a reconnection¹² of the IMF and geomagnetic field. This phenomenon increases near phase 0.5 compared with phase 1.0. This must be followed by intensification of geomagnetic activity near the ends of the even cycles in comparison with ends of the odd cycles.

The variation of geomagnetic activity near solar cycle minima has been described elsewhere¹³. The geomagnetic activity index¹⁴ M used for the interval 1884–1967 was based on a regression of M with the Curie indices, and is not homogeneous throughout the whole hundred year interval. Therefore quantitatively comparing the mean M values in periods with different levels of solar activity is not reliable. The difference in mean Curie values in even and odd solar cycles has also been noted by Russell¹⁵.

The aa index introduced by Mayaud^{5,6} is a better index for long-term studies. Mayaud defined the aa index as the average of 3-h K indices converted to the amplitude of the field at two antipodal observatories. The Greenwich and Melbourne Observatories are roughly antipodal and have been gathering data since 1868. Comparing the aa indices with the planetary a_m indices shows¹⁶ that the correlation coefficient between the mean monthly and the mean annual values attains 0.994 and 0.998, respectively. Thus the aa index obtained from the magnetograms of two antipodal observatories gives a good picture of the mean monthly planetary geomagnetic activity.

For years of low solar activity ($W < 27$), the comparison of mean annual aa indices with Zürich Wolf number (W) for 1868–1975 (refs 17, 18) yielded the following linear approximations:

$$aa = (0.31 \pm 0.08)W + (6.98 \pm 1.12) \text{ nT}$$

$$aa = (0.25 \pm 0.11)W + (12.7 \pm 1.11) \text{ nT}$$

for the ends of the 11-yr cycles with odd and even numbers, respectively. The correlation coefficients between aa and W are 0.88 ± 0.06 and 0.42 ± 0.25 for odd and even minima respectively. So, the geomagnetic activity can be seen to be higher near phase 0.5 than near phase 1.0. When the Wolf Numbers vanishes ($W \rightarrow 0$) the aa index for the even cycles remains almost double that for the odd cycles. The dependence of aa on W for the sunspot minimum, together with the aa index averaged over intervals of W at sunspot maxima are shown in Fig. 1.

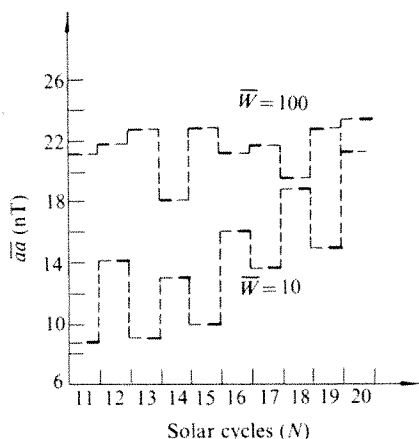


Fig. 2 The variations of the $\overline{aa}$ indices for the maxima ($\bar{W} = 100$) and the ends of the solar 11-yr cycles ($\bar{W} = 10$).

For the years near the extrema of 11-yr cycles the averaged aa and $\bar{W}$ value are used. For maxima we used the data of the three years interval, the year of maximum W and two neighbouring years, in the cycles nos 15 and 16 the fourth year with a large W was also added. For the minima data with average annual values of $W < 27$ and $aa < 20$ were used. The year 1976 is not suitable for this criterion but as it was the minimum of the solar cycle it was taken as being characteristic of the unusual cycle no. 20. For the end of cycle no. 15 we did not use the 1921 and 1922 data as

W (for 1921) and aa (for 1922) were close to the chosen limiting line. The insertion of these years gives $\overline{aa} = 14.0$ and $\bar{W} = 15.7$ for the 1921–1924 interval. The reduction to $\bar{W} = 10$ gives $\overline{aa} = 12.2$, but that does not change the relationship between geomagnetic disturbance and even polarity of a cycle as shown in Fig. 2.

Table 1 Variation of Wolf numbers with annual aa indices

No. of solar cycle (N)	Maxima			Minima		
	Years	$\overline{aa}$	$\bar{W}$	Years	$\overline{aa}$	$\bar{W}$
11	1870–1872	22.5	117.3	1875–1879	8.8	10.0
12	1882–1884	18.3	62.3	1887–1890	13.8	8.3
13	1892–1894	20.7	78.6	1897–1903	10.6	15.2
14	1905–1907	14.6	59.8	1910–1914	12.4	7.8
15	1916–1919	20.6	76.3	1923–1924	10.2	11.2
16	1926–1929	18.4	68.9	1931–1934	16.4	11.7
17	1937–1939	21.9	104.3	1944–1945	17.0	21.3
18	1947–1949	23.0	141.0	1954	17.3	4.4
19	1957–1959	29.4	177.9	1964–1965	15.6	12.6
20	1968–1970	21.6	105.4	1976	23.8	12.6

The $\overline{aa}$ and $\bar{W}$ values for the cycles from no. 11 to no. 20 are given in Table 1. This shows at sunspot minima, despite the differences in $\bar{W}$, the $\overline{aa}$ indices are greater at the ends of the even cycles than at the ends of the neighbouring odd cycles. At sunspot maxima one can see only the 90-yr variations of $\bar{W}$ with essentially no differences between even and odd cycles.

Using the relationships between aa and W (Fig. 1) the aa indices were reduced to values for $\bar{W} = 10$ and $\bar{W} = 100$, at the minima and maxima of solar cycles respectively. These reduced aa values are shown in Fig. 2. The large differences in aa values seen at sunspot minima in passing from odd to even cycles are absent for sunspot maxima. The difference between the geomagnetic activity during the even and odd minima for cycles 11–20 is given by the ratio $\overline{aa}_{2k}/\overline{aa}_{2k-1} = 1.53 \pm 0.05$ (where $N = 2k$ and $2k - 1 = \text{No. of the cycle}$).

The 11-yr and 22-yr mean values of the aa index are given by Russell¹⁹ for the period 1872–1967. However, he used mean values of the cycles, and did not normalise for the difference in the levels of solar activity as measured by sunspot number. Therefore an enhancement of the aa index at the ends of even cycles as compared with the odd cycles was not confirmed.

Our investigation shows evidence of the existence of an extended solar magnetic field and its variation within a 22-yr period. The physics of this phenomenon is as follows. The temperature (and the density) of the solar corona is lower at high latitude near the sunspot minima. The weakened solar wind in this region does not affect the magnetic field. Thus the magnetic field in the vicinity of the Sun must be nearly a dipole, particularly at high latitudes. This is consistent with the structure of a sunspot minimum solar corona. Near the solar equatorial plane the kinetic and thermal energies become more than the energy of dipole magnetic field, that is $\beta = (1/2\rho U^2 + 3N_e kT)/B^2/8\pi \approx 1$ at a distance of $9R_\odot$. Consequently, the solar wind attracts the field lines near the solar equator plane at a distance of $\sim 9R_\odot$. Thus the field has one polarity in the hemisphere north of the solar equatorial plane and the opposite polarity in the hemisphere south of the equatorial plane. Assuming that the total flux for the entire polar region reaches 3×10^{21} Mx and that the magnetic field B is proportional to r^{-3} for $r < 9R_\odot$ and B is proportional to r^{-2} for $r > 9R_\odot$, we obtain $B \sim 1$ nT near the Earth's orbit.

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A radiocarbon date from Lake Bogoria, Kenya Rift Valley

RADIOMETRIC dates from faunal remains deposited in lacustrine and fluvio-lacustrine sediments at numerous locations in and around the lakes of the Kenya Rift Valley confirm a period of extended lake development related to a wetter climatic phase ~10,000 yr ago¹⁻⁷. Thereafter, a drier climatic regime has prevailed and the lakes have contracted, although fluctuating in rapid response to sequences of wetter or drier years. Lake Bogoria (Hannington) is the last of the Kenya Rift Valley lakes to yield a radiocarbon date for this early Holocene high stand. Although a general synchronism of maximal lake development has emerged, as we report here, there is considerable variation in the dates obtained from each lake basin (Fig. 1).

Saline-alkaline Lake Bogoria lies in a fault-controlled trough just north of the Equator⁸⁻⁹, extends north-south for 17 km, varies in width from 500 to 3,500 m (Fig. 2), and has a maximum depth of ~9 m¹⁰. It is sharply delimited by alkaline lavas of Miocene to Pleistocene age⁸⁻⁹, except on its northern margin, where the 1-10 km wide, swamp-covered, Lobo Plain leads for 20 km north across a low watershed to fresh water Lake Baringo. Short ephemeral streams and the semi-permanent Sandai River drain into Lake Bogoria, which also receives water from three groups of hot springs and small geysers. Lake Bogoria has no proven outlet, although underground seepage to the north is likely⁸. Fluvio-lacustrine sediments fringe the lake margins, but are seldom more than a few tens of metres wide except at the northern end. A grey, stromatolitic limestone that coats *in situ* bedrock and clastic sediments up to ~11 m above present lake level, and fossiliferous silts, are the principal evidence for a formerly more extended lake. Excepting algae, no organic life is known to exist in Lake Bogoria today, although crocodiles and hippopotami were photographed swimming in

the lake in 1927 (ref. 11). Mollusc shells, crocodile, hippopotamus and fish bones, and diatoms in fluvio-lacustrine sediments up to 5 m above present lake level, indicate a formerly richer fauna/flora and suggest a fresher water environment. Today the pH of Lake Bogoria exceeds 10.8 and the Na⁺ concentration reaches 28,400 p.p.m.^{8,12}.

A 250 g sample of *Melanoides tuberculata* was collected from the lower part of a 2 m thick fluvio-lacustrine unit of feldspathic silts with pebble bands, the base of which varies from 2.95 to 3.10 m above the July-August 1976 level of Lake Bogoria (Fig. 2). The state of preservation of the shells was good and they were believed to be *in situ* rather than derived. After dissolving and discarding the outer layers of the shells (~20%), dates of 10,560 ± 170 yr BP and 10,320 ± 150 yr BP (Birm-883) were obtained for the inner and middle fractions respectively. In each case the final figure is a conventional radiocarbon date, calculated using a half life of 5,570 ± 30 yr and has been normalised to account for isotopic fractionation effects with respect to a -25‰ enrichment on the PDB limestone scale. The two results are scarcely significantly different and there is therefore little likelihood of isotopic replacement having taken place after deposition (R. E. G. Williams, personal communication).

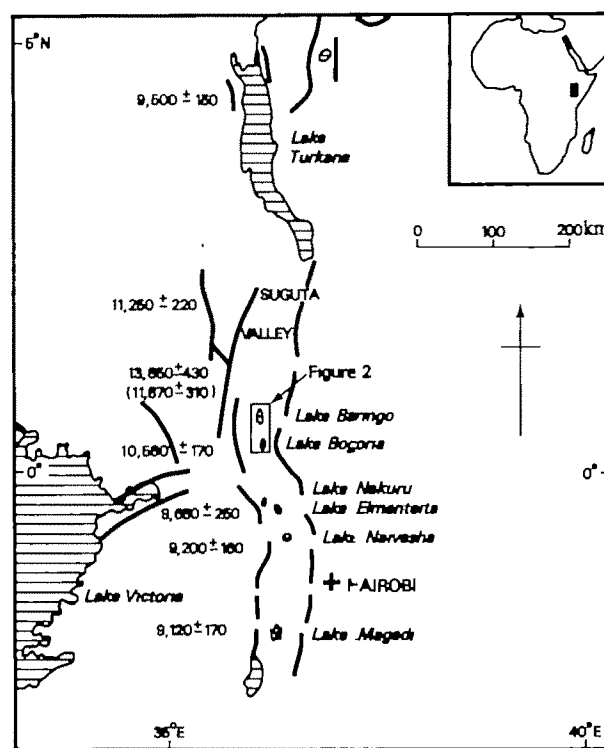


Fig. 1 Lakes of the Kenya Rift Valley. Radiocarbon dates (yr BP) of maximum Holocene extension of the lakes are indicated to the west (left) of the individual lakes or locations.

The possibility of a greater Lake Baringo that may at times have included Lake Bogoria has been discussed frequently^{8-9,13-17}. Fluvio-lacustrine Lobo Silts, which form the bulk of the sedimentary mass of the Lobo Plain have traditionally been considered of Holocene age⁸. Bogoria Silts, which are deposits of a later transgression, have recently been recognised overlying Lobo Silts in the northern Bogoria basin¹⁷. They may correlate with the early Holocene Eokwob Formation^{16,18,19} of the northern Baringo Basin, thus making the Lobo Silts of late Pleistocene age¹⁷. Whichever interpretation is correct, it has been unclear whether only one early Holocene lake existed ('Kobwob Lake') or whether the two lakes rose and fell independently of each other^{9,13,16,17}.

Radiocarbon dates for samples of *M. tuberculata* collected from a series of strandlines up to 15.85 m above the 1973 level

of Lake Baringo provide evidence of a more extended lake between 10,000 and 14,000 yr BP (Birm-541, 542, 544, 545) (Fig. 2). Shells from the highest strandline on the western margin of Lake Baringo yielded dates of $13,670 \pm 320$ yr BP (inner fraction) and $13,850 \pm 430$ yr BP (middle fraction) (Birm-542). In contrast, dates of $11,870 \pm 310$ yr BP (inner fraction) and $10,860 \pm 280$ yr BP (middle fraction) were obtained for shells from a strandline at the same height (W. W. Bishop, personal communication) immediately north of the Bogoria/Baringo watershed (Birm-541). There is no evidence of Holocene faulting in the area; and unless a lake which attained the same

maximum at different times or fluctuated around that maximum for $\sim 2,000$ yr is proposed, one of the dates seems to be spurious. The Birm-544 and 545 dates were obtained for shell samples from lacustrine sediments in lower locations, 15.54 m and 10.06 m respectively above the level of Lake Baringo.

The Birmingham dates for the Baringo and Bogoria basins are generally $\sim 1,000$ yr older than those obtained for the other Kenya Rift Valley lakes (Fig. 1). Isotopic replacement seems to have occurred in the samples from Lake Baringo, resulting in discrepancies between middle and inner shell fractions (W. W. Bishop, personal communication), and in both basins, there is the possibility of hard water effects due to ancient CO_2 derived from hot spring waters and some of the lake water bicarbonate could have been derived from alkaline lavas.

Complete correlation between the two lakes remains impossible because of a lack of accurate inter-basin altimetric evidence and conflict in the presently available heights of key locations in the two basins. The most recently available heights of the surface of Lake Bogoria and Lake Baringo are 989.5 m (ref. 10) and 967.0 m (ref. 13) respectively. Both lakes are subject to seasonal and longer-term fluctuations of several metres. The height of the relatively inaccessible watershed between the lakes is of critical importance and we suggest that it may lie at ~ 10 m above the 1976 level of Lake Bogoria, which is intermediate to two other recent estimates^{13,17}. Holocene tectonic activity is not considered likely although a northward tilt of the basin floor may have occurred^{6,15}. Whilst not resolving the problem of the existence of a single combined Kokwob Lake, the new date suggests high stands of both lakes at a period $\sim 10,500$ yr BP and makes a single large lake, or an extension of the intervening swamp a considered possibility. The relationship of the $+11$ m limestone to the early Holocene lake levels remains uncertain.

The range of dates for maximum extension of the lakes in the Kenya Rift Valley may reflect differences in the topographical and hydrological properties of each basin, as well as difficulties in comparing directly radiocarbon dates from different laboratories. In addition, the approximately linear north to south decrease in the dates (Fig. 1) may be partly explained by early Holocene regional climatic variations between 2°N and 2°S .

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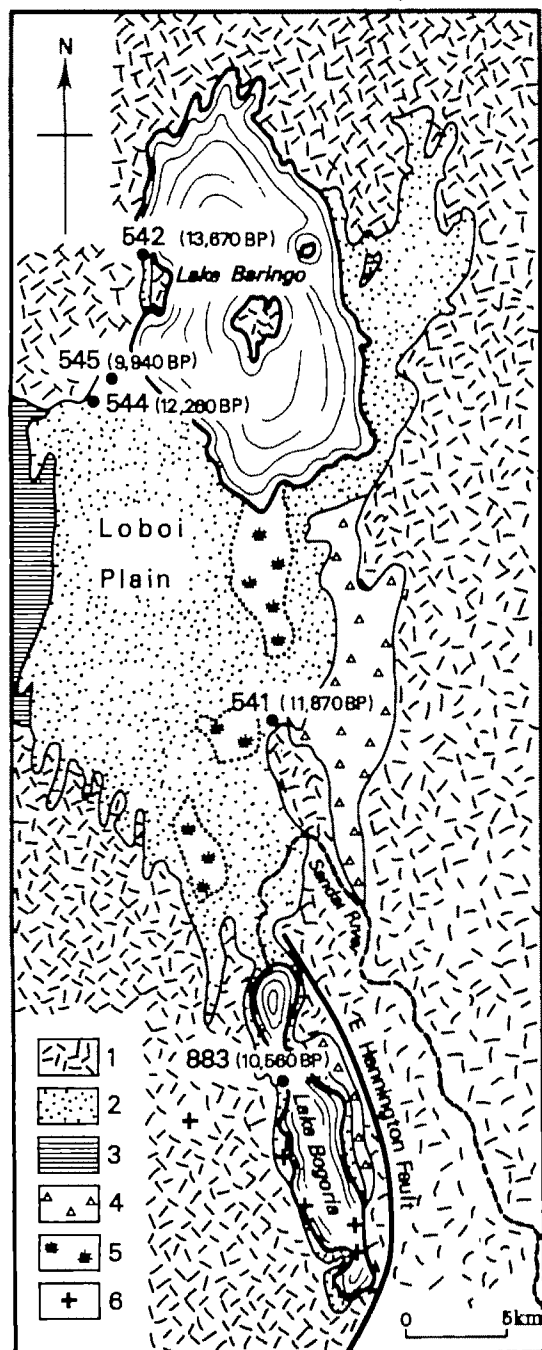


Fig. 2 Simplified geological setting of the Baringo-Bogoria basin 1, Miocene-Pleistocene lava uplands; 2, fluvio-lacustrine sediments; 3, silts and gravels of Pleistocene Kapthurin Formation; 4, fanglomerate; 5, swamp; 6, hot springs. Radiocarbon dated localities are indicated by their (Birm-) numerical prefix and the dates for the inner shell fraction are shown in parentheses.

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Phytoplankton growth and cyclonic eddies

AROUND the British Isles thermal fronts¹ develop during the summer months between cold, well mixed waters that are relatively rich in inorganic nutrients and warm, well stratified waters that are nutrient depleted². The surface standing crop of phytoplankton is generally high in the marginally stratified frontal zone, particularly where the water is deep (for example, off Ushant and around Orkney and Shetland)², although these surface waters are generally low in inorganic nutrients. To

Table 1 Surface (2m) hydrographic data and phytoplankton cell counts for stations representative of stratified, frontal and mixed waters (the station positions are shown in Figs 1 and 4a)

Station	Stratified W	Frontal X	Mixed Y	Mixed Z
Temperature (°C)	16.42	14.53	13.02	12.82
Salinity (‰)	35.22	35.25	35.26	35.26
Nitrate ($\mu\text{g at } 1^{-1}\text{-N}$)	1.4	1.7	1.9	3.8
Silicate ($\mu\text{g at } 1^{-1}\text{-Si}$)	1.3	1.3	1.6	1.8
Chlorophyll <i>a</i> (mg m^{-3})	0.55	2.25	7.22	0.81
Phaeopigment (mg m^{-3})	0.11	0.13	0.33	0.07
Diatoms (cells ml^{-1})				
<i>Chaetoceros</i> spp.*	+	164	23	59
<i>Nitzschia</i> spp.†	8	51	16	63
<i>Rhizosolenia delicatula</i> Cleve	0	8	3	10
<i>Thalassionema nitzschioides</i> Hust.	0	6	1	1
Dinoflagellates (cells ml^{-1})				
<i>Gyrodinium aureolum</i> Hulburt	0	6	169	5
<i>Prorocentrum micans</i> Ehrenb.	3	13	5	1
<i>Scrippsiella trochoidea</i> (Stein)	+	8	8	+
Loeblich III				

* Mainly *C. danicum* Cleve and *C. lacinosum* Schütt.

† Mainly *N. delicatissima* Cleve and *N. seriata* Cleve.

understand the growth of these frontal blooms, therefore, the scales³ of non-tidal motion that determine the exchange of chemical and biological properties in frontal regions need to be identified. Although the frontal boundaries are almost in geostrophic balance, they are unstable and are characterised by cyclonic eddies with a spatial extent of 20–40 km which can persist for a few days⁴. These eddies probably have an important influence on phytoplankton growth through the transfer of nutrients as well as phytoplankton across the frontal zone. Furthermore, as the division time of the plant cells is relatively short (~1 d), growth of the population may actually occur in the eddy transfer process. In this letter a series of observations on the Ushant frontal system, planned to investigate this problem, are described.

An infrared satellite image (Fig. 1), taken on 14 July 1978, shows a series of eddies in the frontal region between the cold, well mixed waters close to the coast of France and the warm, well stratified waters of the Celtic Sea [such baroclinic eddies are comparable with the well-known atmospheric cyclones that occur in the westerlies on a one-hundred times larger scale]. The area defined by the inset was chosen for detailed study (Fig. 2) after a preliminary hydrographic survey (13–14 July) had indicated that eddy formation was most likely in this region and, accordingly, a current meter mooring was laid at station 038.

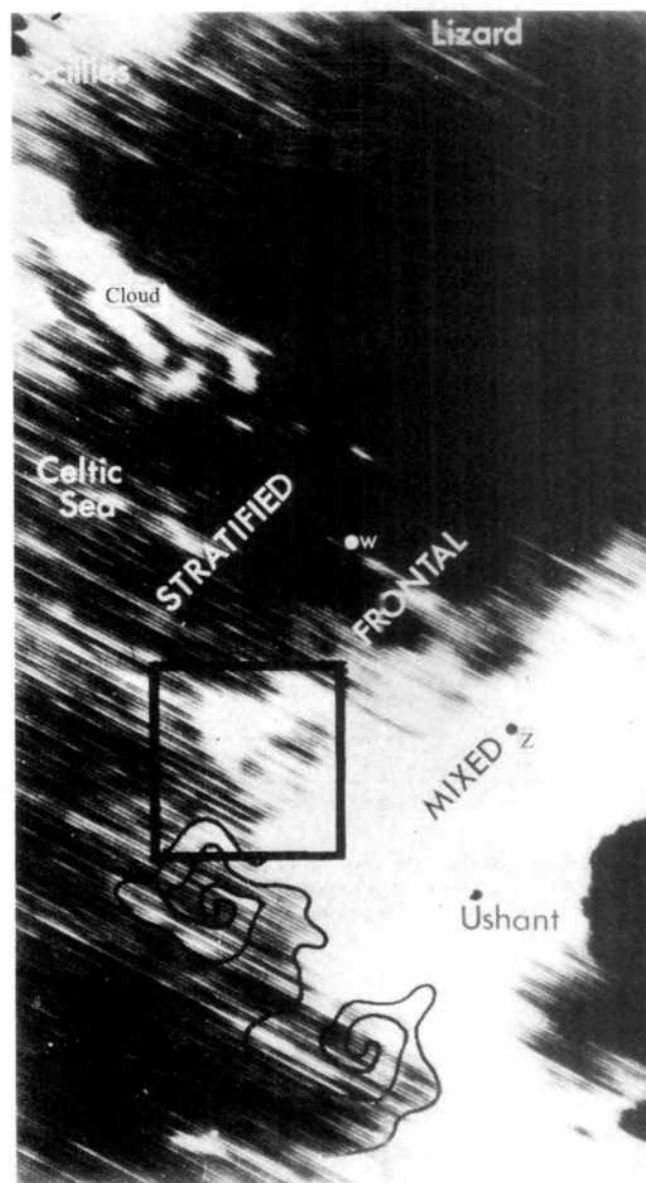


Fig. 1 Infrared satellite image of the southwestern approaches to the English Channel, taken at 10.40 GMT on 14 July 1978. Mooring 038 was located at 48°42' 75" N, 05°43' 85" W (see Fig. 2a). The inset outlines the area shown in Figs 2 and 4, and stations W and Z are referred to in Table 1.

These current records did not reveal eddy motions (Fig. 3a) but allow tidal corrections to be made to plots of the ship's track.

The surface distributions of temperature, salinity and chlorophyll *a* all reflect the cyclonic eddy structure seen in the infrared image (Fig. 2) and, as expected, the temperature profiles (Fig. 3b) showed that the cold arm of the eddy was less stratified than the adjacent warmer water. This detailed survey

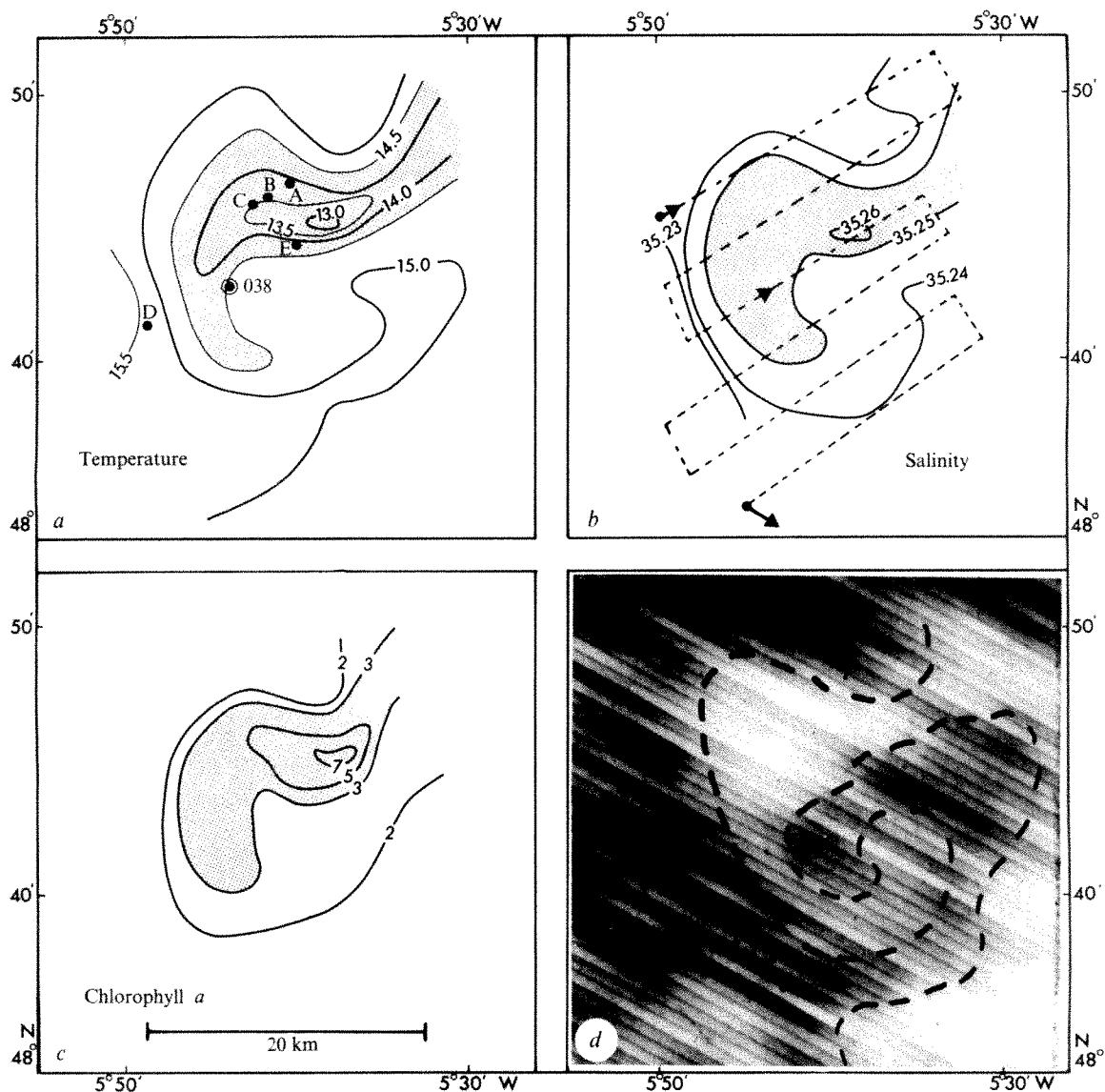


Fig. 2 *a, b, c*, Surface (2m) distributions of temperature (°C), salinity (‰) and chlorophyll *a* (mg m⁻³) between 18.30, 14 July and 03.20 GMT 15 July 1978. *d*, Infrared satellite image, enlarged from Fig. 1. The ship's track, shown in (*b*), was corrected for tidal displacement before contouring the hydrographic data. Expendable bathythermograph (XBT) casts were made at positions A–E (see Fig. 3*b*).

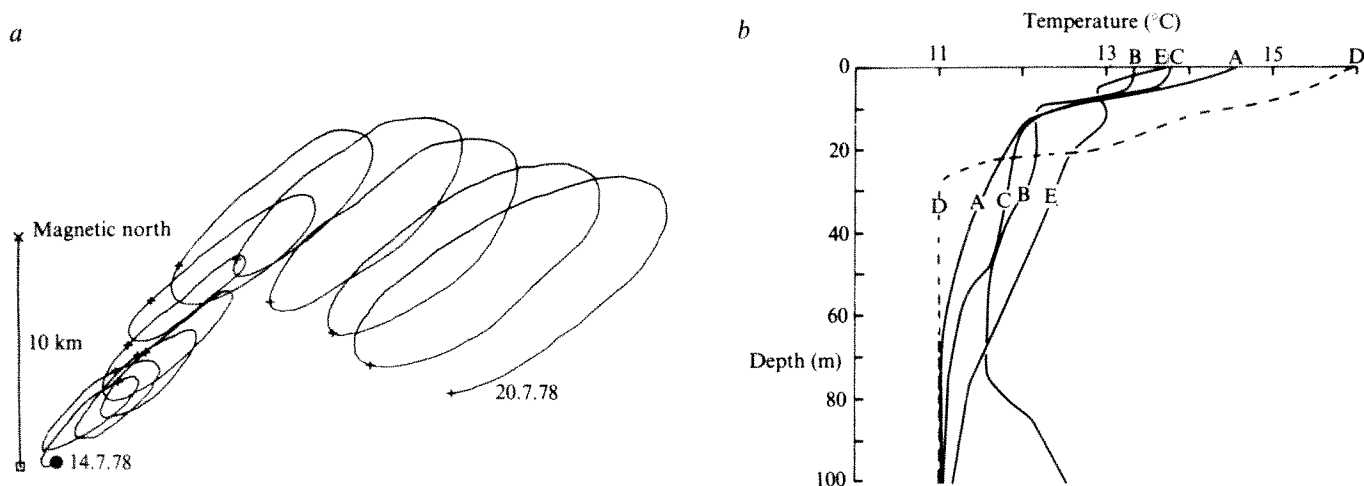


Fig. 3 *a*, Current records at 15m (98m off bottom) from mooring 038 (see Figs 1 and 2). The mooring was laid during neap tides and recovered during spring tides. The crosses mark the midnight and midday time intervals. *b*, XBT records at positions A–E (see Fig. 2*a*). The discrepancies in surface temperature shown here and in Fig. 2*a* are due to differences between 0 and 2m and the inherent smoothing effects of the deck bucket system used for continuous measurements whilst steaming (see ref. 7).

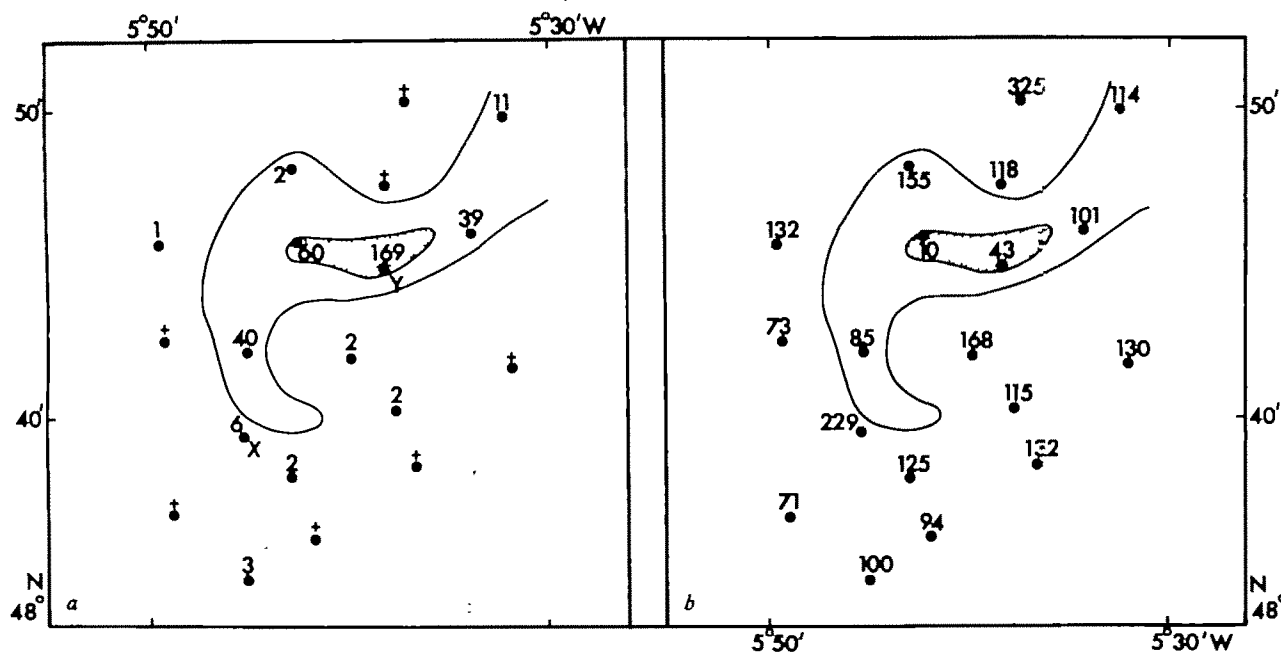


Fig. 4 Surface (2m) cell counts (ml^{-1}) for a, *Gyrodinium aureolum* and b, the diatom species listed in Table 1. (+) indicates <1 cell ml^{-1} . The position of the eddy is marked by the 14.5° and 13.5° temperature contours.

was repeated daily between 17 and 20 July, but over this period the surface temperature structure gradually became more diffuse and no new eddy structures were found, or could be detected subsequently by examination of satellite images. Surface chlorophyll *a* levels remained very variable, with high values (up to 20 mg m^{-3}) associated with horizontal temperature gradients as well as with temperature minima.

The data presented in Fig. 2 give the first confirmation of the suggestion that eddy structures seen on satellite pictures are intrusions of cold, mixed water into warmer, more stratified water. However, the coldest, saltiest region was isolated within the spiral arm of the eddy, suggesting that some of this water may have had a more local origin through upward displacement of the thermocline. The strongest evidence in support of this hypothesis comes from phytoplankton cell counts (Fig. 4) which show that the dominant organism in the cold water patch was *Gyrodinium aureolum* Hulbert, a dinoflagellate characteristic of chlorophyll-rich layers in shallow thermoclines⁵. (Vertical profiles close to the position of the eddy gave sub-surface chlorophyll *a* concentrations and cell counts for *G. aureolum* as high as 200 mg m^{-3} and 10^4 ml^{-1} respectively.) In contrast, diatoms which persist in the well mixed waters throughout the summer⁵ were relatively scarce within this cold water patch.

The distributions of phytoplankton species and inorganic nutrients across the frontal region are summarised in Table 1. It seems that the higher chlorophyll levels in water of frontal properties were due partly to a general increase in the abundance of several diatom and dinoflagellate species and partly to localised patches of *Gyrodinium aureolum* derived from the thermocline. The latter may initiate through a 'seeding' effect the development of widespread blooms of *G. aureolum* that have been observed occasionally in previous summers in the western English Channel⁶. Further experimental studies at sea are now required to establish quantitatively whether mixing of nutrients across frontal regions, due to cyclonic eddies, has a significant effect on annual primary production.

We have been concerned primarily with the influence of eddies on the transfer and growth of phytoplankton in the frontal regions around the British Isles³. The ideas developed here are also important in understanding the cross-frontal transfer and dispersal of other properties, such as heat, radioactive material, hydrocarbons and industrial wastes which are now causing much public concern in our heavily exploited shelf seas.

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Flight capability and the pectoral girdle of *Archaeopteryx*

AS the earliest birds known, the late Jurassic specimens of *Archaeopteryx* have been the object of great speculative interest. Ostrom^{1–3} has argued that *Archaeopteryx* was a terrestrial, cursorial predator that represents a preflight stage in the origin of birds in which the forelimbs were used as nets to trap insects. So far, this has been challenged mainly on the grounds that such activity would have caused excessive feather wear⁴. The principal evidence for regarding *Archaeopteryx* as flightless, or at best an inept non-flapping glider, has come from interpretations of the structure of the pectoral girdle. The absence of an ossified sternum for attachment of flight muscles has long been cited. More recently, it has been argued that the structure of the coracoid of *Archaeopteryx* would not have permitted the supracoracoideus muscle to function as a wing elevator^{2,5}. Because the asymmetrical remiges of *Archaeopteryx* prove that

the wing had an aerodynamic function⁶, we now hope to show that neither of the preceding points precludes a capacity for powered flight in *Archaeopteryx*.

There are several generally held misconceptions concerning the pectoral girdle of modern birds. The most prevalent of these is that the carina of the sternum is the principal site of origin of the massive pectoralis muscle, which provides the power stroke of the wing. This is not so. In most birds, *m. pectoralis* originates to a greater extent from the furcula and the coraco-clavicular membrane (Fig. 1). As it passes posteriorly its fibres originate on the sternum only from those areas not pre-empted by the underlying *m. supracoracoideus*. Typically, these areas consist of a narrow band on the ventral margin of the carina and the most lateral and posterior portions of the sternal plate (Fig. 2). In such birds as the *Dendrocolaptidae*, in which the carina is reduced to facilitate tree-trunk foraging⁷, the pectoralis muscle becomes thin and broad, spreading out laterally and dorsally well past the sternum and on to the rib cage. Once anchored to the furcular area, it would seem that *m. pectoralis* could expand posteriorly and attach to any underlying structure that happened to be present.

Apart from feathers, the character of *Archaeopteryx* most often cited as being bird-like is the well developed furcula. Relative to modern birds of the same size, the furcula of *Archaeopteryx* is actually hypertrophied. There has been no satisfactory explanation of this structure which has received mostly perfunctory treatment. Ostrom merely asks: "Did it function as a transverse spacer between the shoulder sockets?"² Why such a structure would be needed in *Archaeopteryx* and not in any of its suggested ancestors is never dealt with. We consider that the extremely robust furcula of *Archaeopteryx* is best interpreted as having been the site of origin of a well developed pectoralis muscle.

When it is observed that *m. pectoralis* arises to a large extent from the furcular area, it can then be seen that the main function of the ossified sternum and carina in modern birds is to provide attachment for the supracoracoideus muscle (Fig. 2). Although the belly of *m. supracoracoideus* is situated ventrally, it serves to raise the wing because its tendon passes above the glenoid facet and over the acrocoracoid process of the coracoid to insert on the dorsal aspect of the humerus (Fig. 2). In modern birds it is the largest of the muscles that effect the recovery stroke of the wing. For this reason, Ostrom² has emphasised the absence of an acrocoracoid process in *Archaeopteryx*, suggesting that if *m. supracoracoideus* had been present it could not have functioned to raise the wing. Ostrom interprets this as evidence for *Archaeopteryx* having been flightless, the implication being that *m. supracoracoideus* is essential for flight. That this is not the case has been conclusively proven by Sy⁸, who cut the supracoracoideus tendons in living examples of crows (*Corvus*) and pigeons (*Columba*) and found that the birds were still capable of normal, sustained flight. It is of considerable interest that the only capacity lost was the ability of the pigeon to take off from level ground.

It has thus been proved that in modern birds the dorsal elevators (principally *m. deltoideus major*⁸) are completely capable of effecting the recovery stroke of the wing. These

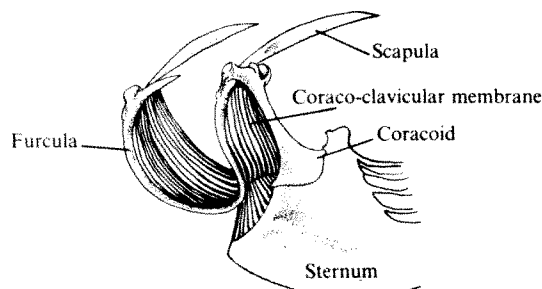


Fig. 1 Left side of pectoral girdle of the duck *Bucephala clangula* showing the furcula and extensive coraco-clavicular membrane from which *m. pectoralis* has its main origin (redrawn from Sy⁸).

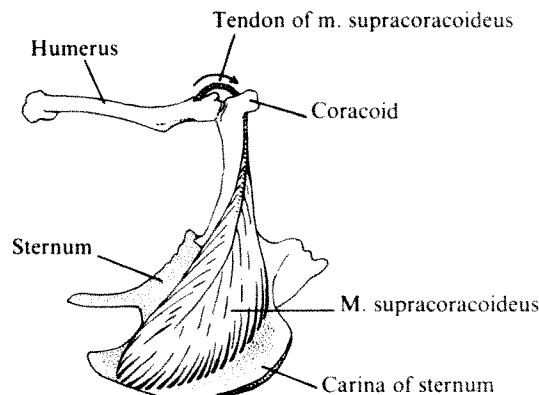


Fig. 2 Right side of pectoral girdle of the pigeon *Columba livia* to show the action of *m. supracoracoideus* and the extensive area of the sternum that this muscle occupies. *M. pectoralis* attaches to the sternum only on those areas shown in stipple. (Modified from Ostrom², after George and Berger¹⁰.)

muscles originate mainly from the scapula, which in *Archaeopteryx* forms an acute angle with the coracoid, as is true in adults of modern flying birds. The acute angle shortens the distance through which the dorsal elevators must act, thus giving greater power. In most flightless birds, on the other hand, the acute angle is lost and the scapula is more nearly vertical⁹.

The dorsal musculature is present and is used to raise the forelimb in virtually all vertebrates; furthermore, it is the dorsal musculature that elevates the wing in such volant forms as bats. Therefore, it is logical to assume that in the evolution of avian flight the recovery stroke of the wing would first have been carried out by the dorsal elevators. The enlarged, ventrally situated *m. supracoracoideus*, the acrocoracoid process, and the ossified sternum with a keel, constitute a single functional complex that is not a requisite of flight but merely a refinement that was superimposed in later birds on an apparatus probably already capable of full flight. The enlarged *m. supracoracoideus* probably evolved to counter the action of an increasingly enlarged *m. pectoralis* and, as evidenced by Sy's experimental pigeons, may have been necessary for birds to adopt purely terrestrial habits.

In conclusion, the robust furcula of *Archaeopteryx* would have provided a suitable point of origin for a well developed pectoralis muscle. Furthermore, the supracoracoideus muscle, and hence an ossified sternum, is not necessary to effect the recovery stroke of the wing. Thus the main evidence for *Archaeopteryx* having been a terrestrial, cursorial predator is invalidated. There is nothing in the structure of the pectoral girdle of *Archaeopteryx* that would preclude its having been a powered flier.

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A new kind of neural superposition eye: the compound eye of male Bibionidae

IN neural superposition eyes the signals of receptors pointing to one region in space are neurally pooled. The optical and neural organisation of these eyes yields an enhanced absolute light sensitivity while minimising the loss of visual acuity that accompanies pooling. In the compound eyes of flies (Diptera), this is achieved in the following way. The distal endings of seven retinula cells lie near the back focal plane of each lens¹. Their light-sensitive structures, the rhabdomeres, are optically isolated from each other. The rhabdomeres of one ommatidium have different optical axes. The optical axes of seven rhabdomeres in seven adjacent ommatidia are parallel (Fig. 1a, ref. 2). The axons of such retinula cells (except the central ones, which pass through to the second optic ganglion) converge onto the same cartridge of secondary neurones in the first optic ganglion, the lamina^{3,4}. Thus, each lamina cartridge 'looks at' one point in space. In one suborder of flies (Cyclorhapha), to which the housefly *Musca* and the fruitfly *Drosophila* belong, the arrangement of rhabdomeres is asymmetrical (Fig. 1a). Eyes organised in this way are one form of neural superposition eyes². Here, I provide evidence that a different kind of neural superposition eye exists in the Bibionidae, a family of flies of the suborder Nematocera. Bibionids are peculiar in being extremely sexually dimorphic in their visual system: the males have, in addition to small 'female' eyes, a pair of large dorsal compound eyes, functionally correlated presumably with the male habit of swarming and chasing females. These dorsal eyes differ from the eyes described in more advanced flies in two respects: the rhabdomeres are symmetrically arranged and the rhabdomeres with parallel optical axes are found in ommatidia $\sqrt{3}$ times further apart than adjacent ommatidia. The course of the retinula cell axons in the lamina differs accordingly.

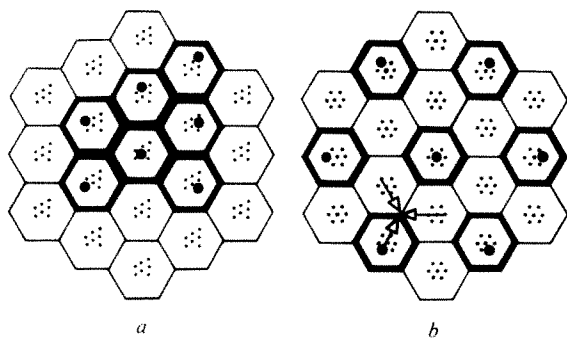


Fig. 1 Arrangement of rhabdomeres and optical organisation of the compound eyes of *a*, cyclorhaphan flies (after Kirschfeld²) and *b*, nematoceran flies (Bibionidae). Rhabdomeres marked with large dots have parallel optical axes. Arrows in *b* show the initial course of retinula cell axons in the lamina, as predicted from optical data.

The technique of antidromic illumination⁵ was used to investigate the optical organisation of the eye. The flies are immobilised with beeswax, and light shone into the ventral side of the head, where a small window has been cut into the cuticle. In this experimental situation, light is diffusely scattered inside the head and travels antidromically up the rhabdomeres, which function as light guides. The distal tips of the rhabdomeres thus become light sources in the back focal plane of each lens. Real and virtual images of the rhabdomere tips can be observed with a microscope, focused at different levels above and below the cornea. Anatomical data were derived from reduced silver preparations⁶.

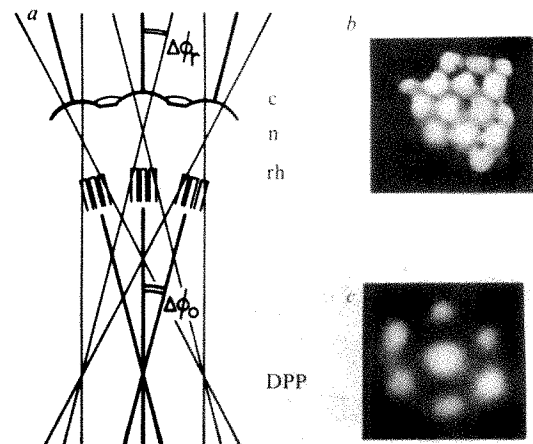


Fig. 2 *a*, Geometrical construction to show the relationship between size and depth of the deep pseudopupil (DPP), the inter-ommatidial angle, $\Delta\phi_0$, and the angle between rhabdomeres, $\Delta\phi_r$. *c*, Cornea; *n*, nodal point; *rh*, distal tips of rhabdomeres. Not drawn to scale. Histological data for *Bibio marci* are as follows (after correction for 15% shrinkage). Radius of curvature of lens, 23.1 μm ; distance of rhabdomere tips from the lens surface, 82.8 μm ; separation of rhabdomere tips, 3.0 μm . The angle subtended by the rhabdomeres at the nodal point is 2.88° , assuming the nodal point to lie in the centre of curvature of the lens (this is the case when the air-cuticle interface alone provides the refractive power of the lens). This agrees well with the optical data listed below. *b*, Pattern of lenses at the level of the cornea. Except for the area shown, the eye is covered with black ink to reduce stray light. Mean distance of adjacent facets, 32.96 μm . The inter-ommatidial angle is given by $\tan \Delta\phi_0 = 32.96/1,100$. $\Delta\phi_0 = 1.72^\circ$. The separation of facets $\sqrt{3}$ times further apart (like those marked in Fig. 1*b*), 55.86 μm . The angle, $\Delta\phi'_0$, between these facets is 2.91° . *c*, Deep pseudopupil. Superposition of virtual images of the distal tips of rhabdomeres at the centre of local curvature of the eye, 1,100 μm below the cornea. Mean distance of the peripheral images from the central one, 55.48 μm . The angle between rhabdomeres is given by $\tan \Delta\phi_r = 55.48/1,100$. $\Delta\phi_r = 2.89^\circ$. Photographs taken through a Leitz Orthoplan microscope, antidromic illumination, male *Bibio marci*.

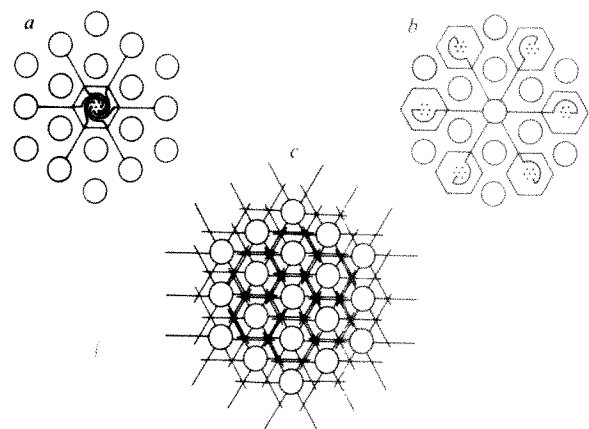


Fig. 3 Hypothesised course and distribution of retinula cell connections in the lamina of male bibionids. Circles represent horizontal views of cartridges. *a*, Divergence of axons from one ommatidium to six different cartridges, corresponding to six different optical axes. Note that the axons have to turn $\sim 180^\circ$ and that they have to pass between two neighbouring cartridges to reach the appropriate one. *b*, Convergence of axons transmitting information about the same region in space, that is, axons of retinula cells having parallel optical axes. Note that these converging axons take the same routes as the divergent ones. *c*, Divergent and convergent axons drawn in one diagram. The fibres form a hexagonal lattice with the same orientation as the corneal lattice of lenses. Histological examination (Fig. 4) indicates that it is not the axons, but their collaterals, that form this network of connections.

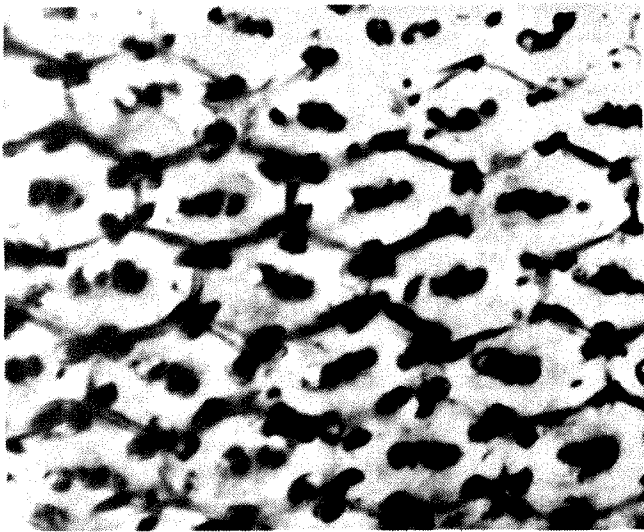


Fig. 4 Transverse section through the lamina, showing the network of axon collaterals of retinula cell axons. Triads in the corners of the lattice are profiles of main axons running perpendicular to the plane of sectioning down into the lamina. The pattern of fibres is strikingly different from that seen in other flies^{3,4}. Bar, 10 μ m; Bodian silver; *Bibio marci*.

The optical axes of the ommatidia (that is, of their central rhabdomeres) intersect at the centre of curvature of the eye (Fig. 2a). At this point (1,100 μ m beneath the cornea in the case of the male eye of *Bibio marci*) the microscope visualises an enlarged image of the receptor endings, originating from the superposition of virtual images of the rhabdomere tips thrown by each lens along its optical axis (Fig. 2b). This image has been termed the deep pseudopupil (DPP, refs 7, 8). The distance of the DPP from the cornea is determined by the inter-ommatidial angle, $\Delta\phi_0$, and its size is a function of the inter-rhabdomere angle, $\Delta\phi_r$ (Fig. 2a, refs 7, 8). In bibionids, which have a symmetrical arrangement of rhabdomeres typical of nematoceran flies^{9,10}, the size of the DPP exactly matches the size of the lens pattern shown in Fig. 1b (Fig. 2b). This means that for the arrangement of ommatidia marked in Fig. 1b the inter-ommatidial angle, $\Delta\phi'_0$ (which is $\sqrt{3}$ times the angle, $\Delta\phi_0$, between adjacent ommatidia), is equal to the angle between rhabdomeres. Every seven rhabdomeres, having relative positions like those marked in Fig. 1b, point in the same direction in space. It follows that the optical requirements of a neural superposition eye are fulfilled.

It remains to be shown, however, that the neural conditions are met: that is, that the axons of retinula cells with parallel optical axes converge onto the same cartridge (set of secondary neurones) in the lamina. Given the optical organisation outlined above, a hypothesis can be advanced about the anatomical organisation of the retina-lamina projection (Fig. 3). Two outstanding and easily testable features of the projection emerge from this hypothesis: (1) to reach their respective cartridges, the axons of the six peripheral retinula cells in one ommatidium have to twist through 180° and diverge in opposite directions, and (2) the axons or their collaterals have to pass between cartridges, forming a hexagonal network.

In horizontal histological sections the axons of the retinula cells, leaving the retina in tight bundles, can be seen to rotate about 180° on entering the lamina. In crossing the superficial layer of cell bodies of lamina neurones, the axon bundles open up. This brings three axons of three adjacent ommatidia into close proximity (see Fig. 1b). The clusters of three axons run down into the lamina, parallel to the long axes of the cartridges, marking the corners of a hexagonal lattice in horizontal sections. In the course of running down into the lamina, the axons give off collaterals, which pass between adjacent cartridges towards the ones in the next row. These collaterals form a hexagonal

network of fibres which have the same orientation as the corneal pattern of hexagonal lenses (Fig. 4). Therefore, it seems to be these collaterals which establish connections with the appropriate cartridges. At the level of light microscopy, the anatomical structure of the lamina therefore agrees with the predictions made, based on the optical structure of the retina.

Up to now neural superposition eyes have been found in only one other order of insects. Although in this case (Hemiptera¹¹) the retina-lamina projection has not been described in detail, the optical organisation is quite different from that of dipteran eyes. The rhabdomeres with parallel optical axes lie in ommatidia even further apart (3–4 facets) than in the case of the nematoceran flies described here. This raises several questions about the evolution of neural superposition eyes. Within Diptera, at least, we may ask what functional and/or structural reasons might have caused the topological rearrangement from the symmetrical, widely spaced organisation of a nematoceran eye to the asymmetrical one of flies considered to be more advanced in evolution.

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The role of inner hair cells in hearing

DISCUSSION about functional differences between the inner and outer hair cells (IHCs and OHCs) in the mammalian organ of Corti^{1–5}, has arisen as a result of several anatomical and electrophysiological observations. The proportions of afferent and efferent fibres supplying the two types of cells are quite different^{6–8}; the afferent neurones associated with them are distinct cytologically as well as in their susceptibility to retrograde degeneration^{6–8}; the electrophysiological responses following preferential loss of OHCs are not what would be expected on the basis of a similarity of function^{2,5,9–11}. Attempts at the elucidation of these differences^{2,9} have made use of the ototoxic antibiotic kanamycin, which causes preferential damage to OHCs. This technique is far from ideal: the damage may be patchy and IHCs may also be affected. As to the selective elimination of IHCs, no procedure has been available. Mutant genes frequently provide material that cannot be produced experimentally, but although a large number of genes affecting the inner ear in various ways are known in the mouse^{12–14}, there

has been none so far that clearly discriminated between IHCs and OHCs. We now report a new mutation in the mouse which selectively eliminates IHCs. As the mutant animals are almost totally deaf, it would seem that hearing is predominantly dependent on IHCs.

The new mutation occurred at Albert Einstein College of Medicine, Bronx. It is an autosomal recessive gene with full penetrance. The homozygotes show an abnormality of behaviour that is known as the waltzing syndrome¹², and to conform with general usage the gene has been named *bronx waltzer* and assigned the symbol *bv*. The hearing ability of *bv/bv* mice is severely impaired. The affected animals either give no response to sound at all or respond very slightly during a short period following the normal time of onset of hearing (at about 13 d). The hearing tests were based on Preyer's pinna reflex.

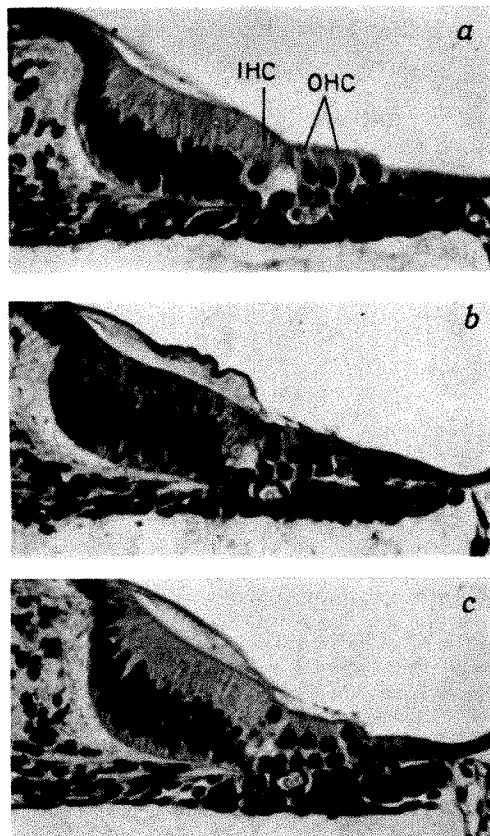


Fig. 1 Organ of Corti in newborn mice. *a*, $+/+$; IHC normal. *b*, *bv/bv*; IHC absent. *c*, *bv/bv*; IHC abnormal.

This method is far from refined, but experience has shown that it is reliable enough for preliminary studies. Moreover, the results of these tests were subsequently confirmed by the severe degeneration of the spiral ganglion, observed even in very young animals.

Altogether, 31 *bv/bv* animals, ranging in age from newborn to 281 d old, and 14 normal controls ($4+/bv$, $10+/+$), ranging from newborn to 253 d old, were used in this study. They were fixed in Witmaack's fluid by intravital perfusion, and the heads were decalcified in 1% nitric acid. The otic capsules, with the surrounding tissues intact, were embedded in celloidin, impregnated with paraffin wax, and sectioned at 10 μ m in a plane parallel to the modiolus. The sections were stained with Ehrlich's haematoxylin and orange G containing a trace of erythrosin.

The gross structure of the cochlea in *bv/bv* mice is normal. Histological abnormalities occur in the organ of Corti and the spiral ganglion. Other structures, including the stria vascularis, seem to be normal. In the organ of Corti the pillars and IHCs may be absent or malformed. As the pillars are formed well after birth, their abnormalities appear later, but those of IHCs are clearly visible even in the newborn (Fig. 1). A large proportion

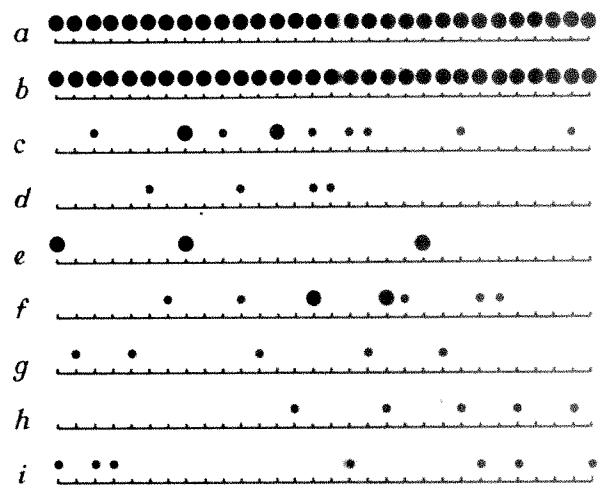


Fig. 2 Distribution of normal (large spot), abnormal (small spot) and missing (no spot) IHCs in 30 consecutive sections from the middle part of the left cochlea. *a*, $+/+$; newborn. *b*, $+/+$; 241 d old. *c*, *bv/bv*; newborn. *d*, *bv/bv*; 3 d. *e*, *bv/bv*; 6 d. *f*, *bv/bv*; 9 d. *g*, *bv/bv*; 29 d. *h*, *bv/bv*; 150 d. *i*, *bv/bv*; 244 d.

of IHCs are not formed at all, and of those that are formed the vast majority are grossly abnormal. They may be very small and rounded or they may occur in strikingly abnormal positions. There may in some cases be two abnormal cells side by side instead of a single normal one. However, occasional IHCs are observed that are normal in appearance and position. Further study would be required to determine whether they actually are normal. These apparently normal cells had a tendency to disappear with age, although some remain even in the oldest animals studied. To determine the distribution of the normal, abnormal and missing IHCs, 30 consecutive sections from the middle part of the cochlea were examined in both the ears of 12 animals ranging in age from newborn to 244 d old. Figure 2 shows the distributions observed in the left ears of a representative sample. The two sides were generally very similar.

The density of the neurones in the spiral ganglion seems to be approximately normal in the newborn, but their degeneration is so rapid that the ganglion is obviously thinner by 6 d, and after about 4 months very few cells remain in it (Fig. 3). Specialised techniques would be required to determine the type of the remaining cells and whether they are normal, but as far as the extent of the degeneration is concerned, it is consistent with Spöndlin's⁶⁻⁸ finding that in the cat >90% of all spiral ganglion cells innervate IHCs, and are subject to retrograde degeneration.

As to OHCs, some may be a little irregular in shape, and an occasional cell may even be missing, but generally they seem to be normal. It is assumed that they are also normal in function,

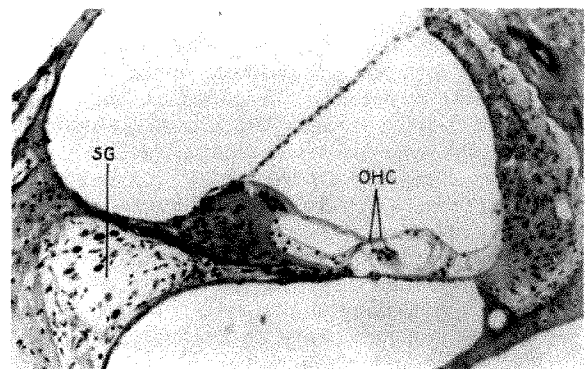


Fig. 3 Organ of Corti and spiral ganglion (SG) of a 150-d-old *bv/bv* mouse showing the absence of IHC and degeneration of SG.

not only because they do not degenerate but also because the tunnel fibres leading to them are intact even in the oldest animals.

These findings suggest that 'basic' hearing is predominantly, if not wholly, dependent on IHCs. OHCs may take part in further discriminatory processes or may even govern the IHCs in some way, but they are incapable of leading to the development of normal hearing ability on their own. Further work on this mutant using specialised techniques may lead to a clearer understanding of the functional differences between IHCs and OHCs.

This gene also affects the vestibular part of the inner ear, but this is not believed to be the cause of the 'waltzing' behaviour. Investigations on a large number of other mutants with this type of behaviour suggest that its cause should be sought in the central nervous system and not in the inner ear¹⁵.

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Dopamine inhibits adenylate cyclase in human prolactin-secreting pituitary adenomas

THERE is evidence for the existence of multiple forms of dopamine (DA) receptors¹⁻³. In particular, some of these receptors are coupled to adenylate cyclase (DA-stimulating enzyme activity), whereas others seem to be independent³⁻⁵. These two classes of receptors, which have been designated D-1 and D-2, respectively, would also have different pharmacological properties⁶. Pituitary mammothroph DA receptors regulating prolactin (PRL) secretion are considered to be typical D-2 receptors. A DA-stimulated adenylate cyclase has not been detected in normal anterior pituitaries^{3,5,6}; furthermore, several studies indicate that cyclic AMP is stimulatory and not inhibitory to PRL secretion^{7,8}. We report here that in homogenates of human PRL adenomas, in which dopaminergic agonists act as inhibitors of PRL secretion, basal adenylate cyclase is inhibited by DA. The DA receptors mediating this inhibition have the

same pharmacological properties as those regulating PRL secretion.

Adenomas were surgically removed from hyperprolactinaemic patients through the transnasosphenoidal route. The adenomatous nature of the surgical fragments was confirmed both by histological examination and by SDS-polyacrylamide gel electrophoretic analysis⁹ of the homogenates used for the adenylate cyclase assay. The latter revealed in all cases a prominent PRL content, whereas growth hormone, which in the electrophoretograms of normal human anterior pituitaries gives a major band (our unpublished observations), was absent. The preoperative plasma levels of PRL and the effects on these levels of dopaminergic agents are shown in Table 1. In six out of the seven cases examined, dopaminergic drugs were clearly inhibitory. Moreover, *in vitro* perfusion experiments carried out in separate adenomas revealed that such inhibition is due, at least partially, to a direct effect of DA on adenoma cells (Fig. 1).

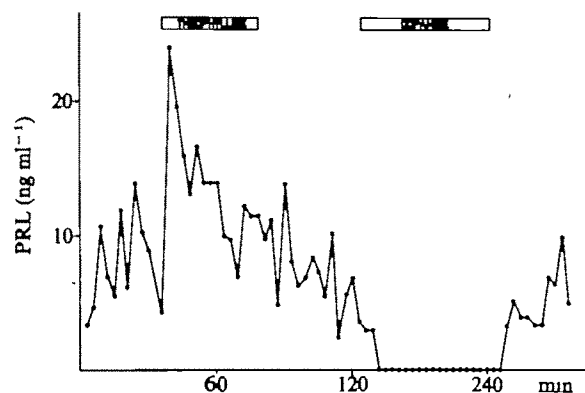


Fig. 1 *In vitro* release of PRL from a human PRL adenoma. Adenoma fragments were immersed, immediately after surgical removal, in minimum essential medium (MEM) at ice temperature and kept as such until they were mounted in a superfusion apparatus³⁰ (within 2 h of the operation). Perfusion was carried out at 37°C with MEM gassed with an oxygen/carbon dioxide (95%/5% w/v) mixture. Fractions were collected every 3 min starting at 90 min after the beginning of the superfusion. During the periods indicated by the horizontal bars the superfusion medium contained 5.5 mM theophylline or 10 µM DA. Duplicate aliquots of the superfusate fractions were analysed for the PRL content by radioimmunoassay. Theophylline, which increases the intracellular levels of cyclic AMP through a blockade of phosphodiesterase, is stimulatory on secretion, whereas DA is strongly inhibitory. Before adenomectomy the plasma PRL concentration was 70 ng ml⁻¹ and this was reduced by more than 50% by the administration of dopaminergic drugs (see legend to Table 1). The radioimmunoassay for PRL was carried out according to Sinha *et al.*²⁸.

Table 1 also shows the effect of 10 µM DA on basal adenylate cyclase in homogenates of individual adenomas. The inhibitory effect of DA on adenylate cyclase *in vitro* parallels the effect of dopaminergic agents on PRL secretion *in vivo*. The action of DA was dose dependent (Fig. 2a, b), with IC₅₀ values of 3 × 10⁻⁷–10⁻⁵ M in individual cases. The inhibitory effect of DA on adenylate cyclase is mimicked by the dopaminergic ergot, CH-29-717 (Sandoz) (a potent water-soluble analogue of CB-154, (ref. 10)), and by (-)-noradrenaline (NA) (Fig. 2b, c). In agreement with *in vitro* studies on PRL secretion and on binding of dopaminergic drugs to pituitary membranes carried out in animal pituitaries¹¹⁻¹⁵, NA is less potent than DA, but CH-29-717 is effective at even lower concentrations than DA. The specificity of the DA action was confirmed from the effects of the dopaminergic antagonists. In the responsive cases, low doses of drugs of the phenothiazine group (trifluoperazine and fluphenazine) had no effect on basal adenylate cyclase, but

Table 1 Effect of dopaminergic drugs on PRL secretion *in vivo* and of DA on basal adenylate cyclase *in vitro*

Case	PRL plasma levels before adenomectomy		Adenylate cyclase activity		
	Basal levels (ng ml ⁻¹)	% Reduction induced by administration of dopaminergic drugs	Cyclic AMP formed (pmol per mg protein per 3 min)		% Variation induced by 10 μ M dopamine
			Control	Dopamine	
1	95	>50%	165.52 $\pm$ 13.76	111.76 $\pm$ 8.31*	-32.48
2	120	>50%	204.56 $\pm$ 17.14	144.40 $\pm$ 4.76*	-29.41
3	90	>50%	193.60 $\pm$ 1.60	118.08 $\pm$ 1.16*	-39.41
4	65	>50%	53.44 $\pm$ 0.78	45.84 $\pm$ 2.00*	-14.22
5	72	>50%	112.48 $\pm$ 2.37	89.11 $\pm$ 7.68*	-20.77
6	120	>50%	50.79 $\pm$ 1.59	44.21 $\pm$ 0.66*	-12.95
7	200	No effect	55.69 $\pm$ 1.30	56.98 $\pm$ 1.67	No effect

Patients were infused with DA (2 μ g per kg per min $\times$ 3 h) or given CB-154 (Sandoz) (5 mg orally). Plasma levels of PRL were evaluated either at the end of the infusion, or after the CB-154 administration. For the enzyme assay, adenoma fragments were frozen to -20 °C immediately after surgical removal and thawed only just before the assay (4-15 d later). They were homogenised in 20 vol (w/v) of 2 mM Tris-maleate buffer, pH 7.4, containing 2 mM EGTA. The final incubation mixture (0.1 ml) contained: 80 mM Tris-maleate pH 7.4, 10 mM theophylline, 1 mM cyclic AMP, 0.2 mM EGTA, 0.15 mM [¹⁴C]-ATP (20 mCi mmol⁻¹) (except in cases 1 and 2 where [¹⁴C]-ATP was 0.5 mM), MgSO₄ (2 mM in cases 1 and 2, 0.6 mM in case 3 and 1.5 mM in all other cases), phosphocreatine (1.8 mg ml⁻¹) and creatinephosphokinase (7 U ml⁻¹). Protein concentration varied between 0.4 and 1 mg ml⁻¹. The reaction was started by the addition of the homogenate, carried out for 8 min at 30 °C and stopped by a solution containing 2% SDS, 1.4 mM cyclic AMP and 40 mM ATP. Biochemical assays were carried out according to the following procedures: adenylate cyclase, ref. 27; PRL, ref. 28; protein, ref. 29. Values for adenylate cyclase activity represent the mean $\pm$ s.e.m. of at least three replicate samples.

* $P < 0.05$, † $P < 0.001$; ‡ $P < 0.025$.

competitively antagonised the inhibitory action of DA (Fig. 2a). The DA effect was also blocked by (-)-sulpiride (Ravizza), an antagonist of mammotroph DA receptors^{11,14,15}. In case 7, dopaminergic agents failed to decrease PRL plasma levels, and DA was without effect on the basal adenylate cyclase activity of the homogenate. However, in this case, adenylate cyclase was stimulated by low doses of phenothiazines¹⁶ and such stimulation was competitively counteracted by DA (Table 2). Thus, even in case 7, DA has a potential ability to inhibit adenylate cyclase.

Our results, which indicate a striking similarity between DA receptors mediating adenylate cyclase inhibition and DA receptors inhibiting PRL secretion, suggest that a decrease in the intracellular concentration of cyclic AMP might mediate the action of DA in mammotrophs. Doses required to inhibit adenylate cyclase are higher than those which are known from *in vitro* studies on animal pituitaries to be effective on PRL secretion^{12,13}; however, in other systems also, discrepancies

have often been observed between the drug concentrations which elicit physiological responses thought to be mediated by cyclic AMP, and those required to modulate the adenylate cyclase-cyclic AMP system. Several explanations have been proposed to account for these results¹⁷⁻¹⁹. The consistency of our findings in the adenomas examined suggests that the effect of DA on adenylate cyclase is not due to a fortuitous association with a tumoral clone, but that it might be a physiological characteristic of all PRL-secreting cells. In the normal anterior pituitary the effect of DA on mammotroph adenylate cyclase might go undetected, because basal adenylate cyclase activity is contributed primarily by other cell types. Furthermore, the inhibitory coupling between DA and adenylate cyclase might be more pronounced in tumour cells. In fact, clinical data suggest the existence of a defective dopaminergic control of the pituitary in hyperprolactinaemic patients with adenomas¹³, and this might lead to supersensitivity of DA receptors in adenomatous cells²⁰.

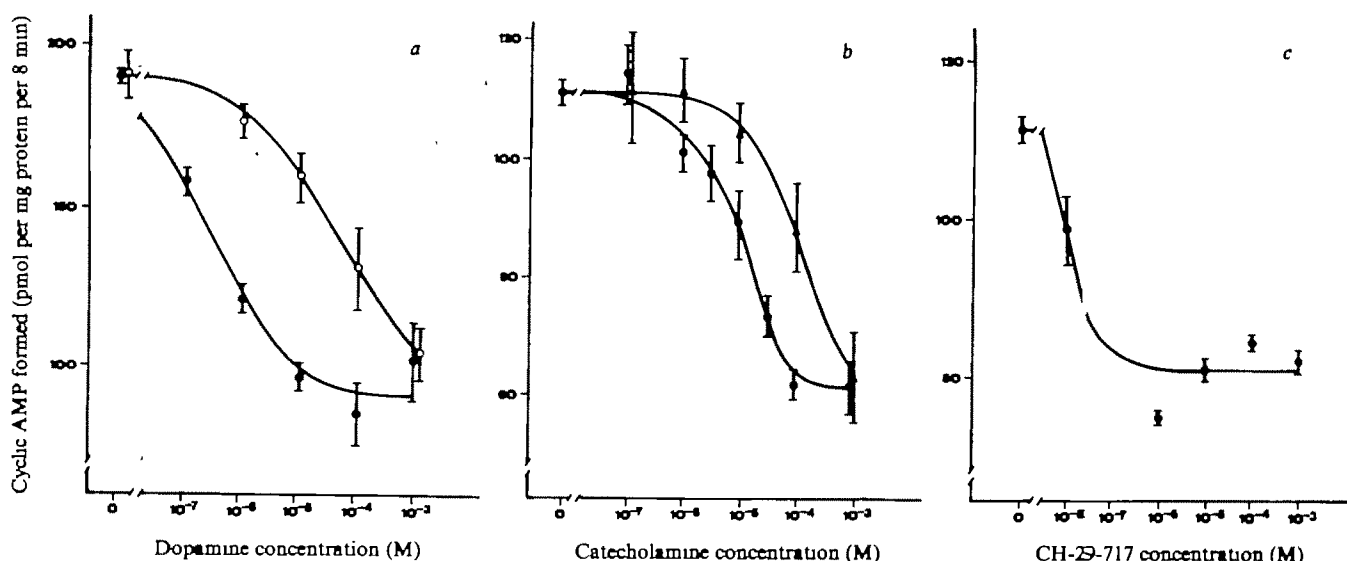


Fig. 2 Effect of drugs on adenylate cyclase activity in homogenates of human PRL adenomas. *a*, Effect of various concentrations of DA in the absence (●) and presence (○) of 0.1 μ M trifluoperazine (Maggioli) (case 3). *b*, Effect of various concentrations of DA (●) and (-)-noradrenaline (▲) (case 5). *c*, Effect of various concentrations of the dopaminergic ergot CH-29-717 (Sandoz) (case 5). See legend to Table 1 for experimental details. Values represent the mean $\pm$ s.e.m. of at least three replicate samples.

Table 2 Case 7: the stimulation of adenylate cyclase by fluphenazine is competitively counteracted by DA

Addition	Cyclic AMP formed (pmol per mg protein per 8 min)
Control	65.84 ± 3.58
Fluphenazine 0.1 µM	82.32 ± 2.72*
Fluphenazine 0.1 µM + DA 10 ⁻⁶ M	77.21 ± 2.66
Fluphenazine 0.1 µM + DA 10 ⁻⁵ M	70.20 ± 1.75
Fluphenazine 0.1 µM + DA 10 ⁻⁴ M	66.24 ± 3.59
Fluphenazine 0.1 µM + DA 10 ⁻³ M	64.78 ± 1.79†

See legend to Table 1 for experimental details. Values represent the mean ± s.e.m. of at least three replicate samples.

* $P < 0.005$ relative to control; † not significant relative to control and $P < 0.001$ relative to fluphenazine (Squibb) 0.1 µM.

Note that at another site where pharmacological evidence indicates the existence of D-2 DA receptors, such as on central dopaminergic terminals²¹⁻²³, cyclic AMP and DA produce opposite effects, the former being stimulatory and the latter inhibitory to the synthesis of the neurotransmitter²⁴⁻²⁶. This suggests that an inhibitory coupling with adenylate cyclase might be a more general property of D-2 DA receptors.

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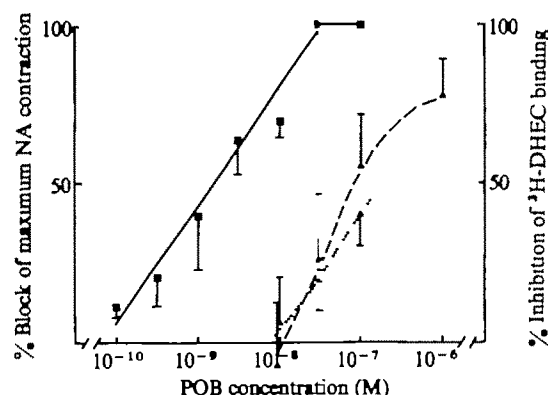
Dihydroergocryptine binding and α -adrenoreceptors in smooth muscle

DIHYDROERGOCRYPTINE is a potent antagonist of α -adrenoreceptors¹ that has recently been labelled to high specific radioactivity with tritium². ³H-DHEC was found to bind to a crude membrane preparation of rabbit uterus, and a portion of this binding was suppressible by phentolamine². The saturability, reversibility and structural and steric selectivity of this specific binding component suggested that it represents α -adrenoreceptors mediating uterine contractions³⁻⁴. In subsequent studies, ³H-DHEC was used to tentatively identify α -adrenoreceptors mediating various biological responses in several tissues⁵⁻¹⁰. The evidence for the identity of ³H-DHEC binding sites in tissue fragments with functional α -receptors in intact tissues is indirect, and is based on the demonstration of similar potencies of various ligands in suppressing ³H-DHEC binding and producing or inhibiting physiological responses. The absolute potency of particular ligands to suppress binding or affect the physiological response, often differs markedly. However, this could be due to changes in the properties of receptors caused by tissue disruption, or the different conditions in which potencies are determined. Therefore, the validity of the use of ³H-DHEC or other reversible ligands to label functionally relevant receptors must be tested in experiments where the above complications can be reduced or eliminated. We report here the use of an irreversible α -adrenoreceptor antagonist which provided a way to bridge the gap between physiological experiments measuring mechanical responses of an intact tissue and binding assays using membrane fragments. α -Receptors mediating contractions of isolated rabbit uterine strips were irreversibly blocked by phenoxybenzamine (POB), and ³H-DHEC binding was then measured in membranes prepared from these POB-treated and from untreated control strips. The results obtained dissociate phentolamine-suppressible ³H-DHEC binding and α -receptors mediating uterine contraction, but indicate that a fraction of the binding with low capacity and high affinity for DHEC, may represent true postsynaptic α -receptors.

Myometrial strips were prepared from uteri of immature New Zealand rabbits and were mounted in tissue baths containing de Jalon solution at 31 °C. Concentration-response curves for noradrenaline (NA) induced isometric contractions were determined before and after a 20-min exposure to various concentrations of POB. A crude membrane fraction was then prepared from the control and POB-treated strips to test the *in vitro* binding of ³H-DHEC.

POB irreversibly blocked the NA-induced contractions of uterine strips. As little as 10⁻¹⁰ M POB produced significant inhibition, and complete block was achieved by 3 × 10⁻⁸ M (Fig. 1). Spare receptors were not evident in the rabbit uterus, as the threshold blocking concentration of POB (10⁻¹⁰ M) significantly reduced the maximal response to NA. The high blocking potency of POB and the absence of spare α -receptors have also been observed in other smooth muscles^{11,12}. POB treatment of the intact strips significantly reduced the specific binding of ³H-DHEC (8 nM) as compared with the binding to

Fig. 1 Effects of phenoxybenzamine (POB) on noradrenaline (NA)-induced uterine contractions and on the specific binding of ^3H -DHEC to uterine membrane fragments. Suppression of the maximal contractile response to NA (■), suppression of specific ^3H -DHEC binding after incubation of POB with intact strips (Δ) or with membrane preparations (▲). Points and bars represent mean $\pm$ s.e. of triplicate determinations in 4–9 experiments. Uteri were removed, and the two horns separated and longitudinally halved. The strips were trimmed of fat and endometrial tissue, and suspended in tissue baths containing de Jalon solution (in mM: NaCl, 154; KCl, 5.6; CaCl₂, 0.3; NaHCO₃, 6.0; glucose, 2.8; pH 7.4) at 31 °C, gassed with 5% CO₂ in O₂. Isometric contractions were recorded by force-displacement transducers (Grass FTO3C) and a polygraph. Contractions were induced by single graded doses of (–)noradrenaline, after which all but one strip were exposed to various concentrations of POB for 20 min. After a 1-h washout period NA concentration–response curves were redetermined and corrected for any spontaneous change as tested in the strip not exposed to POB. After thorough washing with de Jalon solution for 90 min, the strips were dismantled and a crude membrane fraction was prepared³. The strips were minced and homogenised in ice-cold buffer (0.25 M sucrose, 1 mM MgCl₂, 5 mM Tris HCl, pH 7.4, at 25 °C) with a Brinkman polytron (4 $\times$ 6s bursts). The homogenate was filtered through gauze and centrifuged twice at 500g for 10 min at 4 °C. The pellet was discarded and the supernatant centrifuged at 27,000 g (10 min, 4 °C) and washed twice with incubation buffer (10 mM MgCl₂, 50 mM Tris HCl, pH 7.5 at 25 °C), in which the final pellet was resuspended at 3–4 mg ml^{–1} protein. The same method was used to obtain membranes from freshly removed uteri. Aliquots of such membrane preparations were incubated with POB at 25 °C for 12 min (ref. 3). The suspension was then diluted with 50 vol incubation buffer and centrifuged and washed twice at 27,000 g. A control aliquot was treated the same way except for the addition of POB. The binding of ^3H -DHEC (NEN, 27.3 Ci mmol^{–1}) to uterine membranes was assayed by a rapid filtration method³. Incubations were in polyallomer tubes at 25 °C for 15 min and binding was terminated by adding 2 ml of incubation buffer to the 150- μ l aliquots containing 8 nM ^3H -DHEC, and immediate filtering through Whatman GF/C glass fibre filters. The filters were washed for 15 s with 20 ml of the same solution at 25 °C. Specific binding is the difference between binding in the absence and presence of 10 μ M phentolamine, and it was 30–50% of total binding. Control membrane preparations bound 90.6 ± 10.5 fmol ^3H -DHEC per mg protein.



membranes prepared from control strips not exposed to POB. However, the concentration of POB needed to suppress ^3H -DHEC binding was approximately 50 times higher than that required to produce equivalent block of the mechanical response to NA (Fig. 1), and even 10^{-6} M POB did not completely suppress binding. As this discrepancy may have been due to diffusion barriers that limited the access of POB to receptors inside the intact tissue, in a group of experiments POB was incubated with uterine membranes rather than with intact strips. Preparation of the membranes from the freshly removed uteri, incubation with various concentrations of POB and the subsequent assay of ^3H -DHEC binding were as described by Williams and Lefkowitz⁴. Suppression of ^3H -DHEC binding by POB in these preparations was practically identical to the suppression observed after exposure of intact strips to POB (Fig. 1). The 40% decrease in ^3H -DHEC binding by 10^{-7} M POB is comparable to the 45% suppression observed in similar experiments by Williams and Lefkowitz⁴. These observations eliminate the possibility that the dissociation of the effects of POB on the physiological response and on ligand binding could have been due to diffusion barriers in the intact tissue or to breakdown of POB in the incubation medium⁴.

Differences between the results of binding studies and experiments with functioning tissues could also be related to the fact that the ionic composition and temperature of the media used are usually very different. As ^3H -DHEC binding in the present experiments and in earlier studies^{2–5} was tested in Tris buffer at 25 °C, we repeated the last group of experiments using de Jalon solution at 31 °C for the final resuspension of membranes before their incubation with POB and the assay of ^3H -DHEC binding. One-half of each membrane preparation was exposed for 20 min to 3×10^{-6} M POB, the lowest concentration that produced complete block of contractions in strips, and binding of various concentrations of ^3H -DHEC was then tested both in the control and in the POB-treated sample (Fig. 2). POB did not significantly alter specific ^3H -DHEC binding at higher concentrations of the labelled ligand, thus confirming dissociation of these binding sites from α -receptors mediating smooth muscle contraction. However, the same concentration of POB almost completely eliminated specific binding at low concentrations of ^3H -DHEC (≤ 3 nM), and there seems to be a break in the control binding curve at the same ^3H -DHEC concentration. These latter findings suggest that the low-capacity, high-affinity component of DHEC binding (Fig. 2) may

represent true smooth muscle α -receptors with a high sensitivity to blockade by POB.

This possibility was further tested in a 'receptor protection' experiment. Three strips obtained from the same uterus were mounted in tissue baths. After determination of NA concentration–response curves, one strip was exposed for 20 min to 3×10^{-8} M POB alone, the second was exposed to 3×10^{-8} M POB in the presence of 10^{-5} M phentolamine, and the third remained untreated as control. Retesting the effect of NA after repeated washing of the strips for 1 h showed no significant change in the control, complete block in the POB-treated strip and a reduction of the maximal NA contraction from 2.15 to 1.05 g in the strip exposed to both POB and phentolamine (50% protection). The specific binding of ^3H -DHEC (2 nM) in membranes from the same three strips was 40.2, 0 and 22.1 fmol per mg protein, respectively, indicating 55% protection in the last preparation. In two other experiments, methoxamine-induced contractions of strips and suppression of ^3H -DHEC (2 nM) binding in membranes were tested in preparations obtained from the same uterus. Methoxamine was used as agonist, because it is not a substrate for monoamine oxidase, catechol-O-methyl transferase or neuronal uptake¹³ and, in the absence of spare α -receptors in the uterus, its EC₅₀ should be similar to its K_d. The observed mean EC₅₀ of 1.5×10^{-6} M for contraction and K_d of 5×10^{-7} M for binding are in good agreement.

The good correlation between irreversible block of the physiological response and suppression of ^3H -DHEC binding by POB, the similar degree of protection against both effects by phentolamine, and the similar absolute potency of methoxamine for contraction and binding suggest that the high-affinity component of ^3H -DHEC binding does, in fact, represent functional α -receptors in smooth muscle. For the first time, ligand binding and receptor-mediated mechanical responses were determined in the same preparation and could be directly compared. The second, high-capacity, binding component does not represent α -receptors mediating uterine contraction and the present results provide no information about the possible physiological role of these more numerous sites. They may be non-functional 'silent receptors' (ref. 14) for catecholamines, which may have affinities for various adrenergic ligands that are similar but not identical to the affinity of true receptors. Alternatively, the second binding component may be related to release-modulating α -receptors. POB is considerably more

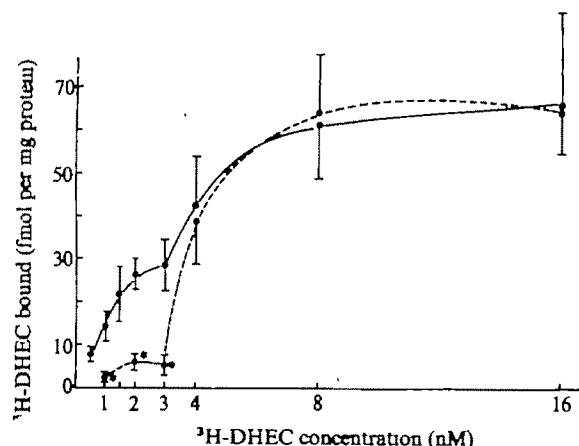


Fig. 2 The effect of phenoxybenzamine on the specific binding of various concentrations of ^3H -DHEC to uterine membranes. Membranes were prepared from freshly removed uteri as described in the legend to Fig. 1, except that final resuspension of membranes before incubation with POB (3×10^{-6} M, 20 min, 31°C) and the binding assay (15 min, 31°C) was in de Jalon solution. Points are means $\pm$ s.e. of triplicate determinations from 4–7 different uteri. POB-treated ($\circ$) and control ($\bullet$) values are matched and membrane material from any one uterus was sufficient to test binding at 4–6 concentrations of ^3H -DHEC. In individual preparations, where at least four points were obtained at or below 3 nM ^3H -DHEC, Scatchard plots of such control values gave a mean K_d of 1.5 ± 0.4 nM and a b_{max} of $36.6 \pm \text{fmol per mg protein}$. In membrane preparations where most points tested were above 3 nM, the K_d of ^3H -DHEC binding was 5.5 ± 1.7 nM and the b_{max} was 97.3 ± 18.8 fmol per mg protein. (1, 2, 4, 8, 16 nM.)

* Indicates significant difference ($P < 0.005$) from the corresponding control value.

potent in blocking post- than presynaptic α -receptor responses¹², and the unexplained high potency of clonidine (K_d 0.23 μM) in suppressing the binding of 8 nM ^3H -DHEC in an earlier study³ is also compatible with the involvement of presynaptic receptors. However, more direct evidence is needed to decide this issue.

The present results should caution against prematurely equating ligand binding sites characterised *in vitro* with receptors subserving a well defined physiological function *in vivo*. ^3H -DHEC binding described in earlier studies in uterine^{2,4} or vascular smooth muscle⁵ differed from the high-affinity binding component in the present experiments: the K_d of ^3H -DHEC was 8–10 nM, and only high concentrations of POB suppressed specific binding⁴. This indicates that the sites involved were not smooth muscle α -receptors mediating contraction. Changes in the density of such ^3H -DHEC binding sites in oestrogen- and progesterone-treated rabbit uterus¹⁵ have been suggested to contribute to or explain the reciprocal change in α - and β -receptor-mediated mechanical responses of such preparations. As the binding sites detected are unrelated to smooth muscle α -receptors, the physiological significance of changes in their density remains to be established.

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Substance P inhibits nicotinic activation of chromaffin cells

THERE is much interest in the possibility that substance P acts as a neurotransmitter and/or a neuromodulator at certain sites in the central and peripheral nervous system (for reviews see refs 1, 2). Although both excitatory^{3–6} and inhibitory^{6,9–11} actions have been observed *in vivo* and *in vitro*, the cellular mechanism of action of substance P has not been defined. Recent electrophysiological studies in the cat^{9–11} have provided evidence that substance P selectively depresses the nicotinic excitation of Renshaw cells by microiontophoretic application of acetylcholine (ACh). We show here that substance P inhibits the ACh-induced nicotinic response of adrenal chromaffin cells in culture to release ^3H -noradrenaline.

Intact and functional chromaffin cells were isolated from bovine adrenal glands by retrograde perfusion of the dissected medullae with collagenase (type 1, Sigma) in a Locke's buffer (pH 7.0) and purified by density gradient centrifugation on

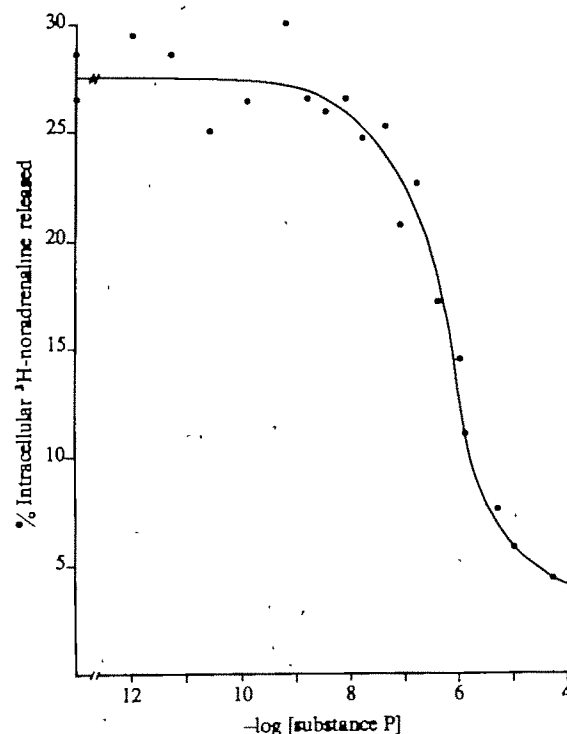


Fig. 1 Inhibition by substance P (10^{-4} – 10^{-12} M; Beckman) of the release of ^3H -noradrenaline from cultured bovine adrenal medullary cells evoked by ACh (5×10^{-5} M). The percentage of the total cellular ^3H -noradrenaline released by 5×10^{-5} M ACh in the absence of substance P is shown on the ordinate. The results of a typical experiment are shown, where each point represents the release from a single, separate culture dish.

Table 1 Effect of substance P (SP) and Ca^{2+} on the release of ^3H -noradrenaline from cultured adrenal chromaffin cells

Secretagogue	Addition	% Intracellular ^3H -noradrenaline released mean $\pm$ s.d. (n)
5×10^{-5} M ACh	2.2 mM Ca^{2+}	20.9 $\pm$ 3.1 (47)
	5×10^{-5} M SP + 2.2 mM Ca^{2+}	3.9 $\pm$ 1.3 (5)
	No Ca^{2+}	1.8 $\pm$ 0.4 (3)
	1 mM EGTA	0.9 $\pm$ 0.1 (3)
5×10^{-6} M nicotine	2.2 mM Ca^{2+}	16.8 $\pm$ 2.4 (5)
	5×10^{-5} M SP + 2.2 mM Ca^{2+}	2.1 $\pm$ 0.4 (3)
	2.2 mM Ca^{2+}	15.3 $\pm$ 1.5 (7)
	5×10^{-5} M SP + 2.2 mM Ca^{2+}	15.1 $\pm$ 0.9 (2)
56 mM K^+	2.2 mM Ca^{2+}	2.3 $\pm$ 0.2 (3)
	No Ca^{2+}	2.3 $\pm$ 0.2 (3)

Percoll as described previously¹²⁻¹⁴. The purified cells were plated on collagen-coated plastic dishes (Corning 35 mm, 10^6 cells per plate) in Dulbecco's modified Eagle's medium (DMEM; Gibco), pH 7.2, in conditions shown to favour sympathetic neurite outgrowth in culture¹⁵. The cells attached firmly within 3 h, and by 8 h had extended processes with growth cones which eventually made contacts with other cells^{13,16}. The cells and their varicose processes contained high concentrations of catecholamines, as demonstrated by the glyoxylic^{14,17} and formaldehyde-glutaraldehyde^{14,18} fluorescence histochemical methods. By day 4 in culture they exhibited many of the morphological features of noradrenergic neurones in culture.

The pharmacological experiments were carried out on chromaffin cells after 4 and 5 days in culture. The plates were loaded for 2 h at 37 °C with ^3H -noradrenaline as described by Greene and Rein¹⁹ for sympathetic nerve cultures. In this technique any unbound amines and metabolites are washed out in a subsequent 2 h period and the washout medium replaced with HEPES-buffered Krebs-Ringer saline (KRH) for 15 min²⁰; however, instead of the 2.6 mM Ca^{2+} concentration used by Greene and Rein we used 2.2 mM Ca^{2+} as our previous work¹² has shown this concentration to effect maximum stimulation of ACh-induced catecholamine release from isolated adrenal medullary cells. After this adaptation period, the plates were incubated in KRH (5 min); KRH containing nicotinic and muscarinic agonists and/or antagonists (5 min); and KRH alone (5 min). Substance P, when tested, was present during the first two 5-min incubation periods. The radioactivity in each of the three incubation media and that remaining in the cells was determined as described by Greene and Rein¹⁹ using a Nuclear Chicago Isocap 300 scintillation counter, with external standards ratio. The results are expressed as the amount of ^3H -noradrenaline released in the 5-min stimulation sample expressed as a percentage of the total radioactivity originally present in the cells.

Pharmacological studies with nicotinic (nicotine, ACh) and muscarinic (methacholine) agonists and their antagonists (mecamylamine HCl; hexamethonium and atropine, respectively) showed that the adrenal chromaffin cells contained only nicotinic receptors. Methacholine (10^{-10} – 10^{-4} M), a muscarinic agonist, did not stimulate the cells to release ^3H -noradrenaline, and the ACh-induced release of ^3H -noradrenaline (ED_{50} 5×10^{-5} M) was not inhibited by low concentrations (10^{-5} – 10^{-10} M) of atropine. By contrast, substance P produced a dose-dependent inhibition of ACh (5×10^{-5} M) stimulated ^3H -noradrenaline release (Fig. 1) in the range 10^{-8} – 5×10^{-5} M, with an ID_{50} of approximately 5×10^{-7} M.

A similar inhibition of noradrenaline release by substance P was obtained when nicotine (5×10^{-6} M) was used as the agonist, but not when K^+ (56 mM) was used to depolarise the cells (Table 1). Omission of Ca^{2+} reduced the release induced by 5×10^{-5} M ACh and by 56 mM K^+ to 1.8% and 2.3%, respectively, of the total intracellular ^3H -noradrenaline, and addition of EDTA (1 mM) or EGTA (1 mM) reduced it to the basal

release level of 0.9% (Table 1). Substance P (5×10^{-5} – 10^{-10} M) by itself did not have a significant effect on the basal release rate of ^3H -noradrenaline from these cells in the presence of 2.2 mM Ca^{2+} .

As pharmacological studies have shown that isolated chromaffin cells^{12,21,22} contain only nicotinic receptors, our present results provide the first direct evidence at a cellular level that substance P acts as an inhibitory modulator of the nicotinic ACh response. Taken with the earlier microiontophoretic studies *in vivo*⁹⁻¹¹, our results support a role for substance P as an inhibitory neuromodulator at nicotinic receptor sites in the nervous system²³.

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Efficient induction of immediate tolerance to contact sensitivity by hapten-modified spleen cells requires Ia⁺ cells compatible with recipient

IMMUNOLOGICAL distinction between 'self' and 'non-self' is important in maintaining normal homeostasis: a breakdown in this regulation probably results in auto-destructive processes. Recent developments in cellular immunology have shown that the induction of an immune response (sensitisation) involves the recognition of antigen in combination with products of the major histocompatibility complex (MHC)^{1,2}. In this context, it is pertinent to ask whether the induction and maintenance of specific immunological unresponsiveness (tolerance) to foreign antigens is also under genetic control. Contact sensitivity and tolerance to simple chemical compounds (haptens) in mice has provided insight into the complex cellular interactions involved in the unresponsive state³⁻⁵. Parenteral injection of the reactive

Table 1 Efficient induction of immediate tolerance to contact sensitivity of DNFB-modified spleen cells requires Ia-bearing cells

Treatment of DNP-SC tolerogen	DNFB sensitisation	DNFB challenge	Ear swelling (inches $\times 10^{-3}$) $\pm$ s.e.m.	% Tolerance
—	+	+	79.2 $\pm$ 3.7	0
NMS + C	+	+	25.8 $\pm$ 3.3*	71
Anti-Ia + C	+	+	59.5 $\pm$ 4.8*	26
—	—	+	3.6 $\pm$ 1.5	—

CBA/J erythrocyte-free spleen cell suspensions were prepared in Hank's balanced salt solution (HBSS). The cells were resuspended to 10^8 cells per ml in HBSS and either NMS or anti-Ia (A.TH into A.TL) antisera added at a concentration of 0.1 ml per 10^8 cells followed by incubation on ice for 60 min. Rabbit complement was then added and the suspension incubated at 37 °C for 30 min. Dead cells were removed by Ficoll-Isopaque gradient centrifugation⁹. Living cells were collected, labelled with DNFB as previously described⁴, and 3×10^6 DNP-SC injected on day 1 intravenously into age-matched CBA/J recipients. The recipient animals were sensitised with either 0.5% DNFB in acetone: olive oil (4:1 v/v) or acetone: olive oil (negative control) on days 0 and 1. On day 5, the recipients were ear challenged with 0.2% DNFB and swelling measured 24 h later. The values represent the mean 24-h ear swelling (4 members per group) $\pm$ s.e.m. The amount of tolerance obtained was calculated by comparing the response of each experimental group to that of positive (sensitised and ear challenged) and negative (ear challenged only) control groups: % Tolerance = [(positive - experimental)/(positive - negative)] $\times$ 100%.

* $P < 0.001$.

haptens 1-fluoro-2,4-dinitrobenzene (DNFB) or its sulphonate (DNBS) before epicutaneous sensitisation with DNFB results in unresponsiveness^{3,4}. This process seems to be mediated by at least two mechanisms, clonal inhibition and induction of suppressor T cells (T_s)⁶, both of which can be induced by either DNBS or DNFB coupled *in vitro* to red blood cells (DNP-RBC). The maximum tolerant state then develops 4–7 d after injection. However, another tolerogen, DNFB coupled *in vitro* to spleen cells (DNP-SC), produces maximum unresponsiveness immediately. This rapid induction of unresponsiveness was shown to be independent of T_s and thus was referred to as clonal inhibition⁶. The dichotomy between syngeneic DNP-SC and DNP-RBC in their ability to tolerate immediately was first thought to be due to either different homing characteristics or cell surface components⁴. With respect to the latter, an intriguing difference between the two tolerogens is the lack of I-region associated (Ia) antigens (of the MHC) on the surface of red blood cells^{7,8}. We have investigated this difference by tolerising mice with DNP-SC depleted of Ia⁺ cells or with DNP-SC from donors which are I-region incompatible with respect to the recipient animal. The results reported here indicate that the induction of immediate tolerance requires I-region compatibility between the donor of the DNP-SC tolerogen and the recipient.

Mice were tolerised with DNP-SC pretreated with either normal mouse serum (NMS) or anti-Ia antiserum plus rabbit complement. After the serum treatment and before labelling

with DNFB, dead cells were removed by differential centrifugation in Ficoll-Isopaque gradients⁹. One day after tolerisation the recipient mice plus appropriate controls were sensitised by two paintings with DNFB. All animals were ear challenged 5 d later and ear swelling measured 24 h later. The results are shown in Table 1. DNP-SC treated with NMS induced 71% tolerance. In contrast, DNP-SC which had been depleted of Ia⁺ cells induced only 26% tolerance, which is equivalent to the amount of unresponsiveness induced by another Ia-negative population of cells, DNP-RBC⁴. This decrease in tolerogenicity of DNP-SC depleted of Ia⁺ cells indicated that Ia⁺ cells are required to produce efficient immediate tolerance.

Further experiments were then done using the proper congenic strains to ascertain whether or not the Ia antigens of the tolerogen had to be identical with the recipient. For this analysis, it is necessary to keep K and D ends of the MHC compatible between the two so as to minimise any allogeneic effects. As seen in Table 2, when the tolerogen was incompatible with the recipient at the I region (DNP-A.TL into A.TH), there was no immediate induction of tolerance (0%) as compared to a syngeneic tolerogen (DNP-A.TH into A.TH), which produced immediate tolerance (53%). These results indicate that if the tolerogen and recipient share identical K and D regions, then I-region compatibility is required to induce efficient immediate tolerance.

The ability to induce unresponsiveness rapidly was also shown in the picryl chloride (Picl) system to depend on the cell population which was haptenated with picryl sulphonic acid (PSA) *in vitro*⁵. Whereas hapten-coupled spleen and lymph node cells were highly efficient at inhibiting contact sensitivity to Picl, PSA coupled to erythrocytes and thymocytes were ineffective. As the latter populations are thought to be deficient in Ia antigens^{7,8}, these results support the idea that induction of immediate tolerance requires hapten on Ia-compatible cells.

It must be pointed out, however, that the requirement for Ia-bearing cells is not seen when the sensitisation regimen is delayed. Miller and H.N.C.⁴ could not detect any differences between several haptenated cell populations (spleen, lymph node, peritoneal exudate cells and thymus) in their ability to induce tolerance to DNFB if the animals were tolerised 7 d before sensitisation. A possible explanation for this apparent inconsistency would be that host reprocessing has occurred during the longer time interval, such that the tolerogen is now expressed on the proper MHC background.

Host processing might also explain the observation that completely allogeneic DNFB-labelled cells (that is, K, D and I region incompatible) are as efficient as a syngeneic tolerogen in their ability to induce tolerance¹⁰. It is possible that alloreactivity might accelerate or somehow alter normal host processing, thereby allowing re-expression of the tolerogen on a self-MHC background. Therefore, I-region compatibility between the DNP-SC donor and the recipient is not required when K and D regions are different.

It is unlikely that the tolerant state induced here by DNP-SC is due solely to the production of a significant number of T_s because of the rapidity with which the unresponsiveness develops^{4,6}. Further evidence for the delay in the development

Table 2 Efficient induction of immediate tolerance to contact sensitivity by DNFB-modified spleen cells requires I-region compatibility between the tolerogen and recipient

Donor of DNP-SC tolerogen (KID)	DNFB sensitisation	DNFB challenge	24-h ear swelling (inches $\times 10^{-4}$) $\pm$ s.e.m.	% Tolerance	MHC compatibility between tolerogen and recipient
—	+	+	60.0 $\pm$ 3.8	0	—
A.TH (asd)	+	+	35.2 $\pm$ 2.0*	53	KID
A.TL (skd)	+	+	60.7 $\pm$ 2.9*	0	KD
—	—	+	13.3 $\pm$ 3.1	—	—

Donor spleen cells were prepared and labelled as described in Table 1. 3×10^6 DNP-SC were injected intravenously into A.TH recipients. The recipients were then sensitised and challenged as in Table 1.

* $P < 0.001$.

of T, is the fact that immune lymph node cells are not inhibited when transferred into animals tolerised 1 d previously with DNP-SC⁶. Also, cyclophosphamide pretreatment (which eliminates T_H precursors) does not alter the kinetics of immediate tolerance induction⁶. Therefore, we believe that DNP-SC is immediately inducing clonal inhibition. The tolerogen may act by blocking or inhibiting the T-cell receptors, thus preventing expansion of the specific clones after antigenic stimulation.

The exact role of Ia-bearing cells in the induction of immediate tolerance is unclear. However, one interpretation is that some T-cell clones which are required for the development of contact sensitivity may recognise DNFB in association with I-region gene products. Other investigators have reported that T cells recognise antigen plus surface MHC structures in both the induction and/or elicitation of immune responses, including delayed hypersensitivity¹¹, T-cell-B-cell collaboration¹², cytotoxic T-cell killing of virus-infected¹³ or hapten-modified target cells¹⁴, and T-cell proliferation *in vitro*^{1,2}.

The above mentioned studies indicate that the presentation of antigen in association with macrophage Ia determinants activates the T cell. In contrast, our results suggest that perhaps antigen, in association with an Ia-bearing cell, is capable of tolerising T cells. Thus, the decision for either tolerance or immunity depends not only on the nature of the ligands presented (antigen with Ia) but also on either their pathway of presentation (perhaps the tolerogenic action of DNP with Ia⁺ cells is due to the fact that it is not presented on macrophages) or their delivery with or without a second signal. This second signal would be required to activate the T cell; in its absence, tolerance results¹⁵.

It should be noted, however, that the relationship between hapten and Ia determinants has not been completely resolved. The hapten PSA has been shown to derivitise murine H-2 antigens *in vitro*¹⁶ but not the Ia-like structure in strain 13 guinea pigs¹⁷. Therefore, although both the hapten and Ia are required in immediate tolerance induction, the hapten may not be covalently attached to Ia but rather associated with some other structure on the Ia⁺ cells. In this case, the interaction is between the hapten-bearing cell and the target cell. Further experiments are underway to determine the exact role of Ia in the induction of immediate tolerance.

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Serum amyloid P-component is an acute-phase reactant in the mouse

THE acute-phase reaction in mammals is characterised by an increase in the concentration of certain plasma proteins which starts within hours or days of most forms of acute tissue damage or inflammation, and persists with chronic inflammation and malignant neoplasia¹⁻⁴. Among acute-phase reactants are protease inhibitors, coagulation factors, complement components and transport proteins as well as proteins without known *in vivo* roles, such as the classical reactant, C-reactive protein (CRP)¹⁻⁴. The pathophysiological significance of the acute-phase reaction is not known, but it is a usual accompaniment of tissue damage, and it is of clinical value in the assessment of disease activity⁴. We now report that in the mouse, serum amyloid P-component (protein SAP)⁵, which is a stable plasma protein in man⁶, is a major acute-phase reactant. In addition, our observations facilitate investigation of the function of protein SAP and of the pathogenesis of amyloidosis, which is a serious disease in man.

Mouse SAP was isolated in different experiments from normal serum, acute phase serum following croton oil injection and ascitic fluid induced either by repeated intraperitoneal injection of Freund's complete adjuvant or by growth of the lymphocytoma McPc 1748 (ref. 7). Mouse CRP was isolated from the same acute-phase sera and ascitic fluids. The isolation procedures were those previously described for the human counterparts^{8,9} and are detailed specifically for the murine proteins elsewhere¹⁰. The SAP from the different sources was indistinguishable. Its behaviour on gel filtration was comparable to that of human SAP¹⁰, and it was homogeneous in gradient

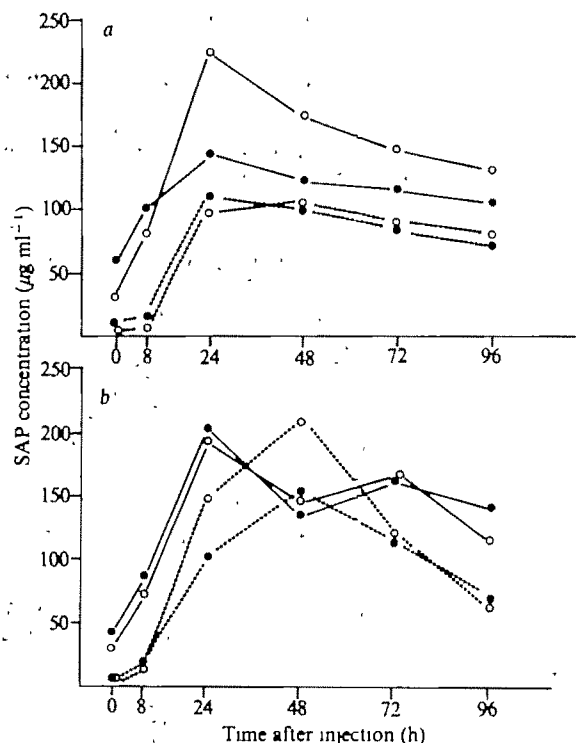


Fig. 1 Effect of acute inflammation on murine serum levels of protein SAP. *a*, Subcutaneous injection of 0.2 ml of a 1% croton oil in liquid paraffin, *b*, intraperitoneal injection of 100 µg of *S. enteritidis* lipopolysaccharide W (Difco). Solid lines, CBA/Lac mice, broken lines, C57BL/6 mice; ○, males; ●, females. There were 5 mice in each group and the values shown are the arithmetic means of all animals tested individually in each group. Standard deviations are not plotted but the coefficient of variation was generally <10% except at the peak values when it was 15-25% in all groups.

polyacrylamide gel electrophoresis (PAGE) and reduced SDS-PAGE¹⁰, with a single subunit of molecular weight 25,300 (ref. 11). In the electron microscope mouse SAP was seen as pentameric disks, 8 nm in diameter, with a tendency to interact in pairs face-to-face (E. A. Munn, A. Feinstein and M.B.P., unpublished observations); this closely resembles human SAP⁹. Amino-terminal analysis revealed a blocked N terminal (C. Milstein, A. Feinstein and M.B.P., unpublished observations). These features, and the serum concentrations reported below, distinguish mouse SAP from serum amyloid A (SAA) and other known proteins.

Immunisation with the isolated SAP induced a monospecific rabbit antiserum which was used in an electroimmunoassay¹² of serum SAP calibrated with the pure protein. Murine serum CRP was measured by electroimmunoassay using a monospecific sheep anti-human CRP which cross-reacted with mouse CRP; isolated mouse CRP served as the standard.

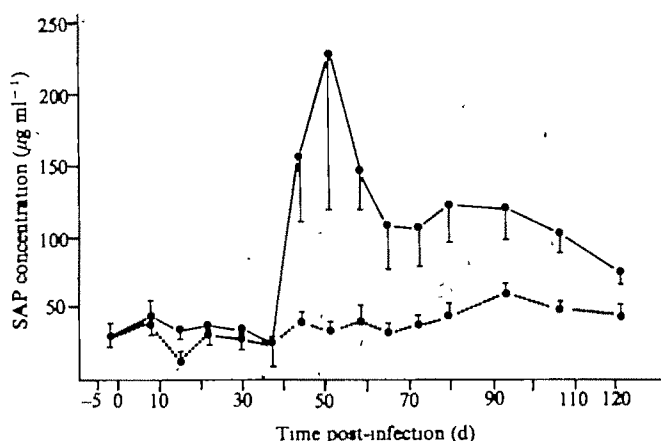


Fig. 2 Effect of *S. mansoni* infection on serum levels of protein SAP in male CBA/Lac mice. Each point represents the arithmetic mean with 1 s.d. of 6 animals in each group. Solid lines, mice infected percutaneously with 20 cercariae¹³ on day 0; broken lines, non-infected age-matched controls.

SAP concentrations rose four- to six-fold in the sera of CBA mice and up to 50-fold in C57BL/Cbi mice within 24–48 h of injection of *Salmonella enteritidis* lipopolysaccharide W (LPS) or croton oil (Fig. 1). On day 32 after the injections, the levels were at pretreatment values in the LPS experiment but were still elevated in the mice given croton oil (100 µg ml⁻¹ in CBA, 10–20 µg ml⁻¹ in C57BL/Cbi mice). Forty days after infection of CBA mice with cercariae of *Schistosoma mansoni*¹³ the serum levels rose sharply and remained elevated (Fig. 2). This coincides with the time of incidence of granulomatous inflammation in the liver. Mice which had received implants of isogenic or xenogenic tumours also had raised serum SAP concentrations (Fig. 3).

The elevation of murine SAP levels after both acute stimuli and more protracted events, such as parasitic infestation and tumour growth, differs from the behaviour of SAP in man. The normal human serum level of SAP is 30–40 µg ml⁻¹; it does not alter significantly after major surgery and is only slightly increased during chronic active diseases⁶. On the other hand, the level of human CRP in disease ranges up to 600 µg ml⁻¹ (refs 6, 14), whereas, although the mouse CRP concentration rose in the present experiments, it did not exceed 2 µg ml⁻¹. These data agree with the results of a new fluorimetric immunoassay for CRP¹⁵. In man, CRP and SAP share substantial homologies of amino acid sequence, similar subunit composition and comparable appearance in the electron microscope¹⁶, and they both undergo calcium-dependent ligand binding^{8,9}. However, the specific interaction of CRP with pneumococcal C-polysaccharide⁸ and of SAP with agarose⁹ has been used to isolate

them in pure form, and also to identify very similar proteins in the sera of fish, amphibia, birds and non-primate mammals¹¹. These proteins from lower animals share amino acid sequence homologies with their human counterparts and each other (C. Milstein, A. Feinstein and M.B.P., unpublished observations). The acute-phase behaviour of murine SAP suggests that there may also be a functional overlap between CRP and SAP in different species.

The consistent differences in serum level of protein SAP observed in healthy adult CBA and C57BL/Cbi mice before acute-phase stimulation (Fig. 1) were confirmed in animals of the same strains bred and housed in other institutions. Sera from other healthy normal mice showed a consistent pattern of interstrain variation over a 50-fold range (Fig. 4). F₁ hybrids of high (DBA/2) and low SAP (C57BL/6) strains bred in two different institutions had low SAP levels. Outbred nude (athymic) mice and T-deprived CBA/Lac mice¹⁷ had levels comparable with those of intact animals (Fig. 4), suggesting that T-dependent immunocompetence is not required for maintenance of normal levels. The commensal flora are also not essential, as serum SAP concentration was not significantly different in conventional and germ-free CBA/Lac mice (Fig. 4). The observed strain variation suggests a genetic influence on SAP production but it remains to be established whether this is a direct primary effect on the protein synthetic and/or catabolic rates or whether it is secondary to hereditary influences on susceptibility to prevalent acute-phase stimuli such as microbial infection or physical factors.

The normal role of protein SAP *in vivo* is not known, but this protein is a minor constituent of amyloid deposits¹⁸ both in man and in experimental animals. We have recently observed sustained elevation of the serum SAP level during induction of amyloidosis in mice¹⁹, suggesting that it may have pathogenetic significance.

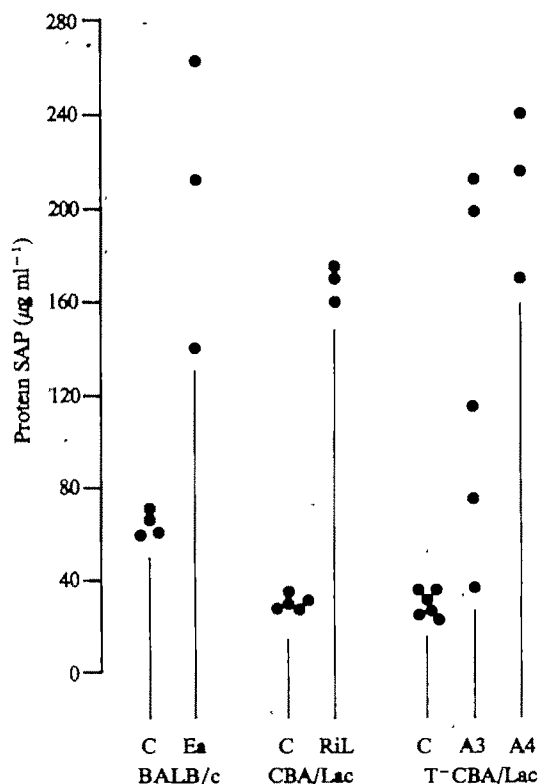


Fig. 3 Effect of tumour implantation on murine serum levels of protein SAP. C, control non-implanted mice; Ea, Ehrlich's ascites tumour, 5 d post-implantation; RiL, radiation-induced leukaemia, 6 d post-implantation; A3, human astrocytoma grade III, 6 d post-implantation; A4, human astrocytoma grade IV, 3 months post-implantation; T-CBA/Lac, T-deprived CBA/Lac mice¹⁷.

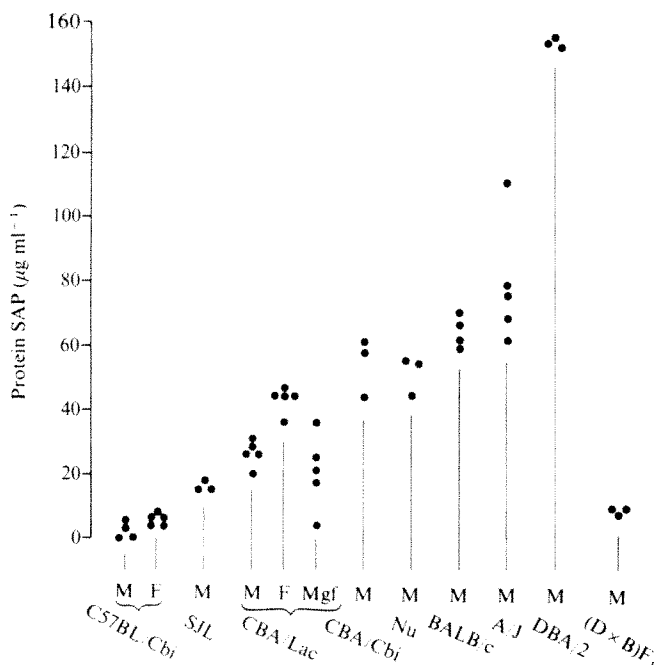


Fig. 4 Serum levels of protein SAP in healthy adult mice of different strains. Each point represents a single estimation in an individual animal. M, males; F, females; Mgf, germ-free males; Nu, outbred nude mice; (D×B)F₁, (DBA/2×C57BL/6)F₁ hybrids.

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T antigen is bound to a host protein in SV40-transformed cells

THE early region of the small DNA tumour virus, simian virus 40 (SV40), is known to code for at least two polypeptides, the t and T antigens ('small t' and 'large T'). Both these polypeptides are expressed in cells transformed by the virus¹⁻⁴, and the T antigen has been shown to be essential for both the initiation and maintenance of the transformed state⁵⁻⁹. We therefore need to know how this T protein interacts with components of the host cell in order to understand the mechanism of SV40-induced transformation. We report here that the T antigen in a line of SV40-transformed mouse cells forms an oligomeric complex with a specific cell coded protein.

When an extract of the SV40-transformed mouse cell line SVA31E7 is immunoprecipitated by as little as 1 µl of a rabbit antiserum raised against purified T (ref. 10), two polypeptides are clearly specifically immunoprecipitated (Fig. 1, tracks 0, 1). The most slowly migrating species has a molecular weight of 94,000 and is identical to the T antigen precipitated from extracts of SV40 lytically infected monkey cells^{10,11}. The faster migrating species has an approximate MW of 53,000 (53 K) and has not been detected in SV40 lytically infected monkey cells. These same two polypeptides were also specifically immunoprecipitated by other anti-T sera: hamster anti-T sera (raised by inoculating the animals with the SV40-transformed hamster cell line, H65), mouse anti-T sera (raised by repeatedly immunising BALB/c mice with the SV40-transformed mouse cell line, SVT2 (ref. 12)), and anti-U sera, which were raised in monkeys and are specific for the C-terminal end of T (refs 13,14).

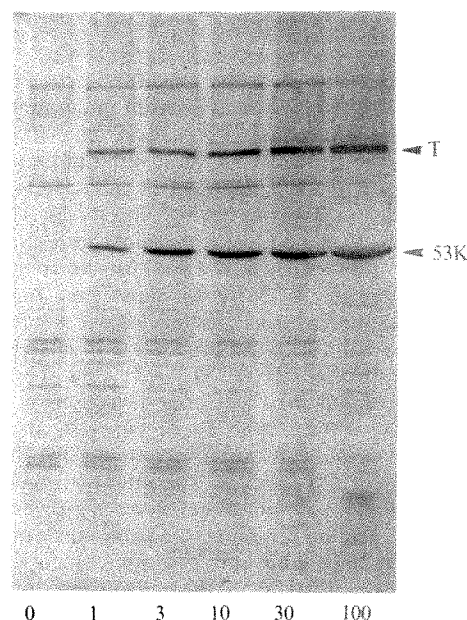


Fig. 1 Quantitation of T and 53K immunoprecipitation by rabbit anti-T serum. An equivalent aliquot of an NP40 cell extract of ³⁵S-methionine-labelled SVA31E7 cells was added to each of 6 tubes followed by normal rabbit serum and rabbit anti-T serum in the following respective amounts: track 0, 30 µl, 0 µl; track 1, 29 µl, 1 µl; track 3, 27 µl, 3 µl; track 10, 20 µl, 10 µl; track 30, 0 µl, 30 µl; track 100, 0 µl, 100 µl. After 3 h incubation at 4 °C, 100 µl of a 10% suspension of fixed *Staphylococcus aureus*²⁰ was added to each tube and following a further 10 min incubation the bacteria were washed 3 times in NET buffer²⁰ and collected by centrifugation. Bound proteins were eluted in 55 µl of sample buffer and 10 µl of each eluate loaded on a 7-20% linear gradient acrylamide gel. The dried gel was autoradiographed for 24 h.

The immunoprecipitation of the 53K protein by the rabbit anti-T serum has two possible explanations, either that the 53K protein shares common antigenic determinants with the gel-purified T antigen used to immunise the rabbit or that the protein is bound in a complex to another species that has such antigenic determinants. The 53K protein could then be specifically immunoprecipitated by the anti-T serum in a 'piggy-back' fashion, in the same way, for example, as antisera to human $\beta 2$ -microglobulin immunoprecipitate HLA antigen¹⁵.

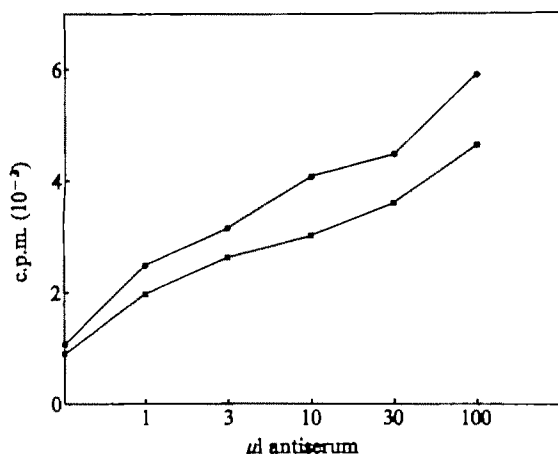


Fig. 2 The strips of dried polyacrylamide gel containing the T antigen (●) and 53K protein (■) from each track were excised, using the autoradiograph as a template, from the gel shown in Fig. 1. Each strip was solubilised²¹ and counted. The c.p.m. present in each band in each track are plotted against the input of rabbit anti-T serum used to immunoprecipitate the cell extract.

To distinguish between these two possibilities we first quantitated the ability of the rabbit anti-T serum to immunoprecipitate the 53K protein, and the T antigen from an extract of SVA31E7 cells. The precipitation ratio for the two proteins (c.p.m. in T/c.p.m. in 53K) remained essentially constant over a 100-fold range of anti-T serum input (Figs 1, 2). Therefore, either the serum has a very similar titre against both species, that is, the 53K protein and T share the majority of the antigenic determinants recognised on T by this antiserum, or the 53K protein is being immunoprecipitated because it is bound to T.

Normal rabbit serum does not precipitate a detectable 53K band from untransformed mouse 3T3 A31 cells. This may be due to one or both of two reasons. Either the cells do not contain the 53K protein or, as there is no T with which it can complex, the 53K protein present is not precipitated by the rabbit antiserum specific for T. A similar experiment to that shown in Figs 1 and 2 with a lower input of cell extract showed that the ratio of T to 53K protein remained the same in serum excess.

It is known that the rabbit anti-T serum, which was raised against T antigen purified by SDS-polyacrylamide gel electrophoresis, recognises a set of determinants on T that are very stable, being resistant to heat, SDS and disulphide bond reduction (ref. 10 and D.P.L. and L.V.C., unpublished). Therefore, we used a direct binding radioimmunoassay to determine whether the isolated 53K protein possessed these antigenic determinants. The results of this assay (Fig. 3) indicate that 53K protein separated from T by SDS-gel electrophoresis is no longer precipitated by the rabbit anti-T serum, whereas the T antigen isolated in the same way is still fully reactive. This shows that the determinants recognised by the rabbit anti-T serum on T are absent from the 53K polypeptide. The immunoprecipitation of the 53K protein from cell extracts by this serum must therefore be due to the fact that it exists in a complex with T. Preliminary gel chromatography results are consistent with this

idea. T and the 53K protein eluted together from Sephacryl S-200 (exclusion limit 0.25×10^6) in the excluded volume (Fig. 4). Sucrose gradient analysis of extracts of transformed cells has shown that the 53K protein and a fraction of the T antigen sediment together at about 14–16S (F. McCormick, personal communication). Co-elution or co-sedimentation clearly do not prove that T and the 53K protein occur in the same complex but are consistent with the complex idea.

We then tested all the other sera which had immunoprecipitated T antigen and the 53K protein from the cell extract to see whether they could bind to the isolated 53K protein and T antigen. The anti-U serum and several different batches of hamster anti-T sera behaved like the rabbit anti-T polypeptide serum in that they efficiently re-immunoprecipitated the gel-purified T antigen but failed to bind the isolated 53K polypeptide. In contrast, the hyperimmune mouse anti-T serum specifically bound both the isolated 53K polypeptide and the T antigen (Fig. 3a, c). The binding of this mouse serum to the gel-purified T antigen from the SVA31E7 cells could be completely inhibited by an unlabelled extract of SV40-infected monkey cells but this treatment did not reduce its titre against

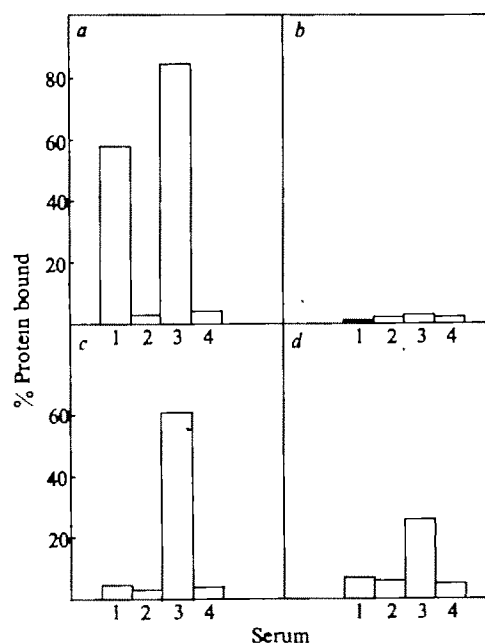


Fig. 3 Serum binding to purified polypeptides. Polypeptides for binding assays were purified from ³⁵S-methionine-labelled cell extracts by immunoprecipitation and SDS-polyacrylamide gel electrophoresis. Cells (PY6 or SVA31E7) were grown in 90-mm dishes in E4 medium with 10% calf serum, and labelled for 3 h with 500 µCi ³⁵S-methionine, then extracted in 0.5% NP40 Tris, pH 8.0, for 30 min on ice¹⁰. To the supernatant of the centrifuged extract, 100 µl of our hamster anti-T serum (for A31E7 cells) or 100 µl of mouse anti-T serum (for PY6 cells) was added. Three hours later 200 µl of *S. aureus* was added and the bacteria washed, collected and eluted as described for Fig. 1. The eluted proteins were separated on 15% polyacrylamide slab gels. The gel fragments containing the polypeptides of interest were located by autoradiography of the dried gel, excised with a scalpel and rehydrated in 2 ml of 0.1 M pH 9 bicarbonate buffer containing 0.1% SDS. Binding assays were carried out as described previously¹⁰ except that 50 µl of *S. aureus* was used in the final step. a, Binding to T polypeptide from A31E7 cells by: (1) 10 µl rabbit anti-T serum, (2) 10 µl normal rabbit serum, (3) 10 µl mouse anti-T serum, (4) 10 µl normal mouse serum. b, Binding assay control. Binding to a 42K protein from A31E7 cells which is nonspecifically precipitated from cell extracts. Sera as in a. c, Binding to 53K protein specifically precipitated from A31E7 cells by sera as in a. d, Binding to 52K protein, specifically precipitated from PY6 cells with mouse anti-T serum, by sera as in a. Ordinate is the % of input ³⁵S-labelled protein bound to *S. aureus*. Correction for counter background only.

the 53K protein. Its binding of the 53K protein was, however, inhibited by unlabelled extracts of the SV40-transformed mouse cell lines, SVT2 (the cell line used to immunise the mouse) and SVA31E7 (the cell line from which the purified 53K polypeptide was prepared). Therefore, both the 53K protein and T antigen have unique non-cross-reactive antigenic determinants and the mouse serum contains some antibodies specific for the 53K protein and some specific for T antigen.

This conclusion has been supported by the results of *in vitro* synthesis experiments carried out in collaboration with E.

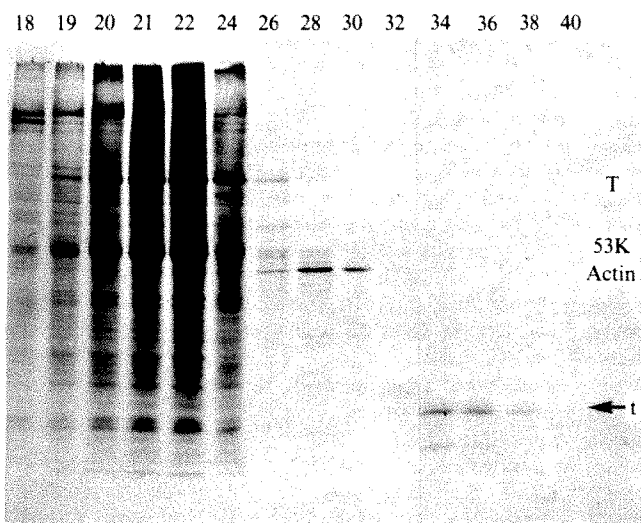


Fig. 4 Gel chromatography of transformed cell extract. Cell extract from ^{35}S -methionine-labelled SVA31E7 cells (10^9 c.p.m.) was applied to a Sephacryl S-200 column ($1.5 \text{ cm} \times 34 \text{ cm}$) and eluted with buffer containing 0.1 M NaCl , 0.01 M Tris , $\text{pH } 8.0$, $1 \text{ mM dithiothreitol}$ and $5\% \text{ glycerol}$. One-ml fractions were collected and 0.1 ml of selected fractions immunoprecipitated with hamster anti-T serum. The immunoprecipitates were analysed by gel electrophoresis as described for Fig. 1. Each track is labelled with the fraction number, indicating the number of ml from addition of the sample to the column. The void volume of the column was estimated to be 20 ml . Much of the T antigen and 53K protein eluted around this position, whereas t was found around 35 ml and the $42,000$ band, thought to be actin, at around 28 ml .

Paucha. When polyadenylated mRNA from SVA31E7 cells was translated in the mouse L-cell protein synthesising system, our hamster anti-T serum immunoprecipitated the T and t polypeptides but not the 53K protein, whereas this same serum could immunoprecipitate both T and the 53K protein from *in vivo* labelled cell extracts. This is not due to a failure to translate the mRNA coding for the 53K protein, as when the same *in vitro* product was immunoprecipitated by the mouse anti-T serum (which contains antibodies to the isolated 53K protein), the 53K protein could then be detected in addition to the T and t polypeptides. This indicates that the T antigen and 53K protein do not assemble to form a complex in the *in vitro* conditions used in the translation reaction.

We have only encountered one other batch of serum able to bind the isolated 53K protein from SVA31E7 cells. This serum was raised in hamsters by inoculation with TSV5 C12 hamster transformed cells. Such reactivity explains why P. May *et al.* (in preparation), in contrast to the previous results of E. Paucha (personal communication), have been able to detect the 53K protein in their cell-free translation of polyadenylated mRNA from SV40-transformed mouse cells.

We postulate that the 53K protein is not virally coded because (1) we could not detect it in lytically infected monkey cells, (2) it shares no detectable antigenic determinants with T, but does have its own set of antigenic determinants (immunogenic in syngeneic mice), and (3) the DNA sequence and mRNA map-

ping data indicate that it would be difficult for the early region of SV40 to encode a third polypeptide of $53,000 \text{ MW}^{16-18}$. If the protein is not coded for by the virus, then it might be detectable in cells transformed by other viruses, by radiation, by chemical carcinogens or even in untransformed cells. In these cases it would not be complexed to T antigen and would therefore be precipitated only by antibodies specific for the 53K protein itself but not by antibodies to T antigen. An initial study supports this idea. When extracts of the polyoma-transformed mouse cell line PY6 were immunoprecipitated with the rabbit anti-T serum, no specific band was detected; immunoprecipitation of the same extract with the mouse anti-T serum (which contains antibodies to the 53K protein) did specifically bring down a band of $\sim 52,000 \text{ MW}$. This cross-reactive protein, when isolated from the gel, was reprecipitated specifically by the mouse antiserum (Fig. 3d). Significantly, it was also specifically bound by the anti-TSV5 C12 serum but not by anti-U serum, rabbit anti-T polypeptide serum (Fig. 3d) or our hamster anti-T serum. The 52K protein from PY6 cells was always precipitated to a lesser extent than the 53K protein. For this reason we believe that the two proteins are related but not identical.

It will be necessary to establish further the nature of the 53K protein, but its specific binding to T antigen, and the presence of an antigenically related protein in polyoma-transformed cells imply that it may have a crucial role in the modulation of the transformed state. Thus, it is possible that it acts to neutralise some of the specific functions of T antigen; for example, it could render the cell non-permissive by preventing the T-dependent initiation of viral replication¹⁸ or it may normally act as a regulator of certain cellular functions related to growth control and itself be neutralised by binding to T antigen. It is of prime importance to determine the level of this T antigen binding protein in untransformed cells and normal tissues and to see if it is induced by other carcinogenic agents.

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Note added in proof: The presence of a middle-T antigen species in SV40 transformed rat cells and SV40 transformed mouse cells has recently been reported²²⁻²⁴ and a preliminary characterisation of the protein carried out²⁵.

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Persisting DNA synthesis in SV40-transformed cells in the presence of cycloheximide

INHIBITION of protein synthesis decreases the rate of DNA synthesis in both prokaryotes and eukaryotes. In prokaryotes, DNA synthesis is dependent on protein synthesis only for the initiation of replication¹. In the case of eukaryotes, reduction of DNA synthesis occurs rapidly after the inhibition of protein synthesis^{2,3}, although the mechanism of the tight coupling between protein and DNA synthesis is unclear. The DNA which is synthesised at a reduced rate in these conditions is basically normal except that the elongation step seems to be modified^{2,5}. Newly replicated DNA synthesised in the presence of cycloheximide seems to be associated with less chromatin protein, because chromatin synthesised in these conditions is more susceptible to digestion by DNases^{3,4}. T antigen of simian virus 40 (SV40) is a product of the A gene of the virus⁵ and has been shown to have a role in the establishment and maintenance of transformation⁶⁻⁹. There is evidence that T antigen is required for the initiation of viral DNA replication¹⁰ and the induction of synthesis of cell DNA¹¹⁻¹⁵. It has also been suggested that T antigen initiates DNA synthesis in transformed cells and thereby serves to retain those cells in the transformed state^{10,16-18}. Recently, T antigen was purified almost to homogeneity, and its microinjection into quiescent mouse cells induced DNA synthesis¹⁹. However, the mechanism of action of T antigen in cell DNA synthesis remains to be clarified²⁰. If T antigen acts on cellular DNA synthesis, SV40-transformed cells which contain T antigen may behave differently from other cell lines in their ability to continue DNA synthesis in conditions of protein synthesis inhibition. We report here that DNA synthesis in SV40-transformed cells continued at an almost normal rate while protein synthesis was inhibited by cycloheximide, whereas DNA synthesis in malignant tumour cells without T antigen did not continue in these conditions.

HeLa and SV80, the former derived from human cervical cancer and the latter a human cell line transformed by SV40²¹, were grown in Dulbecco's modified Eagle's medium (Nissui)

supplemented with fetal bovine serum 10% (Gibco) and streptomycin sulphate (200 µg ml⁻¹). Cells were usually cultured for 3-4 d and used when they had reached subconfluence. Evidence of mitosis was observed under the microscope, indicating active growth. Protein and DNA synthesis in the presence of various concentrations of cycloheximide were studied by measurement of the incorporation of radioactive precursors into acid-precipitable material in cells cultured on 13-mm cover slips. As seen in Table 1a, in both cell lines, protein synthesis was reduced by cycloheximide in a concentration-dependent manner. DNA synthesis in HeLa cells was inhibited in the presence of cycloheximide (Table 1b), which was consistent with observations of cycloheximide inhibition of DNA synthesis in eukaryotes¹⁸⁻²⁰. In SV80 cells, however, DNA synthesis was maintained at an almost normal rate in the presence of cycloheximide (Table 1b), although longer treatment of SV80 cells with cycloheximide reduced DNA synthesis gradually. The ³H-thymidine incorporated into cells treated with or without cycloheximide was located in nuclei, was resistant to alkaline hydrolysis (0.3 M KOH at 37 °C for 18 h), and was completely solubilised by acid hydrolysis (10% perchloric acid at 100 °C for 15 min). This resistance of DNA synthesis to inhibition of protein synthesis was seen not only for SV80 cells, but also for such hamster cell lines transformed by SV40 as SVH93, CHLWT15 and CHLA239L1.

We then carried out experiments to determine whether protein fractions such as histones and/or other proteins which would be required for the maintenance of DNA synthesis were supplied at a normal rate in SV80 cells in the presence of cycloheximide. SV80 cells were labelled with ³H-leucine for 3 h in the presence or absence of cycloheximide. Radioactivity and protein content in cytoplasmic and nuclear fractions of the cells and acid-extractable and residual fractions in nuclei were determined. The acid-extractable fraction contained mostly histones, whereas the residual fraction showed no detectable histones when checked by SDS-polyacrylamide gel electrophoresis. Synthesis of proteins from cells treated with cycloheximide was inhibited to approximately 5% of control regardless of the fractions determined (Table 2). The fluorographic pattern of SDS-polyacrylamide gel electropherograms of cytoplasmic and nuclear proteins confirmed none of specific protein retained a normal rate of synthesis with cycloheximide treatment. In the presence of cycloheximide, DNA in newly

Table 1 Incorporation of ³H-leucine (a) or ³H-thymidine (b) into acid-precipitable material in cells treated with or without cycloheximide

Labelling period (h) after cycloheximide	Cycloheximide (µg ml ⁻¹)			
	0	0.5	2.0	5.0
a				
HeLa (c.p.m. × 10 ⁻² per culture)				
0.5-1.5	24.1 ± 2.2 *	7.48 ± 0.44(31.0)†	3.67 ± 0.12(15.2)	2.97 ± 0.08(12.3)
1.5-2.5	26.7 ± 0.31	7.90 ± 0.70(29.6)	3.98 ± 0.38(14.9)	3.07 ± 0.37(11.5)
2.5-3.5	33.7 ± 2.7	8.17 ± 0.48(24.3)	3.07 ± 0.37(9.1)	3.33 ± 0.06(9.9)
SV80 (c.p.m. × 10 ⁻² per culture)				
0.5-1.5	86.3 ± 4.6	15.5 ± 0.55(18.0)	9.18 ± 1.5(10.6)	8.60 ± 1.2(10.0)
1.5-2.5	86.7 ± 8.2	15.3 ± 0.44(17.7)	9.64 ± 0.40(11.1)	8.27 ± 0.42(9.5)
2.5-3.5	81.9 ± 4.7	14.8 ± 0.25(18.1)	7.92 ± 0.34(9.7)	7.27 ± 0.60(8.4)
b				
HeLa (c.p.m. × 10 ⁻³ per culture)				
0.5-1.5	11.1 ± 0.32	3.94 ± 0.47(35.5)	2.22 ± 0.11(20.0)	1.62 ± 0.04(14.6)
1.5-2.5	12.0 ± 0.28	4.41 ± 0.07(36.8)	2.54 ± 0.06(21.2)	1.78 ± 0.52(14.8)
2.5-3.5	11.7 ± 0.18	5.08 ± 0.21(43.4)	2.77 ± 0.16(23.7)	2.19 ± 0.18(18.7)
SV80 (c.p.m. × 10 ⁻³ per culture)				
0.5-1.5	12.1 ± 0.44	10.9 ± 0.29(90.1)	10.2 ± 2.71(84.3)	11.6 ± 1.12(95.9)
1.5-2.5	11.6 ± 2.2	11.0 ± 1.5(94.8)	9.9 ± 1.39(85.3)	12.3 ± 0.73(106.0)
2.5-3.5	14.0 ± 2.3	10.2 ± 1.2(72.9)	10.4 ± 1.08(74.3)	12.7 ± 0.91(90.7)

Cells were plated on 13-mm cover slips in a multiwell culture tray (Costar) with 1 ml of medium and cultured for 3-4 d until subconfluent. After 0.5, 1.5 or 2.5 h of cycloheximide addition, L-[4,5-³H]leucine (50.6 Ci mmol⁻¹, Amersham) was added at a final concentration of 13 µCi ml⁻¹ and cells were labelled for 1 h. Medium was discarded and the cells were washed twice with cold phosphate-buffered saline, four times with 10% cold trichloroacetic acid, and finally with ethanol. After drying, 10 ml of scintillation cocktail (4 g diphenylloxazole in 1 toluene) was added and radioactivity was counted. b, As in a except that [6-³H]thymidine (26.6 Ci mmol⁻¹, Amersham) was added at a final concentration of 13 µCi ml⁻¹.

* Mean of 3 determinations ± s.e.m.

† % Of control without cycloheximide.

Table 2 Inhibition of protein synthesis by cycloheximide

Fractions	Protein (mg)		Radioactivity (c.p.m. $\times 10^{-4}$)		Specific radioactivity (c.p.m. $\times 10^{-4}$ per mg)	
	Control	Cycloheximide	Control	Cycloheximide	Control	Cycloheximide
Whole cell	6.66	6.58	128.7	4.88	19.33	0.74(3.8)*
Cytoplasm	3.30	3.77	57.4	2.80	17.40	0.74(4.3)
Nuclei	1.50	1.50	47.2	2.03	31.44	1.35(4.3)
Acid extractable	1.28	0.76	10.0	0.30	7.89	0.39(5.0)
Residual	0.28	0.19	19.3	0.56	68.8	2.97(4.3)

SV80 cells were labelled with L-[4,5- ^3H]leucine for 3 h from 0.5 to 3.5 h after addition of $5.0 \mu\text{g ml}^{-1}$ of cycloheximide. Cells were then washed twice with phosphate-buffered saline, homogenised in reticulocyte standard buffer (10 mM Tris-HCl, pH 7.4; 10 mM NaCl; 1.5 mM MgCl_2), and spun down through a 0.88 M sucrose layer to pellet nuclei. Supernatant including sucrose cushion was saved as cytoplasmic fraction. Acid-extractable and residual proteins from nuclei were separated by the 0.25 M H_2SO_4 method²⁷. All fractions were pelleted and washed with 10% cold trichloroacetic acid, except for the acid-extractable fraction which was pelleted by adding 4 volumes of ethanol²⁷. Pellets were dissolved in 0.2 M NaOH. Radioactivity was measured after mixing with scintillation cocktail PCS (Amersham). Protein was assayed by the method of Lowry *et al.*²⁸.

* % Of control without cycloheximide.

synthesised chromatin of both SV80 and HeLa cells was more susceptible to digestion by micrococcal nuclease, as compared with that in control without cycloheximide, suggesting a reduced assembly of proteins into newly synthesised chromatin. These results indicated that DNA synthesis in SV80 cells continued at an almost normal rate under a reduced supply of new proteins in the presence of cycloheximide.

As inhibition of DNA synthesis by cycloheximide was reported to occur very rapidly, within a few minutes^{2,3}, certain proteins with a very short half life were assumed to be required for the maintenance of DNA synthesis, although isolation and characterisation of these proteins have been unsuccessful²². SV80 cells contained high amounts of T antigen among SV40-transformed cell lines²³. T antigen was located almost exclusively in nuclei²⁴ and was bound to double-stranded DNA²⁵ and chromatin²⁶. The fact that T antigen was required for the initiation of each round of viral DNA synthesis⁶ and the fact that the size of replicon units in SV40-transformed cells was smaller than that in normal cells¹⁸ suggested that T antigen may be involved in cell DNA synthesis. These and the following results seem to favour the idea that T antigen may maintain cellular DNA synthesis despite the absence of protein synthesis, directly or indirectly: (1) in all four SV40-transformed cell lines of human and rodent that we have tested, DNA synthesis continued in the presence of cycloheximide; (2) in cells transformed by the ΔTA mutant of SV40 virus, CHLA239L1, which contained temperature-sensitive T antigen, DNA synthesis continued at the permissive temperature, but was inhibited at the non-permissive temperature in the presence of cycloheximide; (3) variation in the resistance of DNA synthesis to inhibition of protein synthesis among SV40-transformed cell lines correlated with the concentration of T antigen in cells. It should be emphasised, however, that these results do not prove a direct action of T antigen on cell DNA synthesis, but may provide us with a new approach for the analysis of the function of T antigen in transformed cells.

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Sodium channels in rabbit cardiac Purkinje fibres

SODIUM channels are essential for normal impulse conduction in the heart and are a major target for the therapeutic action of antiarrhythmic drugs. However, these channels have not been well studied due to the difficulty of measuring cardiac sodium currents under voltage clamp. Previous efforts have not been generally accepted because of doubts about the adequacy of voltage control¹⁻⁴. The absence of direct information on cardiac sodium channels is unfortunate as evidence exists suggesting that they differ significantly from sodium channels in other excitable membranes. First, blockade of sodium-dependent impulses in heart requires tetrodotoxin (TTX) at 10^3 - 10^4 times greater concentrations than in nerve or skeletal muscle⁵. Second, the effect of TTX in heart appears to be voltage-dependent¹⁰, unlike its action on sodium currents in other excitable cells^{11,12}. These findings raise the possibility that cardiac sodium channels may have novel structural properties. Further progress depends on the development of reliable measures of sodium current in the heart itself. We report here voltage-clamp recordings of sodium currents in rabbit Purkinje fibres satisfying a number of criteria for adequate voltage control. The experiments indicate the feasibility of characterising sodium channels in an intact mammalian cardiac preparation and open the way for the direct analysis of antiarrhythmic drug action on cardiac conducting tissue.

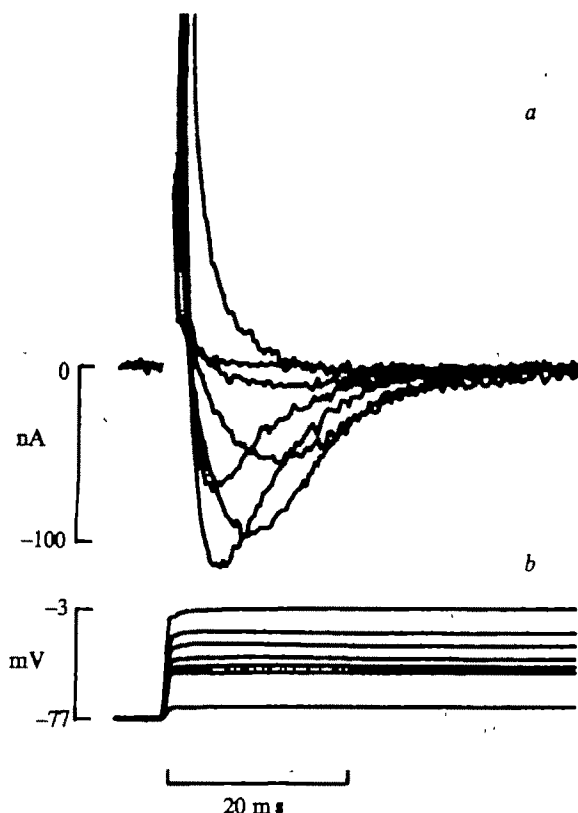


Fig. 1 Sodium currents in the rabbit Purkinje fibre. Membrane currents elicited by depolarisations from a holding potential of -77 mV. Traces are the average of two sweeps (10 kHz filtering) and have been corrected for steady current by computer subtraction. (The size of the steady current is evident in Fig. 2.) Command pulses were exponentially lagged to allow higher clamp gain without ringing and to protect the preparation by limiting the initial surge of capacitive current. Cylindrical surface area, 0.0032 cm²; total capacity, 18 nF. Fibre T46-1, 0 Ca (5.4 mM Mn), 13 °C.

The experiments were carried out in rabbit Purkinje fibre preparations using the two-microelectrode voltage-clamp technique. Johnson *et al.*^{13,14} suggested the choice of the rabbit Purkinje fibres because of its structural advantages over other preparations. It lacks transverse tubules, unlike mammalian ventricular muscle, and it has wide (1 μ m) intercellular clefts, unlike sheep Purkinje fibres or frog myocardium. The absence of narrow or long extracellular spaces is important in avoiding undesirable external voltage drops. The structural features of the rabbit Purkinje fibre are consistent with its electrical properties. Shortened rabbit Purkinje fibres behave like simple, terminated RC cables, with an extremely low external series resistance to transmembrane current flow^{15,16}. In studying the rabbit Purkinje fibre, we avoided the double sucrose gap method used in earlier studies^{14,17} because of the reported poor viability of the preparations and the problem of extracellular shunt currents¹⁴. We chose instead the two-microelectrode method¹⁸, having found that it allows normal resting and action potentials to be recorded over periods up to several hours long^{15,16}. The main obstacle when studying I_{Na} is longitudinal nonuniformity; this was reduced to an acceptable level by using a low external sodium concentration (15 mM), and short preparations (450–700 μ m) to restrict the distance from the current passing electrode to either of the healed-over ends. Reduction of sodium conductance by TTX is less appropriate in these experiments because, as Baer *et al.* have suggested¹⁰, its inhibitory effect is voltage-dependent (T.J.C., C. J. Cohen and R.W.T., unpublished). Temporal resolution of I_{Na} was improved by cooling (13–23 °C). Interference from other ionic currents was not a serious problem as I_{Na} is much faster and larger than other

Table 1 Voltage-dependent inactivation of sodium current

Expt	Solution	Temperature (°C)	V_{test} (mV)	E_h (mV)	k (mV)
T42-3	5.4 Ca	22	-13	-72	5.45
T44-5	5.4 Ca	19	-24	-66	3.83
T45-1	1.8 Ca + 3.6 Mn	19	-24	-65	3.71
T48-2	5.4 Mn	26	-34	-83	6.64
T48-6	5.4 Mn	23	-35	-83	7.17
mean				-74 ± 4	5.36 ± 0.71
$\pm$ s.e.m.					

Analysis of $h_{\infty}(E)$ as shown in Fig. 4. Sodium currents were measured at the test potential (V_{test}) indicated. E_h and k were evaluated for each experiment by fitting the data with equation (2).

components such as the slow inward current or delayed potassium current. These currents and twitch contraction were largely inhibited in most experiments by including manganese ion in the external solution. The composition of the external solution was as follows (mM): 15 NaCl, 135 choline-Cl (Mallinckrodt), 4 KCl, 0.5 MgCl₂, 10 Tris-buffer (pH 7.4), 5 glucose, and either 5.4 CaCl₂ or 1.8 CaCl₂ + 3.6 MnCl₂ or 5.4 MnCl₂. (See Table 1 and Figure legends for concentrations of Ca or Mn.) Records of membrane current and intracellular potential were taken with a PDP 8/e computer after low pass filtering at 10 kHz.

Figure 1 shows sodium currents (a) produced by step depolarisations from a holding potential at -77 mV (b). The traces in a show total membrane current (capacitive plus ionic) with a small component of steady current omitted. The depolarising step to -68 mV evokes an outward capacity transient but no significant time-dependent ionic current. Larger steps to -46 , -41 , -37 and -28 mV produce progressively greater surges of I_{Na} and progressively earlier peak current. The largest inward current is associated with the step to -28 mV. With further depolarisation, the transient current becomes smaller (-19 mV) and eventually reverses (-3 mV).

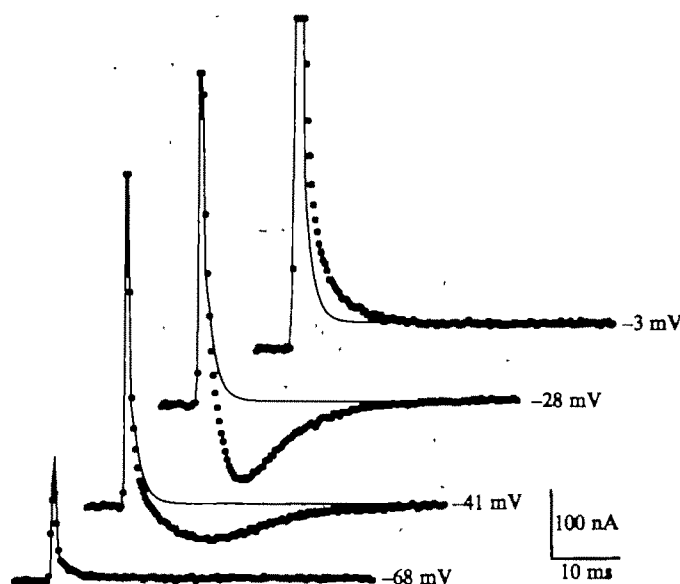


Fig. 2 Procedure for correcting current records for linear capacitive components. The current response to a subthreshold depolarisation to -59 mV was fitted by connecting the first six data points (1.8 ms) and fitting later points by a single exponential ($\tau = 1.3$ ms). The resulting function was then scaled in proportion to the size of the voltage step for other depolarisations (smooth curves), and superimposed on the experimentally measured currents (points). The points and the smooth curves both include a component of steady current. The lowest trace (-68 mV) shows that the fit is reasonable for a smaller subthreshold step. The peak of the capacity transient in the upper three traces (-41 , -28 , -3 mV) was off scale and is not resolved. Fibre T46-1.

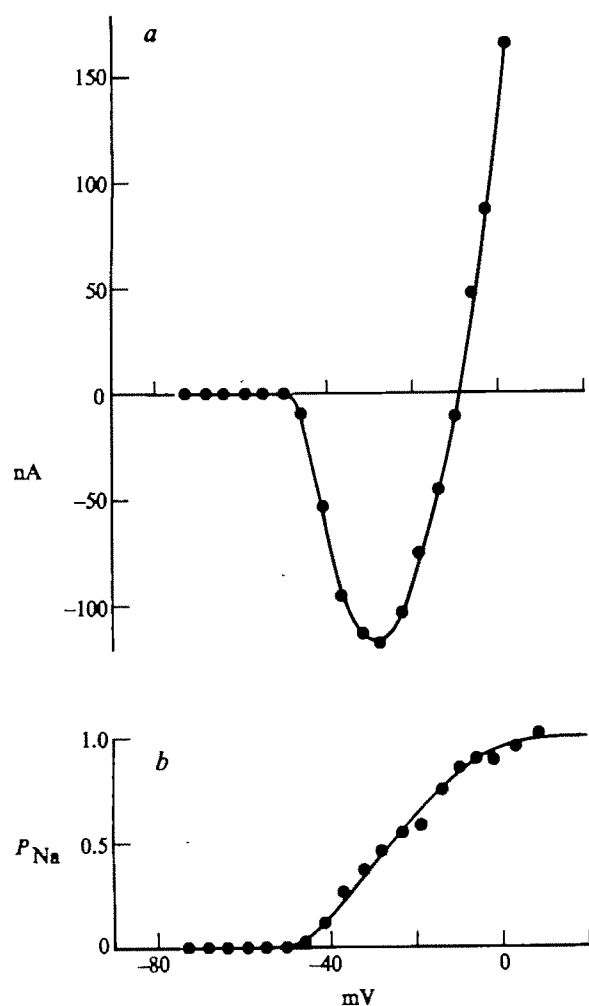


Fig. 3 Voltage dependence of sodium current and sodium permeability in the rabbit Purkinje fibre. *a*, measured currents corrected for capacity and steady current and plotted against membrane potential. Inward values represent peak inward currents. Outward peaks were difficult to resolve accurately because of rapid activation at strong depolarisations. Outward values were therefore measured isochronally at 1.8 ms. *b*, P_{Na} -voltage relationship in the rabbit Purkinje fibre. Sodium permeability calculated from *a* using equation (1) and normalised. When expressed in terms of the external cylindrical surface area of the preparation, maximal P_{Na} is $6.3 \times 10^{-3} \text{ cm s}^{-1}$. This estimate incorporates some resting inactivation at the holding potential, -77 mV. Fibre T46-1.

Sodium current was separated from capacitive current by the procedure shown in Fig. 2. The time course of the capacity transient was obtained from the response to an 18-mV step which was below the threshold for I_{Na} . The smooth curves show this time course, after scaling appropriate to the amplitude of other voltage steps. As expected, the smooth curve provides a good fit to the capacity transient during an even weaker step (record labelled -68 mV). At stronger depolarisations, I_{Na} can be seen as the difference between the smooth curve and the data points. The activation of I_{Na} can be largely separated from the decay of the capacity transient during the step to -41 mV. Temporal resolution is less satisfactory with stronger depolarisations where I_{Na} turns on more quickly. Nevertheless, the difference signal clearly shows outward ionic current at -3 mV.

Peak ionic currents obtained by this analysis are plotted in Fig. 3*a*. The voltage-dependence of peak I_{Na} is qualitatively similar to that found in other excitable tissues. The reversal of the ionic current takes place near -9 mV. This value is reasonably close to -3 mV, the value calculated from the Goldman-Hodgkin-Katz potential equation^{20,21} with P_{Na}/P_K set at 12 (ref. 22) and Na and K activities estimated by ion-sensitive microelectrodes^{23,24}. The calculated value is close to zero membrane

potential because the external Na activity is close to one-twelfth of the internal K activity.

Figure 3*b* shows the voltage-dependence of the sodium permeability P_{Na} , calculated from the data in Fig. 3*a* by the following equation^{20,21}

$$P_{Na} = \frac{I_{Na}}{(Na)_o} \frac{RT}{EF^2} \frac{\exp(EF/RT) - 1}{\exp((E - E_{Na})F/RT) - 1} \quad (1)$$

where E is the membrane potential (inside minus outside), E_{Na} is the measured reversal potential, and R , T and F have their usual meanings. P_{Na} shows a sigmoid dependence with membrane potential, varying from almost no activation at -50 mV to nearly full activation at 0 mV. The smooth curve is drawn for half maximal activation at -26 mV and a maximal P_{Na} of $6.3 \times 10^{-3} \text{ cm s}^{-1}$. This estimate is close to that reported by Adrian and Marshall for rat skeletal muscle²⁵.

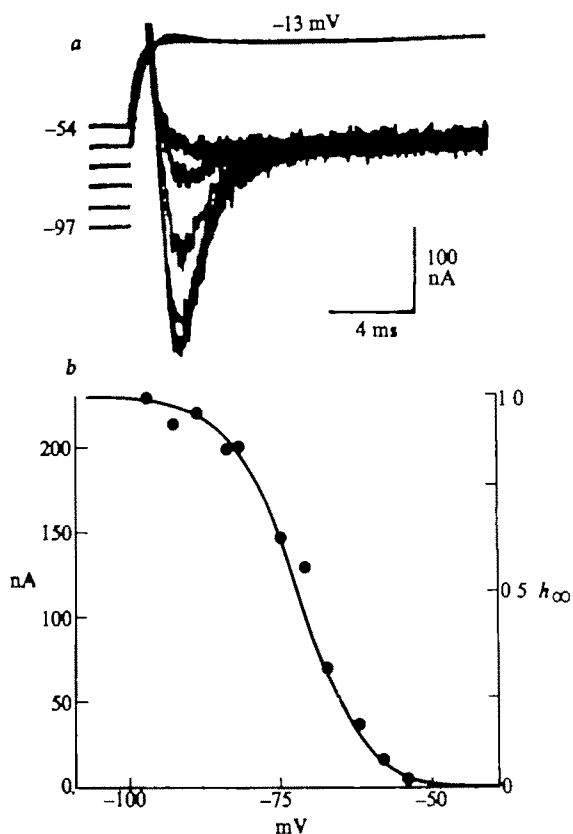


Fig. 4 Influence of membrane potential on I_{Na} inactivation. *a*, membrane currents elicited by depolarisations to -13 mV following 200-ms prepulses to -54, -62, -71, -80, -89 and -97 mV. Only the last 2 ms of the prepulse are shown. Peak currents following prepulses to -89 and -97 mV largely superimpose. *b*, Sodium inactivation curve obtained by plotting the amplitude of the inward current (corrected for capacity transient) against prepulse potential. The normalised data were fitted by the equation $h_{\infty} = 1/(1 + \exp(E + 72/5.45))$. Holding and test potential, -13 mV; pulsing rate, 0.2 Hz. External cylindrical surface area, 0.0024 cm^2 . Fibre T42-3, 5.4 mM Ca, 22 °C.

The phasic nature of the sodium permeability is attributed to a process termed sodium inactivation²⁶. Figure 4*a* shows one method for studying the voltage-dependence of inactivation. I_{Na} was elicited by step depolarisations to a fixed test potential (-13 mV). The test pulse was preceded by 200 ms prepulses to various voltage levels. As the current records indicate, the amplitude of I_{Na} was largest following a prepulse to -97 mV, while almost no sodium current was seen following a prepulse to -54 mV. The analysis of these data and others from the same

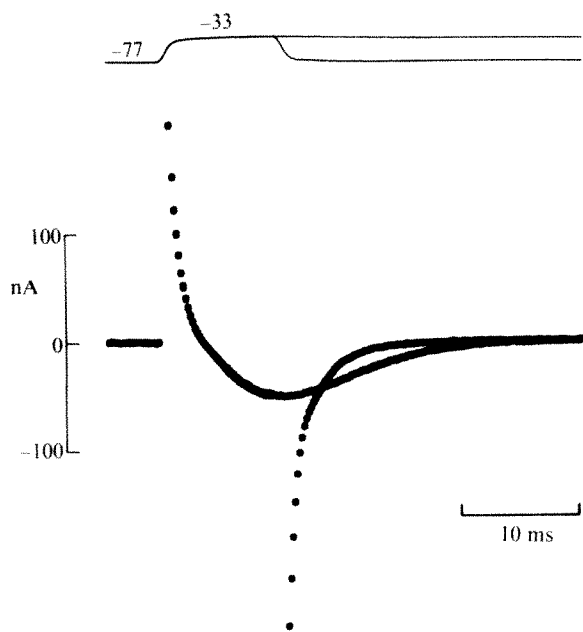


Fig. 5 Test of voltage control during I_{Na} . Superimposed records of recorded potential (top) and membrane current (bottom) during long and short clamp pulses. Brief pulse was terminated at peak P_{Na} . Good voltage control is indicated by the rapid decay of inward current following repolarisation. Traces are the average of two sweeps. Fibre T46-1.

experiment is shown in Fig. 4b. The amplitude of the I_{Na} transient displays a sigmoid dependence on prepulse potential. After normalisation, the data were fitted by the function

$$h_{\infty} = \frac{I_{Na}}{(I_{Na})_{\max}} = \frac{1}{1 + \exp((E - E_h)/k)} \quad (2)$$

where $E_h = -72$ mV and $k = 5.45$ mV. Table I presents data from a total of five experiments where $E_h = -74 \pm 4$ mV and $k = 5.36 \pm 0.71$ mV (mean $\pm$ s.e.m.). Equation (2) and very similar parameter values were used by Weidmann¹⁹ in his studies using maximal upstroke velocity as an index of P_{Na} . Our results resemble Thompson's data for rabbit Purkinje fibres in the double sucrose gap¹⁷, but differ from observations of Dudel and Rüdel in sheep Purkinje fibres²⁷. At 20 °C, Dudel and Rüdel found complete inactivation ($h_{\infty} = 0$) for potentials positive to -90 mV. We have seen similar behaviour in three exceptional cases in which the full inactivation curve was not determined. The data in Table I are representative of over 90% of our preparations, in which substantial I_{Na} is available at -85 mV, the normal resting potential at physiological temperature¹⁶.

The current signals in these experiments satisfy several criteria for adequate voltage control¹⁻⁸: (1) the inward current record shows a smooth decay, with no secondary humps or 'abominable notches' (Fig. 1); (2) the time course of the current remains unchanged when its magnitude is varied by prepulse inactivation (Fig. 4a); (3) the measured reversal potential lies close to the theoretically expected value (see ref. 5); and (4) activation is a gradual function of membrane potential, extending over a range of about 50 mV (Fig. 3b) as in other excitable tissues. There is no indication of the sharp voltage-dependence predicted for certain conditions where voltage control is poor^{2,6}.

Figure 5 shows an additional test for adequate clamp control which has been proposed in experimental^{14,17,28} and theoretical⁴ studies. The depolarising command pulse is terminated just as P_{Na} reaches its peak. If the membrane potential is adequately controlled, the 'tail' current should follow a smooth, rapid decay; if not, the current may be hooked or show the waveform of an inverted action potential. As Fig. 5 indicates, the experimental trace does in fact show a fast shut-off following the repolarisation.

Experimental evidence for adequate voltage control is compatible with rough calculations based on electrical properties of the preparation. Previous experiments¹⁶ provide an upper estimate of the effective external series resistance, $R_s \leq 3.3 \Omega \text{ cm}^2$. With this value, the voltage drop along R_s can be calculated for the maximum I_{Na} in Fig. 3 (120 nA) and the external surface area of the preparation (0.0032 cm^2). Thus $(120 \text{ nA})(3.3 \Omega \text{ cm}^2)/(0.0032 \text{ cm}^2) = 0.12 \text{ mV}$. This radial voltage error is negligible. Longitudinal voltage nonuniformity may be calculated by taking an experimental estimate of the longitudinal resistivity, $R_l = 450 \Omega \text{ cm}$. For the measured radius of $80 \mu\text{m}$, the axial resistance per unit length is $r_l = 2.17 \text{ M}\Omega \text{ cm}^{-1}$. The negative slope region of the I - V relationship in Fig. 3 corresponds to a membrane resistance per unit length of $r_m = -6.3 \text{ k}\Omega \text{ cm}$. From these values, one calculates a longitudinal space constant $|\lambda| = (|r_m|/r_l)^{1/2} = 540 \mu\text{m}$. This estimate of $|\lambda|$ is long relative to the distance from the end of the cable to the current source ($315 \mu\text{m}$). At worst, when the potential at the end of the cable is near maximum inward current, the calculated voltage difference from end to current source is only -1.7 mV . Thus, the longitudinal nonuniformity should introduce relatively little distortion in the measured characteristics.

The success of these experiments is not restricted by the properties of the microelectrodes. Current passing limitations were not a problem during the surge of P_{Na} because of the small preparation size and the low sodium concentration. The speed of clamp control was mainly limited by the product of axial resistance and membrane capacitance. Thus, at strong depolarisations, the capacity transient seriously overlaps I_{Na} activation. For analysis of activation kinetics over a wide voltage range, or effects of intracellular constituents, the method of Lee *et al.*²⁹ is clearly preferable. Our approach is very suitable, however, for analysis of sodium permeability over the voltage range where action potentials are initiated. It offers the possibility of studying sodium channels and their pharmacological modification in intact cardiac tissue free from enzymatic treatment. Further work will be needed to establish how cardiac sodium channels differ from those in other excitable membranes.

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Sodium current in single heart muscle cells

A COMPLETE description of the sodium current, I_{Na} , in heart muscle has not been obtained despite the practical importance of such information as a rational basis for the therapy of arrhythmias. I_{Na} has been examined under voltage clamp using micropipette¹ or sucrose gap methods², but the adequacy of spatial control of voltage has been questioned and temporal resolution of the early kinetics has been unsatisfactory and results therefore inconclusive³. These problems reflect the fact that heart muscle is a syncytium with restricted extracellular compartments. One of the more favourable preparations is rabbit Purkinje fibres⁴ and Colatsky and Tsien⁵ have recently performed voltage clamp studies on them with the two micropipettes technique. They have demonstrated the feasibility of this approach when proper precautions are taken⁵. Quite a different approach has been taken by other investigators such as Mehdi and Sachs⁶ and Powell *et al.*⁷⁻⁹, who have dispersed individual heart muscle cells and examined them with micropipette techniques. In this way the complex geometry is greatly simplified although the enzymes used in dispersion may pose another set of problems. We have followed this second approach and have isolated individual heart muscle cells from adult rats with a modified version of the dispersion method reported by Powell and Twist⁹. To perform electrophysiological studies we have sucked single cells into a suction pipette and used a technique which allows voltage clamp and internal perfusion¹⁰. I_{Na} has been isolated by suppression of other cardiac currents and its kinetics separated from the capacitive current transient. The success of the present approach indicates its potential usefulness in cardiac electrophysiology.

The suction pipette method is similar to that used in snail neurones and the details have been published elsewhere¹⁰. The main difference in the present experiments is that the tip diameter of the suction pipette was smaller, about 20 μm , and the measured tip resistances ranged from 150 to 250 k Ω . Series resistances determined from the peak amplitude of the capacitive current transient have similar values, indicating that the

tip resistance was mainly responsible. For currents less than $\sim 2 \times 10^{-8}$ A, the effects were negligible but for larger currents series resistance compensation¹⁰ was used. The rise time of the voltage clamp step was 10 μs and the capacitive current transient had a single time constant 100–200 μs in duration. Current-time records during single clamp runs were digitised every 5 μs using a 12-bit digital oscilloscope with a 2 K memory (Nicolet 575) and stored on a digital tape recorder (Kennedy Model 9700). Currents produced by voltage steps equal in amplitude but opposite in sign could be added to remove leakage and shunt currents and the linear components of the capacitive current. The external solution for separating I_{Na} had the following composition in mM: sodium isethionate, 120; CsF, 5.4; Mg_2SO_4 , 2.85; NaH_2PO_4 , 0.42; NaHCO_3 , 25; glucose, 5.5; pH 7.5 when equilibrated with 95% O_2 , 5% CO_2 . In the early experiments 5.0 mM tetraethylammonium was added to both internal and external solutions but later its use was discontinued due to its lack of effect. Cs^+ was used to substitute for K^+ in the internal solution which had the following composition (mM): CsF, 130; NaHCO_3 , 16; glucose, 5.5, pH 7.3 when equilibrated with 95% O_2 and 5% CO_2 . Experiments were carried out at 23 °C. The general procedures for obtaining individual, isolated beating adult rat myocytes have been described⁹ and details as well as biochemical characteristics of the cells prepared in this way will be published elsewhere.

The structural characteristics of single dispersed myocytes are similar to those already reported^{11,12}. The individual cells are separated at the intercalated disks, are approximately cylindrical, but have longitudinal grooves and transverse ridges with T-tubule openings near these ridges. Cell dimensions were 15–25 μm radius and about 100 μm length. Under the dissecting microscope the cells were beating in solution before and after aspiration, but during maintained suction visible beating ceased. In low or zero Cl^- solutions resting potentials were –70 to –90 mV (ref. 7) and the cardiac action potential had the shape shown in Fig. 1a. The short duration of the plateau may be related to the rapid heart rate of the rat (4 Hz); similarly shaped action potentials for intact rat ventricle¹³ and dispersed rat myocytes⁸ have been reported. Potassium currents were suppressed by Cs^+ substitution for internal and external K^+ , Ca current was suppressed either by Co^{2+} and/or zero Ca^{2+} solutions and Cl^- current was suppressed by isethionate[–] and F^-

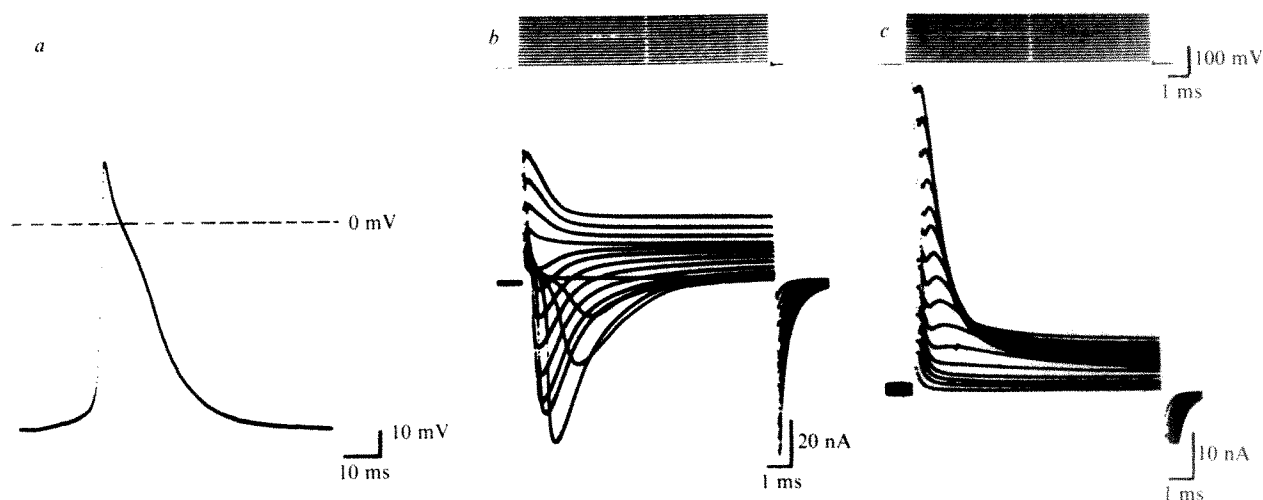


Fig. 1 Membrane potential and I_{Na} in single rat heart muscle cells. Individual myocytes were obtained by exposing rat ventricles to Krebs–Henseleit solutions containing 0.1% collagenase (Sigma type 1) and 0.1% hyaluronidase (Sigma type 1) according to the methods of Powell *et al.*⁷⁻⁹. *a*, Record of an action potential obtained from a single rat heart muscle cell in zero Cl^- solutions using the suction pipette method. Stimulating current was delivered by current clamp. The resting potential is –80 mV. The action potential amplitude is about 100 mV with a 20 mV overshoot and was corrected for the voltage drop across the tip resistance. The plateau phase is shortened and similar configurations in rat heart muscle have been reported^{8,13}. *b*, *c*, I_{Na} records uncorrected for capacitive, leakage and shunt currents. I_K , I_{Ca} and I_{Cl} have been suppressed by substituting Cs^+ for K^+ , Co^{2+} for Ca^{2+} and isethionate[–] and F^- for Cl^- . The traces are from the same cell in 100% (*b*) and 0% (*c*) external Na solutions. In (*b*) I_{Na} is resolved after about 150 μs and a clear reversal of currents is observed. In (*c*) only outward current appears. In both cases, the peak inward or outward current appears and then disappears within the first 2–6 ms depending on the magnitude of the step voltage change. As the step potential becomes progressively higher, peak current appears more quickly. Holding potential, $V_H = -90$ mV. $1-2 \times 10^{-9}$ A of inward current was usually required to achieve this V_H . Each voltage trace represents a 10 mV increment from V_H .

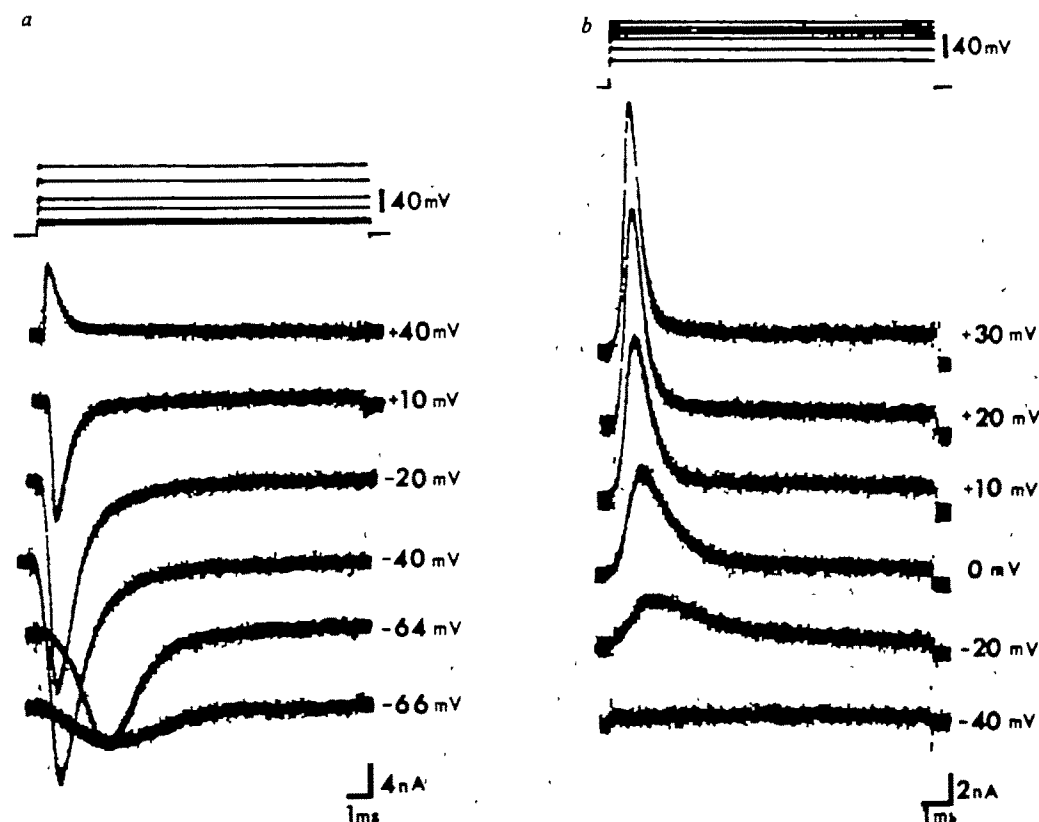


Fig. 2 I_{Na} currents after subtraction of leakage, shunt and linear capacitive currents and their I - V relationships. *a*, I_{Na} values from a single myocyte in 50% $[Na^+]_o$. *b*, Same cell in zero $[Na^+]_o$. $V_H = -90$ mV and corresponding clamp potentials are indicated besides each current trace. Activation and inactivation are apparent and are faster at higher voltages. A small outward current persists in zero $[Na^+]_o$ solutions. The reason for the slower kinetics in (*b*) is explained in the text.

substitution. After suppression of I_K , I_{Ca} and I_{Cl} , the current-time records and associated clamp potentials in 100% Na solutions are shown in Fig. 1*b*. The currents in these records have deliberately not been corrected for leakage or capacitive components and show that early I_{Na} is clearly distinguishable from the capacitive transients. In the photographic reproductions the latter appear mainly as discontinuities after the step depolarisations. As voltage amplitudes are increased above threshold the inward current becomes progressively larger and faster, but then the amplitude decreases as the voltage step approaches E_{Na} . The currents reverse as the steps go positive

with respect to E_{Na} . A small delay preceding activation is present and is more apparent in Fig. 2. I_{Na} also shows inactivation; this process is about 4–8 times slower than activation. After leakage subtraction it appears that inactivation of inward I_{Na} is complete. The tail currents occurring during repolarisation are capacitive and have a single time constant of 100–200 μ s which is similar to that measured at the onset of the step depolarisations. When Na^+ is completely replaced by Tris or isotonic sucrose solution, I_{Na} is outward only, as Fig. 1*c* demonstrates. In the absence of extracellular Na^+ , a small steady outward current persists and this current is often smaller at more

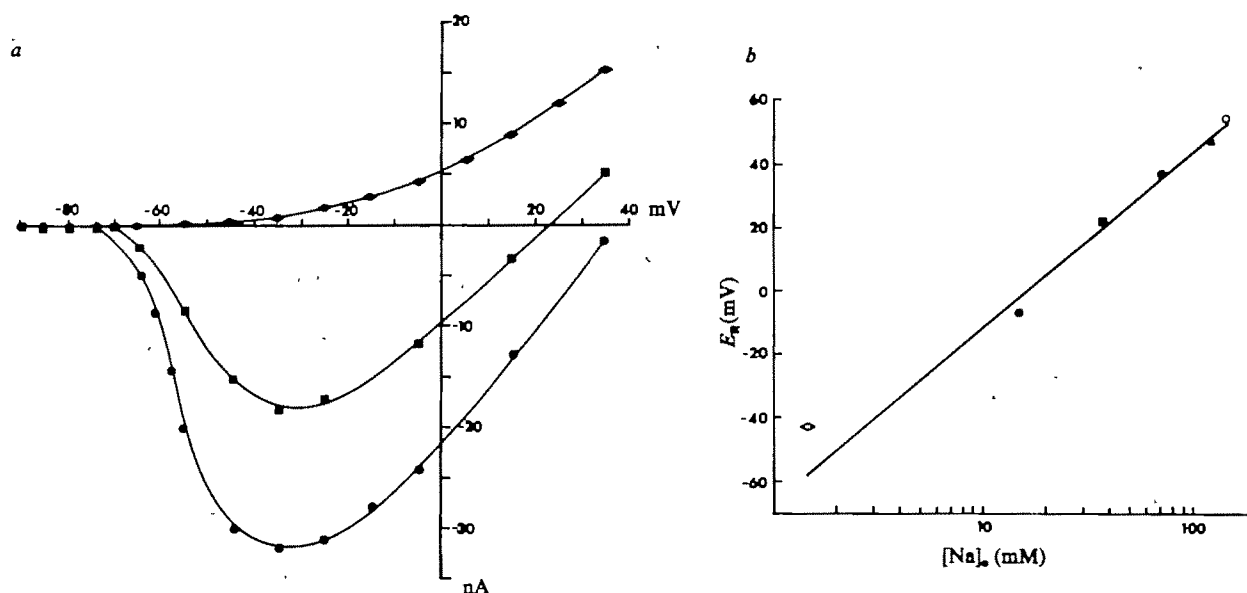


Fig. 3 Effects of changes in $[Na^+]_o$. *a*, Current-voltage relationship of peak inward and outward Na^+ currents in external solutions containing varying amounts of sodium as follows: 50% ($\bullet$), 25% ($\blacksquare$) and zero ($\blacklozenge$). Same cell as in Fig. 2. The peak currents are sensitive to both voltage and $[Na^+]$. The null potential of this current shifts to the left along the voltage axes as $[Na^+]_o$ decreases and becomes totally outward as the sodium in the external solutions is reduced to zero. *b*, Changes of E_{Na} in relation to log changes of $[Na^+]_o$. Each point is from a different myocyte. The solid line represents the Nernst potentials for $[Na^+]_i = 16$ mM, the internal perfusate concentration. Na^+ concentrations are: 100% ($\circ$), 75% (Δ), 50% ($\bullet$), 25% ($\blacksquare$), 10% ($\odot$) and 1% ($\blacklozenge$).

positive voltages leading to a crossover of the steady outward currents at long times. This may reflect the voltage-dependence of the inactivation process but could also be due to a voltage-dependent blocking action of Cs^+ upon I_{Na} similar to that observed in squid axon¹⁴. When I_{Na} was outward, it could be abolished by replacing Na with Cs. The effect appeared 1 to 2 min after solution change and was complete after 10–20 min indicating that internal perfusion can effectively control intracellular ionic composition.

As Na^+ substitutes we used Tris or tetramethylammonium (TMA) ions or isosmotic replacement of NaCl with sucrose. TMA had deleterious effects within a few minutes and the cells did not tolerate exposures to Tris for periods longer than 15 min. At shorter times, however, Tris was a reasonable substitute. The effects of Tris or sucrose substitutions on I_{Na} were similar.

The current-voltage relationship in 100% Na_0 solutions often had a steep negative resistance region indicating inadequate voltage control. This problem seldom occurred when $[\text{Na}^+]_0$ was reduced to 50% or less as shown in Fig. 3a. The relationship between peak I_{Na} and voltage in the different solutions was derived from traces of currents such as those shown in Fig. 2 which were obtained after subtraction of leakage and linear capacitative components. Lowering $[\text{Na}^+]_0$ did not appear to alter the basic Na^+ conduction mechanism. Thus, in low Na^+ solutions currents were reduced in accordance with the independence principle and kinetics and normalised conductance-voltage relationships were similar. The slower kinetics in zero Na_0^+ (Fig. 2b) compared to 1/2 Na_0^+ (Fig. 2a) reflect the effects of experimental times greater than 30 min which were required to complete the studies in several different external solutions. Similar changes may also occur at prolonged times in a single test solution. We used chord conductances for conductance calculations because the instantaneous I - V curves were linear. The G_{Na} - V curve at its steepest increased e -fold for about 10 mV of depolarisation, which is similar to the findings of Colatsky and Tsien⁵. I_{Na} could also be inactivated by depolarising prepulses and the extent of inactivation was determined by the duration and amplitude of the prepulse.

The reversal potentials, E_r 's, for leakage-corrected I_{Na} are similar to those predicted by the Nernst potentials for the internal and external concentrations of Na^+ used (Fig. 3b); this indicates a high selectivity of the conductance for sodium ion.

The degree of complexity of membrane geometry in the present preparation needs to be examined in detail because a major weakness in voltage clamp studies of heart muscle has been an inadequate consideration of such complications³. Towards this end impedance studies are now underway. Calculations of surface area derived from the capacitative current transient are slightly larger than calculations assuming a right cylinder of the dimensions referred to but some of the discrepancy might be due to the longitudinal grooves and transverse ridges present in these cells. A contribution from T-tubules is also possible, but a complicated equivalent electrical circuit may be unlikely because the capacitative current transient is a single exponential function. Spatial control of voltage seems adequate as there are no notches on the current records, the negative slope region of the I - V curves in solutions with 50% Na or less is not overly steep, that is, peak inward current increases gradually with voltage in this range and lowering $[\text{Na}^+]_0$ reduces currents without altering kinetics or voltage-dependence. With the present method it is possible to examine I_{Na} in the outward direction only and avoid negative resistances where voltage control is most difficult (Fig. 3a, zero Na_0^+ curve). In three instances, we were able to compare recorded values for the maximum rate of rise of the action potential dV/dt_{max} with computed values using I_{Na} and C_m calculated from the capacitative current transient, and these were in reasonable agreement. As all other currents were suppressed it cannot be concluded, however, that under normal circumstances dV/dt_{max} and I_{Na} would be equivalent.

The effects of enzyme treatment upon I_{Na} require some comment. The high selectivity of G_{Na} and the similarity of our

G_{Na} - V curve to the P_{Na} - V relationship obtained by Colatsky and Tsien⁵ in the absence of enzymes suggest that enzyme treatment has few effects. Moreover, collagenase treatment of mammalian skeletal muscle had little or no effect on resting or action potentials¹⁵.

In *Helix* neurones, the enzyme trypsin does not alter the electrical properties of I_{Na} but it does abolish tetrodotoxin (TTX) sensitivity¹⁶. We found that myocyte I_{Na} was blocked by TTX but unlike axons, the blockage requires micromolar concentrations and is voltage-dependent, being less complete at more positive potentials. A voltage-dependent action of TTX on dV/dt_{max} in untreated guinea pig papillary muscle cells has been reported¹⁷ as having the large concentration requirements. Therefore the presently reported TTX effects are probably not due to enzymatic actions but result either from differences in all Na channels, or a small number of TTX-resistant channels.

A full description of I_{Na} is clearly feasible as the present report and that of Colatsky and Tsien⁵ indicate. Our methods also provide the capability for simultaneous voltage clamp and internal perfusion of individual cardiac cells. This approach will be very useful for future electrophysiological studies on heart muscle and similar approaches are likely to be successful in other body tissues.

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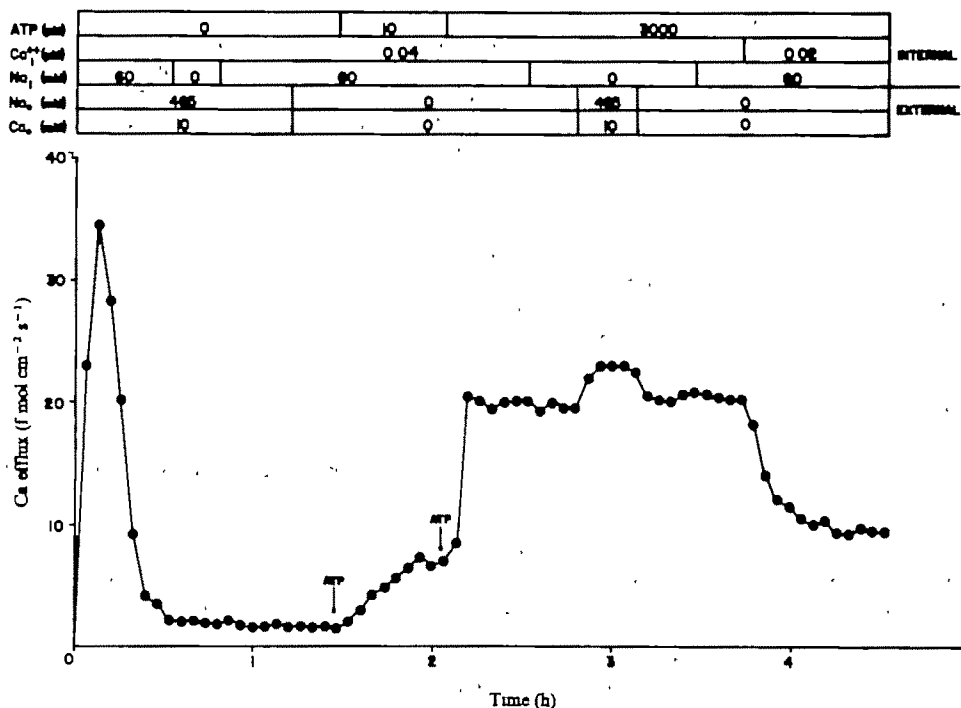
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Physiological role of ATP-driven calcium pump in squid axon

It has been proposed that the regulation of the ionised calcium concentration inside many excitable and non-excitable cells is mediated by a Na-Ca countertransport mechanism where the Na electrochemical gradient provides the energy to maintain low levels of intracellular Ca^{2+} (refs 1, 2). Two recent observations, however, provide evidence consistent with the idea that Ca extrusion in squid axons is mediated, at least in part, by an ATP-driven Ca pump of the type observed in red blood cells^{3,4}. First, in the absence of a Na gradient across the membrane there is an ATP-dependent 'uphill' extrusion of Ca which persists in the absence of external Na, Ca and Mg (ref. 3). And second, in Ca-injected unpoisoned axons, 50–90% of the Ca efflux can occur as an 'uncoupled' flux—that is, Ca efflux not accompanied

Fig. 1 Effects of Na_i , Na_o and ATP on the activation of Ca efflux at physiological Ca_i^{2+} concentration (axon 010678A, 530 μm). The composition of the dialysis solution was (mM): K^+ 310; Na^+ 60; Mg^{2+} 4 in excess of the ATP concentration; Tris^+ 5; Cl^- 79; aspartate 310; EGTA 1; glycine 330; oligomycin 10 $\mu\text{g ml}^{-1}$. The osmolality was 980 mosM and the pH (17 °C) 7.1. ATP and phosphoarginine (PA) were obtained from Boehringer as sodium salts and were stored at -90 °C as stock solutions (250 mM at pH 7.1). The ATP solution contained in addition 250 mM MgCl_2 . The composition of the standard artificial seawater was (mM): Na^+ 440; K^+ 10; Mg^{2+} 50; Ca^{2+} 10; Tris^+ 10; Cl^- 580; EDTA 0.1. The osmolality was 1,000 mosM and the pH (17 °C) 7.6. Tris^+ was used as Na^+ substitute. Removal of Na_i or Na_o and Ca_o was carried out without changing the other constituents. The nominally Ca-free media contained 0.5 mM EGTA and no added Ca. The axon was preincubated for 1 h in KCN before dialysis. At zero time, the dialysis began with the indicated solution plus radioactive Ca. The fluid superfusing the axon (1 ml min^{-1}) contained nominally 1 mM KCN and was collected at 4-min intervals. An appropriate volume of a scintillation mixture was added and the samples counted in a scintillation counter. The reduction following the initial rise in Ca efflux is a consequence of the ATP washout. The arrows refer to addition of ATP + PA to the internal medium.



by the uptake of Na, Ca or Mg (ref. 5). The existence of two apparently separate mechanisms for Ca transport, Na-Ca exchange and an ATP driven Ca-pump, raises the question of whether, in normal conditions, both mechanisms participate in the maintenance of the physiological $p\text{Ca}$. To be of any use in controlling the $p\text{Ca}$ of the nerve, the mechanism responsible must be able to operate at normal Ca_i^{2+} concentrations, $<10^{-7}$ M (ref. 6). We now present evidence showing (1) that in squid axons with physiological levels of Ca_i^{2+} most of the Ca efflux can be accounted for by an ATP-dependent system with high affinity for Ca_i^{2+} and ATP and which can operate in the complete absence of external Na and Ca; and (2) that a Ca transport mechanism with a low affinity for Ca_i^{2+} and ATP and extremely sensitive to Na_i , Na_o and Ca_o can contribute substantially to the total Ca efflux only at Ca_i^{2+} well above the physiological Ca_i^{2+} concentrations.

The present experiments were carried out on giant axons of the squid *Loligo pealei*, using the internal dialysis technique to control the intracellular concentration of low molecular weight solutes. Washout of internal ATP was achieved using glass dialysis capillaries. The amount of ADP produced by hydrolysis of exogenous ATP was minimised by adding 5 mM phosphoarginine to the dialysis solutions.

The dependence of Ca efflux on Na_i , Na_o and ATP was determined at a Ca_i^{2+} concentration of 0.04 μM (physiological levels of Ca_i^{2+} are between 0.02 μM and 0.1 μM). After the washout period a steady Ca efflux of ~ 2 $\text{fmol cm}^{-2} \text{s}^{-1}$ was obtained (Fig. 1). Changes in the Na electrochemical gradient (removal of internal Na at constant external Na and vice versa) had no effect on Ca efflux. In contrast, addition of as little as 10 μM ATP, in the absence of Na_o and Ca_o , led to a significant increase in Ca efflux. In the presence of 3 mM ATP removal of Na_i had no effect on Ca efflux, whereas readdition of external Na and Ca increased Ca efflux slightly and reversibly. Note also that the ATP dependent Ca efflux can operate at very low Ca_i^{2+} , as the levels of Ca efflux at 0.02 μM Ca_i^{2+} are appreciably higher than the basal Ca efflux observed in the absence of ATP (Fig. 1).

Figure 2 shows that the ATP dependent, Na_i and Ca_o independent (uncoupled) Ca efflux constitutes most of the Ca efflux at physiological Ca_i^{2+} concentrations, with a $K_{1/2}$ for Ca_i^{2+} of ~ 0.18 μM . On the other hand, the ATP independent, Na_i

and Ca_o dependent Ca efflux is minimal at physiological Ca_i^{2+} concentrations and shows no sign of saturation up to ~ 1 μM Ca_i^{2+} . When internal ionised calcium was raised to 80–110 μM , the Na_i dependent Ca efflux rose to 680–750 $\text{fmol cm}^{-2} \text{s}^{-1}$ and was highly sensitive to internal Na concentration (data not shown). In *Dorytheutis plei* at physiological Ca_i^{2+} and Na_i concentrations, $>95\%$ of the Ca influx was independent of Na_i and Na_o and increased linearly with the external Ca concen-

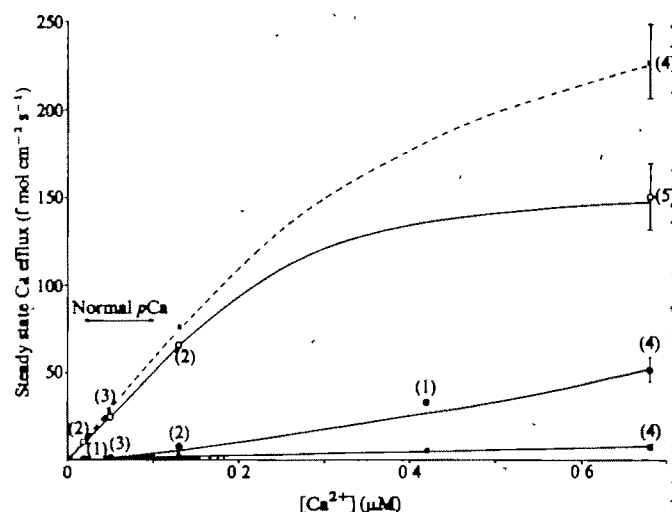


Fig. 2 Effect of internal ionised Ca on the Ca efflux components in dialysed squid axons. $\text{Na}_i = 65$ mM; temperature 16.5 °C. x, Ca efflux measured in ATP-dialysed axons (3 mM ATP + 5 mM PA) bathed in standard artificial seawater. o, Ca efflux measured in ATP-dialysed axons in solutions with Na_i and Ca_o both zero ('uncoupled' component). •, Na- and Ca-dependent Ca efflux measured in ATP-free axons. ■, Ca efflux measured in ATP-free axons with Na_i and Ca_o both zero (leak component). Ionised calcium in the dialysis medium was controlled by varying the ratio (CaEGTA)/(free EGTA) at a constant total EGTA of 1 mM. The apparent dissociation constant for the CaEGTA complex was chosen as 0.15 μM^2 . The numbers above the experimental points represent the number of determinations. The physiological range of Ca_i^{2+} concentration is indicated by the horizontal arrow.

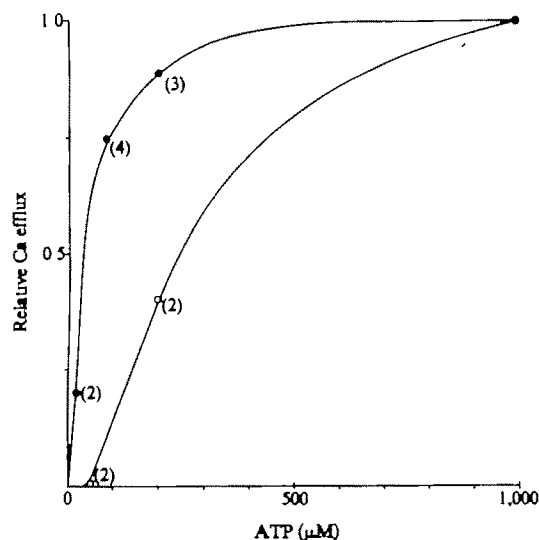


Fig. 3 Effect of internal ATP on the 'uncoupled' and Na-Ca dependent components of the Ca efflux. Ordinate: Ca efflux relative to the maximum efflux obtained with 1 mM ATP. Abscissa: ATP concentration in the dialysate in μM . The ATP in the axoplasm was buffered by adding 5 mM phosphoarginine to the dialysis medium. ●, Steady state Ca efflux level in the absence of external Na and Ca ('uncoupled'). ○, Na-Ca dependent Ca efflux. Half activation was obtained at 30 μM and 230 μM respectively. In most of the experiments shown above, the ATP dependence of both components was tested in the same axon. Once the ATP from the fibre had been removed by dialysis (washout 1–2 h) Na and Ca were removed from the external medium and increasing amounts of ATP (10, 50, 200, 1,000 μM) were sequentially added to the dialysis fluid. Once the ATP dependent 'uncoupled' Ca efflux had reached a steady state corresponding to each ATP concentration, the external Na and Ca sensitivity was tested in square pulse fashion. The number to the right of the experimental points represent the number of determinations. The curves were drawn by eye. The mean temperature was 17 °C.

trations. With 3–4 mM external Ca the influx of Ca was $\sim 38 \text{ fmol cm}^{-2} \text{ s}^{-1}$; this 'leak' influx closely matches the magnitude of the uncoupled Ca efflux observed here and obtained in comparable conditions. With high Ca^{2+} concentrations Ca influx was mainly through the Na-Ca exchange mechanism⁹.

Although ATP is not essential for the Na-Ca exchange mechanism to operate, it is known that the addition of ATP brings about an increase in the rate of that exchange¹⁰. Figure 3 shows several experiments where the relative rate of the 'uncoupled' Ca efflux and the ATP-stimulated Na-Ca exchange are plotted as a function of the ATP concentration in the dialysis solution. It is clear that the 'uncoupled' component shows a much higher affinity for ATP ($K_{1/2} = 30 \mu\text{M}$) than the Na-Ca exchange ($K_{1/2} = 230 \mu\text{M}$).

The experiments described above indicate the existence of two components of Ca efflux in squid axons: (1) uncoupled Ca efflux and (2) Na-Ca exchange. The properties of these components suggest that they indeed represent two different mechanisms for Ca translocation.

The 'uncoupled' Ca efflux, which has all the properties of a Ca pump, operates in the physiological range of Ca_i^{2+} concentration, thus playing a fundamental part in the regulation of intracellular ionized calcium. The Na-Ca exchange becomes more effective when Ca_i^{2+} is increased far beyond the physiological range. Although ATP hydrolysis is almost certainly involved in the operation of the Ca pump, the role of ATP on the Na-Ca exchange is still not clear. It is possible that the high Ca affinity (Ca + Mg)ATPase described by Robinson¹¹ in rat brain microsomes represents the biochemical counterpart of the ATP-fuelled Ca-pump considered here.

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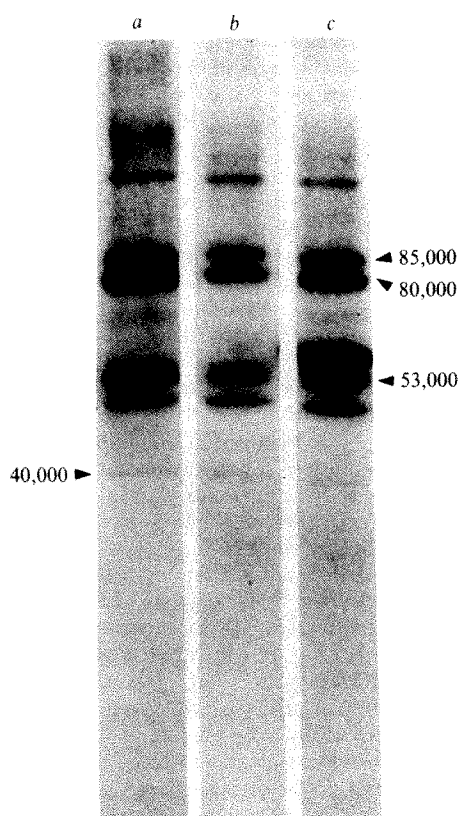
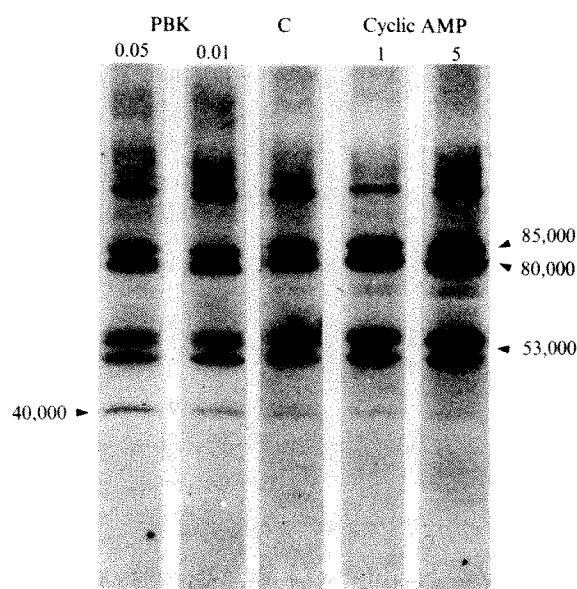
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Phosphorylase kinase phosphorylates a brain protein which is influenced by repetitive synaptic activation

SYNAPTIC systems from mammalian brain can now be studied *in situ* using a brain slice preparation which displays most of the electrophysiological characteristics observed in intact preparations^{1,2}. We have been particularly interested in analysis of long-term potentiation (LTP), a marked and quasi-permanent increase in synaptic efficacy which is found in the hippocampal formation following a brief (1 s) train of repetitive stimulation^{3–6}. Previous work from this laboratory demonstrated a strong correlation between induction of such LTP and the apparent phosphorylation of a 40,000 molecular weight protein in synaptic plasma membranes (SEM)^{7,8}. Both LTP and the stimulation-dependent changes in the 40,000-MW protein were dependent on calcium and this cation has also recently been shown (ref. 9 and unpublished observations) to enhance the endogenous phosphorylation of a protein with a molecular weight of $\sim 40,000$. If potentiation and the phosphorylation of the 40,000-MW protein are related, with calcium being required for both, then it is possible that a calcium-dependent protein kinase might be an intermediary enzyme in the two processes. We have therefore investigated the effect of exogenous phosphorylase kinase (PBK; EC 2.7.1.38), a calcium-sensitive enzyme found in brain^{10,11}, on the phosphorylation of the 40,000-MW protein. Since several laboratories have demonstrated cyclic AMP-dependent phosphorylation of several SPM proteins^{12–15}, it was also of interest to test for the possibility that this nucleotide might be involved in the phosphorylation of the 40,000-MW protein. We report here that PBK produces a marked and highly specific phosphorylation of the 40,000-MW protein that we have previously shown to be affected by high frequency potentiating stimulation.

We assayed the endogenous phosphorylation (in the presence of either cyclic AMP or PBK) of a SPM fraction which was prepared from hippocampal slices maintained in conditions described previously². Figure 1 shows an autoradiograph from a typical experiment. As can be seen, PBK markedly stimulated the phosphorylation of the 40,000-MW protein and had no detectable effect on any other protein band. Cyclic AMP produced a significant stimulation of three bands with MWs of 85,000, 80,000 and 53,000, as has been reported by several investigators^{12–15}, but it had no effect on the phosphorylation of

Fig. 1 Autoradiograph showing the effects of PBK and cyclic AMP on the endogenous phosphorylation of SPM proteins. PBK produced a marked dose-dependent increase, compared to control (C), in the phosphorylation of the 40,000-MW protein. The final concentrations of PBK were 0.01 and 0.05 mg ml⁻¹. Quantification was based on densitometric scans as described previously⁸. Here, 0.01 mg ml⁻¹ PBK increased the phosphorylation of the 40,000-MW protein by 45% and 0.05 mg ml⁻¹ increased the phosphorylation by 140%. Cyclic AMP produced significant stimulation of the phosphorylation of proteins with MWs of 85,000, 80,000 and 53,000, but this nucleotide had no effect on the phosphorylation of the 40,000 protein. The concentrations of cyclic AMP used in these experiments were 1 and 5 μ M. Molecular weight estimates were based on comparisons with the mobilities of standards of known MW. The SPM fractions used were prepared⁸ from hippocampal slices which had been maintained *in vitro*² for ~1 h. After isolation SPMs were preincubated at 30 °C in 40 μ l of the assay buffer (10 mM MgCl₂, 50 mM HEPES, pH 7.4), at a protein concentration of 0.25 mg ml⁻¹, and after 5 min the reaction was initiated by the addition of 10 μ l of [γ -³²P]ATP (10 μ Ci, 20 μ M final concentration) and was terminated after 20 s by the introduction of 30 μ l of a stop solution which yielded concentrations of: 2.3% SDS (w/v), 5% β -mercaptoethanol (v/v), 62.5 mM Tris HCl, 10% glycerol, pH 6.8. Samples were heated in a boiling water bath for 3 min and then run on SDS-polyacrylamide gradient gels¹⁶. The gels were stained for protein with Coomassie blue, dried under vacuum and exposed to Kodak RP Royal X-Omat film for 3–4 d. Kinematic studies, to be described elsewhere, indicate that incorporation of label in this assay reflects the initial velocity of the phosphorylation reaction. Cyclic AMP and phosphorylase kinase (Sigma, in 5 μ l assay buffer) were introduced just before addition of ATP to lessen possible phosphodiesterase attack on the cyclic AMP or proteolytic degradation of the phosphorylase kinase. Addition of 5 μ l of assay buffer just before addition of ATP had no effect on the phosphorylation of control samples. In the endogenous assay of the effects of PBK, no calcium is added but it can be assumed that endogenous calcium remains following the use of 50 μ M calcium in our fractionation solutions. The question of whether calcium can stimulate the phosphorylation of the 40,000-MW protein is obviously important to our hypothesis that a stimulation-dependent calcium influx activates PBK which in turn phosphorylates the protein. Herschkovitz⁹ has shown, using Ca/EGTA buffers, that the phosphorylation of a 41–43,000-MW SPM protein is stimulated by but not totally dependent on calcium. Our preliminary experiments, also using Ca/EGTA buffers, indicate that the 40,000-MW protein is stimulated by but not totally dependent on calcium. It is important to note in this regard that although PBK has an almost absolute requirement for calcium in its phosphorylation of phosphorylase b¹⁰, PBK from tissue other than skeletal muscle retains a considerable proportion (25–40%) of its activity in the absence of calcium¹⁷; in the case of troponin, calcium enhances a basal level of PBL-catalysed phosphorylation of this contractile protein¹⁸.



the 40,000-MW component. It should be noted that several bands which undergo phosphorylation in our assay are apparently not significantly influenced by either phosphorylase kinase or cyclic AMP.

It is possible that the very specific effects of PBK could occur if the kinase were sterically hindered and hence did not have access to other substrates. We therefore tested for the effects of another soluble kinase, a cyclic AMP-dependent protein kinase from beef heart, and the results of adding this enzyme to our assay are shown in Fig. 2. Several protein substrates for this kinase are present in our membrane preparation but little or no stimulation of the 40,000-MW protein was obtained. Thus the specificity of PBK for the 40,000-MW material is presumably a true substrate specificity and not the result of steric factors.

It seems then that PBK selectively phosphorylates an SPM protein which is also strongly influenced by repetitive synaptic

Fig. 2 The pattern of incorporation of ³²P into specific bands of a SPM fraction, assayed in the presence and absence of either the holoenzyme or the catalytic subunit of the cyclic AMP-dependent protein kinase from beef heart (Sigma), is shown in this autoradiograph. In sample a, which contained the catalytic subunit at a concentration of 0.025 mg ml⁻¹, there was a significant stimulation compared to the control (b), of three bands with MWs 85,000, 80,000 and 53,000. Similarly the holoenzyme produced a large stimulation of these same bands and also increased the incorporation of ³²P into a band with a MW of 57,000. This latter band may be the regulatory subunit of the beef heart protein kinase which has been reported to undergo autophosphorylation. Densitometric quantification indicated that the catalytic subunit and the holoenzyme of cyclic AMP-dependent protein kinase produced increases in the phosphorylation of the 40,000-MW protein of less than 5% in this autoradiograph and similar values were obtained in five separate experiments. All enzymes were added just before addition of ATP and reaction conditions were identical to those in Fig. 1.

activation. Further evidence that these two treatments are acting on the same polypeptide was obtained in experiments in which endogenously phosphorylated SPM proteins were analysed in the two-dimensional gel system of O'Farrell¹⁹. As is apparent in Fig. 3, the 40,000-MW material migrates as one (and sometimes two) quite basic polypeptides, and this material shows increased incorporation following the addition of PBK. This very basic spot (or doublet) represents essentially all of the phosphoprotein material found in the two-dimensional gel at the 40,000-MW position. These data indicate that PBK probably phosphorylates the same protein which shows changes in endogenous phosphorylation following repetitive synaptic stimulation, as the kinase increases the phosphorylation of all the 40,000-MW material resolved in the two-dimensional gel analysis. Furthermore, in preliminary studies (nine separate experiments) we have found that repetitive synaptic stimulation reduces the subsequent PBK-induced phosphorylation of the 40,000-MW band.

Studies of protein phosphorylation in brain often concentrate on cyclic AMP-dependent phosphorylation, but our data

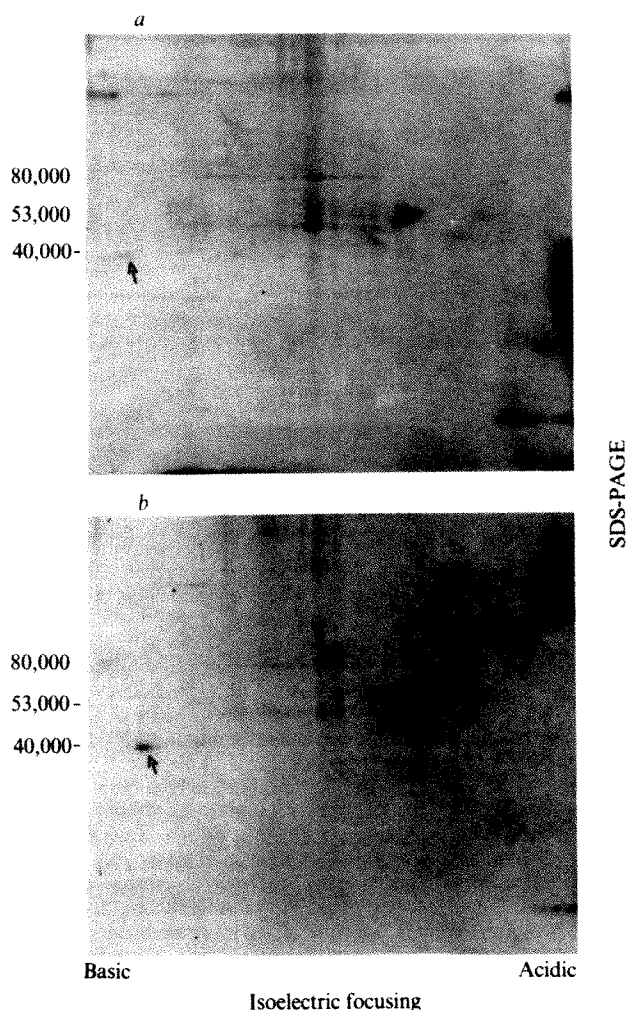


Fig. 3 Autoradiograph demonstrating the distribution in a two-dimensional electrophoretic system¹⁹, of ³²P-SPM proteins which were phosphorylated in the absence or presence of 0.01 mg ml⁻¹ PBK. The arrows indicate the position of the 40,000-MW material which migrates to a quite basic region in the isoelectric dimension of the gel. Note that in the fraction incubated with PBK there is a large increase, compared to control, in the amount of label incorporated into the 40,000-MW material. The tissue was phosphorylated in conditions described in Fig. 1 and 20 s after introduction of the [γ -³²P]ATP a 10 μ l stop solution was added to the incubation to yield final concentrations of 0.1% (w/v) SDS and 1% (v/v) β -mercaptoethanol. The samples were lyophilised, resuspended in sample buffer¹⁹, and subjected to a two-dimensional electrophoresis as described by O'Farrell¹⁹ with the exception that 3% ampholytes (pH 3.5–10.0, LKB) were used throughout.

suggest that this nucleotide is not involved in the change in the phosphorylation of the 40,000-MW protein that follows synaptic activation. We propose that the change produced in the phosphorylation of the 40,000-MW protein is more likely to be mediated by activation of phosphorylase kinase. This activation could result from the intraterminal accumulation of Ca²⁺ that is thought to occur during high frequency stimulation^{20–23}. Support for this hypothesis can be found in recent work which provides evidence that brain phosphorylase kinase is activated following stimulation^{24,25}, during seizures²⁶, and following application of putative neurotransmitters to cortical brain slices²⁷. Such an hypothesis obviously necessitates a function for phosphorylase kinase in brain other than its classical role in glycolysis, as the substrate for this process (phosphorylase *b*) has a MW considerably larger than the 40,000-MW protein. However, there is considerable evidence which indicates that PBK may in fact have several roles. Although controversial²⁸, England *et al.*²⁹ reported that the PBK-catalysed phosphorylation of troponin influences the Ca²⁺ sensitivity of the actomyosin ATPase. Interestingly, the troponin-T subunit, which is preferentially phosphorylated by PBK¹⁸, closely resembles the 40,000-MW SPM protein in MW and in its behaviour in isoelectric focusing gels³⁰. PBK has also been shown to modulate Ca²⁺ transport in the sarcoplasmic reticulum^{31,32} and the plasma membrane of skeletal muscle³³, but no protein substrate has yet been identified in this action although phosphorylase *b* inhibits the effect^{31,32}. Processes such as these are particularly exciting as potential modes of action for PBK in brain, especially in view of the possibility that the activation of PBK underlies the phosphoprotein changes correlated with long-term potentiation.

The relationship between the 40,000-MW protein changes produced by electrical stimulation and the extraordinarily long lasting increase in synaptic strength which follows such stimulation is uncertain. However, our demonstration that PBK selectively phosphorylates the 40,000-MW protein suggests new avenues for investigating the biochemical substrates of synaptic plasticity in brain.

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Mitochondrial calcium uptake and oxygen consumption in cystic fibrosis

CYSTIC FIBROSIS (CF), an autosomal recessive exocrinopathy, is characterised by chronic obstructive lung disease and gastrointestinal malabsorption. Reports of exocrine gland secretory anomalies in CF¹ and increased calcium ion concentrations in some CF secretions²⁻⁵, and the importance of cellular Ca to stimulus-secretion coupling⁶ led us to study cellular Ca in CF. We previously reported an enlarged intracellular Ca pool in skin fibroblasts from subjects with CF and obligate heterozygotes, as measured by ⁴⁵Ca exchange into and out of whole cells⁷. We suggested that the altered Ca pool represented a change in mitochondrial Ca. The finding of such an alteration in cells from obligate heterozygotes, who cannot be detected as possessing the CF gene before the birth of a child with CF, suggests that the Ca finding is related to the basic gene defect in CF. The intracellular site of the altered Ca pool or pools could not be identified by studies with whole cells but required measurement of Ca uptake by isolated cell organelles. Here, we report data on Ca uptake studies with mitochondrial and microsomal fractions isolated from skin fibroblasts of subjects with CF and age-, sex- and passage number-matched controls in which mitochondria from CF cells accumulate more Ca than those from control cells. We also describe subsequent studies which show increased mitochondrial electron transport activity, measured as oxygen uptake by whole cells, in cells from subjects with CF and obligate heterozygotes. All experiments were carried out with cells at passage 4-10.

Cells were grown to confluence in roller bottles as previously described⁷, collected by rubber scraper, washed and pelleted by centrifugation in cold 0.85% NaCl. Homogenisation of the resulting cell pellet (approximately 5 mg protein) was carried out at 4 °C in 5 ml 0.25 M sucrose containing 1 mM EDTA and 10 mM Tris-HCl, pH 7.4. Fifteen strokes with a tight-fitting Dounce homogeniser were sufficient to yield 90% cell disruption as assayed by light microscopy. Three subcellular fractions were recovered by differential centrifugation: unbroken cells and nuclei at 600g for 10 min, mitochondria at 20,000g for 15 min, and microsomes at 105,000g for 45 min. Succinate dehydrogenase (SDH) activity determinations⁸ were carried out on samples of cell homogenates and isolated fractions. SDH activity per mg protein in the mitochondrial fraction was 30-fold that in the cell homogenate and there was no measurable SDH activity in the microsomal fraction. Cell fractionation was carried out simultaneously on CF and control cell lines, and Ca uptake measurements were promptly carried out on the isolated fractions following procedures for mitochondrial⁹ and microsomal¹⁰ fractions.

Mitochondria isolated from normal human skin fibroblasts accumulated Ca (Fig. 1) with a capacity and time course similar to mitochondria from other tissues¹¹. We believe this to be the first report of Ca uptake by mitochondria isolated from a nontransformed human cell type. CF mitochondria accumulated more Ca than control mitochondria (Fig. 1), and Ca uptake in CF mitochondria was about 78% more than in controls at 30 min (nmol Ca per mg mitochondrial protein: CF = 122.7 ± 10.7, control = 69.1 ± 6.0, *n* = 15 pairs). This difference was highly significant (*P* < 0.0005). In contrast to the marked difference in Ca uptake measured between CF and control mitochondria, the microsomal fractions from CF and control cells accumulated equal amounts of Ca (nmol per mg protein measured at 15 min: CF = 23.5 ± 3.1, control = 27.6 ± 3.7, *n* = 8 pairs). These findings indicate an altered mitochondrial capacity for Ca uptake in CF fibroblasts sufficient to account for the previously reported increase in CF whole cell Ca^{7,12}. The microsomal fraction from skin fibroblasts does not contribute to the CF Ca difference observed in these cells.

Ca uptake measured on whole cell homogenates after the method of Ash and Bygrave¹³ further supported the hypothesis of altered mitochondrial uptake in CF fibroblasts. In this experiment Ca uptake was determined in the presence and absence of ruthenium red, a potent and specific inhibitor of mitochondrial Ca uptake, which allowed a breakdown of the total Ca uptake by the cell homogenate into two portions, one attributable to mitochondrial (ruthenium red sensitive) and the other to all extra-mitochondrial Ca accumulation. The 8-min measurement taken after a plateau in Ca uptake was reached revealed a greater total Ca uptake in four CF cell-line homogenates compared with paired control values (110.10 and 93.43 nmol per mg protein, respectively), matched pair Student's *t*-test *P* < 0.01, and a greater mitochondrial Ca uptake in CF cell homogenates compared with paired control values (101.28 and 83.73 nmol per mg protein, respectively), matched pair *t*-test *P* < 0.005. The entire difference in Ca uptake between CF and control cell homogenates is accounted for by the increased Ca accumulated by CF mitochondria.

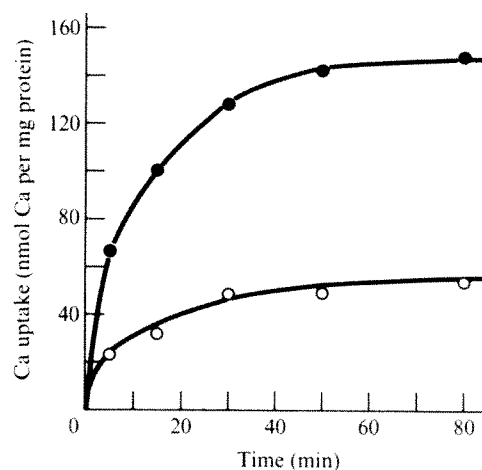


Fig. 1 The time course of Ca uptake per mg mitochondrial protein by CF (●) and control (○) mitochondria. Each point represents the mean of duplicate determinations on two CF and two control mitochondrial preparations. Isolated mitochondrial fractions (~500 µg protein) were washed twice in EDTA-free 0.25 M sucrose/Tris, pH 7.4, then suspended in solution A (Tris-HCl 33.6 µmol, KCl 24 µmol, MgCl₂ 3 µmol, and sucrose 25 µmol per 400 µl, pH 7.4). A 100-µl aliquot of mitochondrial suspension was delivered to each experimental tube (no. 2051 Falcon) containing 400 µl solution A at 20 °C with air as the gas phase. After a 10-min incubation, 100 µl of solution B (sodium succinate 6 µmol, KH₂PO₄ 1.8 µmol, and ATP 0.45 µmol per 100 µl, pH 7.4) was added, and 60 s later 100 nmol ⁴⁵CaCl₂ were added in a 100-µl volume. At each sampling time point a 100-µl aliquot was removed from the buffer and delivered to a microcentrifuge tube (500 µl) containing 100 µl of quench solution (20 mM EGTA and 10 µM ruthenium red). The quenching tubes were centrifuged at 12,000g for 4 min to pellet the microchondria, and a 100-µl sample of the supernatant was taken for ⁴⁵Ca determination.

Mitochondrial Ca uptake and release is vital to Ca homeostasis in many cell types. In such cells, Ca-dependent processes may be fundamentally under the influence of the mitochondrial Ca pool. Release of Ca by mitochondria gives a rapidly available Ca source and sequestration by mitochondria provides a major Ca sink. Cytoplasmic Ca, even in discrete areas of the cell, has been shown to be controlled by local mitochondria¹⁴.

One can hypothesise a mechanism to link the altered mitochondrial Ca in CF fibroblasts and the major signs associated with the disease. Much of the pathology of CF is caused by secretion of highly viscous mucus in the respiratory and gastrointestinal systems¹. Increased viscosity of CF mucus can

be attributed to high levels of Ca in these secretions^{15,16} and Ca in secretory cells is closely associated with secretory vesicles¹⁷. The enlarged intracellular Ca pool present in fibroblasts may also exist in exocrine cells in CF and may account for the increased levels of Ca in some CF secretions. Such an hypothesis demands further substantiation, but it is very significant that altered cell function in CF may be due to altered Ca homeostasis.

Ca uptake by mitochondria is a complex reaction driven by the proton gradient established across the inner mitochondrial membrane by the electron transport system^{18,19}, and this system may form the basis for the observed Ca alteration in CF cells. We have begun to study electron transport activity in CF fibroblasts by measuring oxygen uptake by whole cells.

CF, heterozygote and respective control fibroblasts were collected by trypsin digestion and suspended in Hank's balanced salt solution with 1 mg% bovine serum albumin and 200 mg% dextrose. Oxygen consumption by the suspended cells was measured with an oxygen electrode (model 53, Yellow Springs Instruments) at a cell concentration of approximately 1×10^7 cells per 3 ml buffer. Both CF and heterozygote fibroblasts consumed oxygen at a significantly higher rate than their respective control fibroblasts (n atoms oxygen per min per 10^8 cells: CF = 335.4 ± 28.7 , control = 170.7 ± 28.5 , $n = 5$ pairs, $P < 0.005$; heterozygote = 227.7 ± 17.7 , control = 156.3 ± 15.6 , $n = 8$ pairs, $P < 0.005$). The measured oxygen uptake was completely inhibited by cyanide, indicating that it was mitochondrial. These data suggest that the electron transport system in CF and obligate heterozygote fibroblasts is more active than that in respective controls, in the conditions of this study.

The apparent central role of Ca in several pathogenetic pathways in CF and the data reported here in which both mitochondrial Ca capacity and oxygen consumption are increased in CF suggest that this organelle is a suitable focus for CF investigations.

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Intraspecific hybridisation and the release of mutator activity

It has been suggested by Grant^{1,2} that mutation and hybridisation may not be independent of one another as sources of genetic variation in natural populations. Indeed, it has been shown in both plants and animals³⁻⁶ that hybridisation can stimulate the production of new mutations and chromosome breakage events. The possible role of intraspecific hybridisation in inducing genetic change has recently been discussed in various contexts, especially in relation to the action of mutator genes in natural populations^{1,2,9-11}. We have previously suggested that mutator factors, which are known to induce both visible and lethal mutations and chromosome breakage, are commonly present in natural populations of *Drosophila melanogaster*. Within subpopulations, however, these mutators are genetically suppressed. On hybridisation between subpopulations, genetic suppression breaks down, resulting in the release of mutator activity and, hence, in an explosive increase in genetic variation.

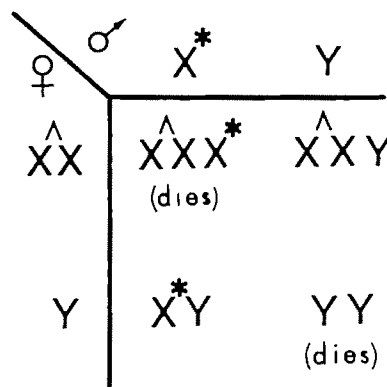


Fig. 1 Progeny from a cross of *D. melanogaster* females with attached-X chromosomes and males from natural population lines or a control line. X* = X chromosomes scored for spontaneous sex-linked visible mutations in male progeny.

Clearly, these explosions of mutation, which have been documented in some natural populations^{12,13}, might have an important role in evolution and speciation. Here, we test this hypothesis of 'hybrid release' directly by determining whether intraspecific hybridisation increases the frequency of mutation above that found in the sampled natural population. Our study differs significantly from other assays of mutator gene effect¹⁴⁻¹⁶ in that mutation frequencies before and after hybridisation are compared directly. The results show that hybrid release of mutator activity does occur as the result of hybridisation, producing a severalfold increase in the frequency of mutation. Thus, hybrid release may be a major mechanism in the induction of genetic variation in natural populations and so may be a driving force in evolution.

Two sets of experiments were carried out, one assaying X-linked visible mutations, the other X-linked recessive lethal mutations. The rationale in both experiments is to determine the frequency of mutation events before and after hybridisation of a wild-type *D. melanogaster* strain with a laboratory strain. The wild-type strains included 'OK1' (which has been observed to undergo recombination in males¹⁷ and frequent chromosome breakage in spermatocytes¹⁸ after outcrossing to laboratory stocks, and is typical of the large number of wild mutator strains that have been collected worldwide⁹), 'GR' males (collected from a Grand Rapids, Ohio, population in June 1978), and 'NO' males (collected from a Noble, Oklahoma, population in July and August 1978). The inbred wild-type laboratory strain Canton-S served as a control. C(1)DX, yf (a marked attached-X stock) and Basc (a marked X chromosome balancer stock)

Table 1 Sex-linked visible and recessive lethal mutation frequencies before and after hybridisation of wild-type strains and a control stock

Males tested	Visible mutants*			Lethal mutants†		
	Mutants	Total	%	Mutants	Total	%
Wild base population	0	13,355	0	31	10,496	0.30
Wild hybrid males	43	16,501	0.26	95	10,288	0.92
Control (Canton-S) base population	0	8,590	0			
Control (Canton-S) hybrid males	0	8,803	0			

Visible mutants were scored using OK1 as the wild population following the breeding programme of Fig. 1; recessive lethal mutations were scored using Grand Rapids males as the wild population and the breeding programme described in the text.

* For wild population $P < 0.001$.

† $P < 0.001$.

were used to manipulate tested chromosomes in the breeding programmes¹⁹.

The experiment to assay X-linked recessive visible mutations is based on the transmission of *Drosophila* attached-X chromosomes (Fig. 1). The only surviving offspring of a cross between females with attached-X chromosomes and wild-type males are attached-X females and males which have received their X chromosome from their father. Thus, in the first generation of such a cross, the phenotypes of male offspring will provide a direct measure of mutation in the homozygous wild-type base population; any mutant male will have been the result of mutation in a sperm formed before hybridisation. These male progeny, however, are hybrids, being heterozygous for each of the autosomes. Mutation events that occur in their developing sperm can be detected by repeating the cross to attached-X females. A comparison of mutation frequency in the second cross (hybrid males) to that in the first cross (base stock males) will show the effect of hybridisation on mutation frequency. Data from these crosses are summarised in Table 1.

No visible mutations were found among 13,355 tested X chromosomes from the base population, but a mutation frequency of 0.26% was found among 16,501 X chromosomes in hybrid males of the second cross. In a smaller sample of chromosomes tested with NO males, no visible mutations were found among 1,726 tested X chromosomes in the first cross, whereas 9 visible mutations were found among 3,483 tested chromosomes in the second cross (0.26%). The absence of visible mutations is apparently typical of first crosses of wild lines. Although a test of second-cross hybrids has not been done with two other wild Oklahoma populations, no visible mutations have been detected in a total of 9,510 tested first-cross chromosomes (0/4,578 from Stratford, Oklahoma and 0/4,932 from Tishomingo, Oklahoma, populations). As a control, the wild-type laboratory strain, Canton-S, which has been observed to be free of mutator factors¹⁸, was also scored for spontaneous sex-linked visible mutations in base stock males and in hybrid males. No increase in mutation frequency was observed after hybridisation (Table 1). The results of this control experiment show that the increase in mutation frequency after hybridisation in the crosses with natural population lines is due to hybrid release of mutator factors in the natural population lines and not in the laboratory stock.

The most common mutations were allelic to the *singed* bristle mutation, although a variety of body and eye colour mutants, rough eye, and bristle size and shape mutations have been found in the hybrid cross. All these mutations bred true in subsequent generations. It is interesting that *singed* was also the most

common mutant type found by Green *et al.*^{20,21} in their studies of mutator genes. In addition, their mutants were unstable, suggesting that they might have been induced by the reversible incorporation of an episome or other factor into the chromosome^{20,21}. No revertants have yet been found in our mutants among only a small sample scored, but one case of a second (*singed*) mutation occurring in a mutant line (yellow body colour) demonstrates that mutation induction is not limited to the first hybrid generation.

As the X chromosomes in the first and second crosses are the same, any difference in mutation frequencies is due to the intraspecific hybridisation between lines. There is no doubt that these data show a marked increase in mutation frequency in hybrids. The visible mutations induced in a line, however, are only a small proportion of the total mutations, the largest class being recessive lethals. Thus, we confirmed these observations by assaying recessive lethal mutation frequencies in crosses equivalent to the first and second crosses described above.

Recessive sex-linked lethal mutations were detected by crossing 51 individual wild-caught GR males to Basc females. From each cross, individual GR/Basc females were then mated with Basc males, and their progeny scored for recessive X-linked lethals by noting the presence (no lethal) or absence (lethal) of wild-type males among the progeny. Any lethals would have occurred during the sperm development of the base population wild males, making this cross equivalent to the first cross described for visible mutations. Hybrid GR males from each of the 51 lines were scored by crossing single viable hybrid GR males (heterozygous for the Basc autosomes) to Basc females and repeating the original crosses.

A comparison of recessive lethal mutation frequencies in base population males and hybrid males is shown in Table 1. The hybrid release hypothesis would again predict that the frequency of lethal mutations in the hybrid males would be higher than that in the base population males. This is precisely what is found. A threefold increase in mutation frequency is observed on hybridisation.

The results from these experiments show that intraspecific hybridisation does increase the frequency of mutations, and provides strong evidence in favour of the hypothesis of hybrid release of mutator activity. It has been well established that mutators are present in many *Drosophila* populations⁹ and probably in other organisms^{4,5,22}. The assumption that mutators are genetically suppressed within subpopulations has been supported by these results and by studies of chromosome breakage in *Drosophila* spermatocytes¹⁸ which were analysed directly in the base population.

Indeed, the genetic suppression of mutator activity within a subpopulation is a consequence that is readily predictable from the action of natural selection^{6,10,11}. However, one would not necessarily expect the same genes to be involved in the suppression of mutation in different subpopulations. Thus, hybridisation would lead to heterozygosity of suppressor genes and to further breakdown by segregation in later generations. This hypothesis makes several predictions, such as the involvement of recessive rather than dominant suppressor loci, which are testable in principle. The increase in mutation frequency and chromosome breakage events observed after hybridisation could also be part of a larger syndrome of genetic and developmental changes that occur with the disruption of adapted gene complexes. Further work in this area should contribute to a better understanding of the role of mutator genes in generating the variability that has a major role in the response of populations to changing environments and to competition from other populations.

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Gene numbers as measured by single-copy DNA saturation with mRNA are routinely overestimates

A MAJOR experimental approach to the elucidation of the role of differential gene expression in developmental processes has been to measure the number of different mRNA sequences in eukaryotic cells¹. This number has been obtained from the percentage of radioactive single-copy DNA rendered double-stranded during hybridisation with homologous mRNA. The percentage, appropriately corrected for the average size of mRNA and the chemical complexity of the unique DNA of the organisms, can be related to the total number of different genes expressed in the functional mRNAs of the cell type or tissue studied². The number and distribution of different mRNA sequences can also be determined by hybridising radioactive cDNA with excess unlabelled mRNA and comparing its kinetics of hybridisation with a suitable kinetic standard³. However, here, I discuss the use of single-copy DNA saturation with mRNA, and suggest that gene numbers obtained in this way are routinely overestimates.

Although both the above methods have disadvantages, especially because of difficulties in driving the reactions to completion, quite consistent measurements have been obtained for some organisms^{4,5}. However, gene numbers as determined by the two experimental approaches often differ appreciably. As shown in Table 1, it is striking that cDNA values obtained even in the most favourable conditions⁶ are generally less than those of single-copy DNA measurements. This may be due to the experimental shortcomings of the cDNA method as suggested by Hereford and Rosbash⁷.

However, we should consider the basic assumptions underlying interpretation of single-copy saturation data⁸. Most single-copy sequences were presumed to be of about the same length as mRNA and to be flanked by repetitive sequences. Single-copy sequences, isolated from randomly sheared nuclear DNA by

repeated fractionation at C_0t levels at which most repetitive sequences should have reassociated, were thought to be a representative probe of total unique DNA. Furthermore, it was thought that coding sequences were rendered double-stranded by complementary RNA sequences over their whole length due to the short tracer length (200-300 nucleotides) relative to mRNA length. Both assumptions, if invalid, would create a bias towards too high gene numbers.

Eukaryotic genomes contain much repetitive DNA⁸, most of which is intimately interspersed with unique DNA⁹. It has been shown that the reassociation analysis of sheared nuclear DNA does not reveal all the unique DNA because of its interspersion with sequence classes with faster kinetics¹⁰. The cryptic unique DNA content of a genome may be estimated by further experiments which allow the extrapolation of reassociation kinetics to zero tracer fragment length. In the first such experiment, gene number estimation was explicitly based on the total genomic unique DNA content. This rationale has also been used in other investigations^{11,12}. ³H-labelled single-copy DNA used for such saturation experiments will, however, be enriched for the fraction of single-copy DNA that is organised in long, continuous stretches. Thus, cryptic single-copy sequences will seldom, if ever, be present in such preparations, whereas sequences from long stretches of unique DNA will be almost exclusively non-cryptic and will be over-represented by a factor depending on the ratio of total unique DNA to the kinetically resolved unique DNA. This is because, from an individual unique sequence of length, L_u , using hydroxyapatite (HAP) assay in reassociation kinetics analysis of total randomly sheared DNA of median length L_m , only the fraction $(L_u - L_m + L_h)/L_u$ will seem to be unique, where L_h is the minimum length of double-stranded DNA able to bind to HAP. If L_h is 40 nucleotides and L_m 300 nucleotides, then unique sequences of L_u below 260 nucleotides will not be seen as unique, and sequences of $L_u = 500$ nucleotides will seem to be 50% unique. At a median length of 1,400 nucleotides about 80% of all sequences will seem to be unique. As the mean length of mRNAs is about 1,500 nucleotides and as almost all mRNAs are coded for by unique sequences^{1,11,13}, it is reasonable to assume that most mRNAs will be complementary to unique sequences which mainly behave non-cryptically during reassociation analysis of moderately long sheared DNA (300-400 nucleotides).

If the proposed assumption is valid, gene numbers in higher animals would need to be reduced by a factor of 0.6-1.0. However, gene numbers in plants would be more seriously affected. Higher plant genomes contain a large proportion of repetitive DNA, often amounting to more than 60% (ref. 14). Repetitive DNA sequences occur at short intervals throughout the majority of all unique DNA¹⁵⁻¹⁹, thus concealing a large fraction of unique DNA during reassociation analysis. Murray *et al.*¹⁹ have demonstrated that the majority of all unique DNA sequences in the pea are only about 300 nucleotides long. This must also be true of parsley, where we have shown that in the reassociation analysis of 300-nucleotide total nuclear DNA, only 40% of all unique DNA was revealed.

Goldberg *et al.*¹¹ have reported the first measurements of the sequence complexity of polyosomal RNA from cells of a higher plant (tobacco leaves). Here, although ³H-labelled single-copy saturation experiments indicated the presence of 27,000 different average-sized mRNAs, cDNA-poly(A)-mRNA hybridisation only revealed the presence of 12,000 different species. This and similar results of our own experiments on higher plants are listed in Table 1.

The strikingly different results obtained by the two methods suggest that the assumptions outlined above may be correct, particularly for plant gene number measurements. It may therefore be necessary to reduce single-copy DNA gene number estimates to take into account the degree of over-representation of mRNA coding sequences in the tracer single-copy DNA preparation. I propose that gene number estimates should be based on the fraction of unique DNA resolvable in reassociation kinetics of 400-nucleotide DNA.

Table 1 Comparison of gene number measurements in various species to show overestimation

Organism	Haploid genome size (nucleotide pairs)	% of scDNA (300 nucleotides) reacting with RNA	% of DNA (300-450 nucleotides) observably unique	% of DNA revealed unique in inter-spersion analysis	% of DNA assumed unique in complexity estimate	Gene number estimate based on assumed unique DNA	Gene number estimate based on observed unique DNA	Gene number estimate based on cDNA hybridisation kinetics
Yeast ⁷	1.3×10^7	20.0	95		95	4,000 (1,500)*	4,000	3,000
Achlya ^{5,24,25}	4.2×10^7	3.76-7.0	82		64	1,850-3,400 (1,150)	2,400-4,400	3,200
Sea urchin								
Embryo ²	8.1×10^8	1.35	50	75	75	14,000 (1,200)	9,300	
Embryo ²⁶ + adult	8.1×10^8	3.0	50	75	75	30,000 (1,200)	20,000	
Chicken								
oviduct	1.23×10^9	1.8	70		70	15,700 (1,900)	15,700	14,000
Tobacco ^{11,17}								
leaves	1.65×10^9	2.6	23	42	42	27,000 (1,240)	15,000	12,000
Mouse ²⁷⁻²⁹								
L cells	2.7×10^9	1.3	70		70	26,000 (1,000)	26,000	10,000
Paraleya ^{18,30}								
Leaves	1.9×10^9	2.54	12	30	30	25,000 (1,400)	10,000	9,200
Callus ^{13,18}	1.9×10^9	3.43	12	30	30	35,000 (1,400)	13,700	11,500
Rat ⁶	2.9×10^9	1.43	70		70	31,000 (1,800)	31,000	23,000
Barley ^{30,31}								
leaves	6.1×10^9	1.46	12	25	25	30,000 (1,500)	14,400	

* Figures in parentheses are nucleotides.

In view of recent findings on gene structure^{20,21}, the assumption that during hybridisation, coding sequences are rendered double-stranded over their whole length, may also be incorrect. The organisation of eukaryotic genes may be based on the pattern of the ovalbumin gene²². Here, quite short coding pieces alternate with longer spacers of noncoding DNA belonging to the same kinetic DNA class, that is, unique DNA. However, this would mean that random shearing of genomic DNA and hybridisation with homologous mRNA would enlarge the fraction of single-copy DNA bound to HAP during the standard assay procedure. Hybridised coding sequences will bear additional single-copy DNA single-strand tails, thus apparently representing a larger coding sequence complexity than they really comprise.

Consider a coding element of DNA (an exon) of length L_e , sheared to a median length, L_m , and separated from other coding sequences by spacers of length L_s . Then, this stretch of DNA after saturation hybridisation to RNA and analysis by HAP chromatography, may form hybrids over a length of $L = L_e + 2(L_m - L_h)$ and statistically will seem to be hybridised over a length of

$$L = L_e + L_m - 2L_h \quad \text{if } L_m - L_h < L_s$$

and

$$L = L_e + L_s - 2L_h \quad \text{if } L_m - L_h > L_s$$

L/L_e will give an estimate of the overhybridisation. For an arbitrary case of an intron 200 nucleotides long, with fragments of mean length 250 nucleotides, a space of length 400 nucleotides, and HAP binding to a minimum length of 40 nucleotides of double-stranded nucleic acid, the overestimation of the length of the intron would be 1.86-fold.

A way of avoiding such overestimates would be to use the S_1 nuclease assay instead of the HAP assay. A procedure adapted for experiments where low background values are of crucial importance has been developed by Maxwell *et al.*²³

If both the above considerations are broadly correct, the new scDNA-based gene numbers would be less than the corresponding cDNA numbers. However, it seems that the two assumptions will not be wholly applicable. There will be at least some small unique DNA genes and also some genes with

intervening repetitive DNA spacer sequences, as suggested by the finding of repetitive sequences in animal hnRNA¹.

However, taking into account these modified assumptions, it seems possible to reconcile results of gene number measurements obtained by different methods. This is particularly so for differences in plant experiments. The results reinterpreted in this way will reduce gene number estimates for plants from some 30,000 to 10-15,000 different active genes in cells and tissues examined.

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matters arising

Holocene reef growth on the edge of the Florida Shelf

LIGHTY *ET AL.*¹ have raised several important lines of speculation concerning the growth of reefs in the Florida and Bahamas areas and elsewhere in the Caribbean. The principal contribution of these authors has been to show an effective growth of a shallow-water coral-frame assemblage dominated by *Acropora palmata* in a period between $9,440 \pm 85$ and $7,145 \pm 80$ yr BP. This extends the evidence previously presented by Macintyre and Glynn², Adey³ and Easton and Olson⁴ for progressive growth over the past 6,000 yr.

The structures described lie within a depth range (15–30 m) which, only 40 km to the south, is accessible to Recent coral growth. One might speculate that, where conditions of growth are more favourable, reefs of the same age as those described by Lighty *et al.*¹ have been buried beneath growth covering the period from ~7,000 yr BP to the present.

Observations by Goreau and Goreau⁵ and Goreau and Land⁶ in Jamaica and by Newall and Rigby⁷ and Shinn⁸ and myself in the Florida–Bahamas province show a remarkable similarity of offshore profiles. The first element is a gentle slope to ~10–15 m depth (visible as a distinctive terrace on Fig. 1 of Lighty *et al.*¹). It is from this that the major reefs in Florida (for example, Caryfort, Dry Rocks, Alligator Reef, and so on) rise and, at least on Andros and Eleuthera, so do the major reef patches. Seawards, the surface slopes more steeply to about 30 m before plunging almost vertically in ‘the wall’ described by Ginsburg and James⁹, Goreau and Land⁶ and others. The illustrations of Goreau and Goreau⁵ seem to apply throughout the Caribbean area and suggest only two shelf-edge growth sites. Figure 1 of Lighty *et al.*¹ suggests a third, from 15 m.

We need to exercise some care in interpreting terrace levels as much of the mobile sand is derived from the excess production of the shallow-water zone (see Neumann and Land¹⁰ and Moore *et al.*¹¹). However, the widespread occurrence within these offshore platforms of exposed Pleistocene limestones indicates that we are not dealing with accretion levels but with surfaces prepared by erosion during glacial periods (compare Purdy¹²).

Now, although there are no readily

available figures for growth rates at 30 m depth there is certainly subjective evidence that they are slow. There are commonly <2 m of ‘frame’ projecting above the sand surface. In effect, relief from present-day surfaces in these depths is no greater, and may perhaps be less, than on the ‘inactive’ structures to the north.

Thus, it seems reasonable to assume first that surfaces which could have supported active coral growth at a time of rising sea level did not and, second, that the structures which we now see at 30 m are in effect the surfaces of frames which were first formed 7,000–10,000 yr ago. There is an obvious incentive to test them, using the methods described by Macintyre¹³, bearing in mind the suggestion of Macintyre¹⁴ that structures on terraces at 30–80 m depth in the eastern Caribbean do indeed represent an older phase of accretion.

We may now rephrase the question posed by Lighty *et al.*¹ What event, taking place ~7,000 yr ago, inhibited all shallow-water reef growth in the Caribbean? This growth was never able to resume in their northern area, presumably for the reasons which they accept pertaining to cold surface waters. In southern areas, however, growth began on new sites along the edge of the inner break in slope, forming the present shelf edge patches.

Lighty *et al.*¹ provide figures to show that an *Acropora palmata* reef is capable of keeping pace with rising sea level. There seems little reason to doubt this capability, but did it in fact do so? I believe that it did not and that, far from creeping upwards along the surface of a submerging slope, the principal locus of growth was established on a new and much shallower site. We are left with the conclusion that although sea level rose throughout the Caribbean there was a relatively abrupt change at ~7,000 yr BP. The broad outlines of reef morphology may have been defined at some earlier date but it was this event which determined the areas in which corals would grow. Sadly, in the area described by Lighty *et al.*¹ growth was no longer possible.

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LIGHTY *ET AL.* REPLY—The points raised by Braithwaite have been discussed in several studies of Caribbean Holocene reefs. As mentioned in our letter¹, for example, core data from a relict reef (depth 18 m) off southeastern St Croix indicated that a flourishing, shallow-water *Acropora palmata* (Lamarck) barrier reef was killed about 8,000 to 9,000 yr BP, when the shelf was flooded². Increased water turbidity related to the erosion of soil cover was thought to be responsible for the demise of this early Holocene reef. By the time water conditions returned to normal, the depth of water was too great for an *A. palmata* reef to be re-established. Subsequently, the substrate was colonised by a slowly accreting, deeper water community consisting of coral heads and octocorals.

That study² and others^{3,4} have proposed that turbidity, related to shelf erosion accompanying rising sea level, has generally prevented the continuous accumulation of Holocene reefs on wide shelf platforms of the Caribbean that are deeper than 10 m below present sea level. Although impressive reef structures are present on platforms <10 m below present sea level, these structures cannot be older than 4,000–7,000 yr BP, which is the period of initial flooding of these platforms. In south Florida, for example, the present reef tract has developed over the past 7,000 yr (ref. 5) on shallow areas of the shelf, behind the relict, shelf-edge reef of early Holocene age⁶.

Some deeper shelf areas isolated from the effects of shelf erosion might have continuous Holocene reef sections dating back to 10,000 yr BP, but no such section has yet been found.

Note, however, that in protected areas,

the fragile and rapidly growing *Acropora cervicornis* (Lamarck) is capable of constructing extensive reef structures in depths of 10–15 m (ref. 7). A Holocene reef section 33.5 m thick that consists primarily of this coral has been drilled off the lee side of the Alacran reef complex^{7,8}.

Evidence thus far indicates that Holocene reef history in the Western Atlantic is governed by factors related to shelf depth, extent of Pleistocene sediment cover on these shelves before flooding, degree of protection allowing the accumulation of *Acropora cervicornis* in intermediate water depths, and water temperatures in areas near the northerly limits of present-day reef development.

There is no evidence to indicate that a major 'event' 7,000 yr ago inhibited reef growth in the Western Atlantic. The death of shelf-edge reefs in this region is related to the time of shelf flooding, which varies from area to area, depending on local bathymetry. Some reefs (such as Galeata Reef⁹ and South Florida reef¹⁰) were, in fact, established on shallow-shelf areas around 7,000 yr BP. Another point to clarify is that relict reefs died when the rate of rising sea level was decreasing, rather than increasing, so that their death cannot be related to their inability to keep pace with the rising seas.

Braithwaite implies that reef growth, if uninterrupted, would accumulate by "creeping upwards along the surface of a submerging slope". Evidence suggests, however, that the relict, early Holocene reefs, in response to relatively rapid changes in sea level, favoured vertical over lateral growth. Reef accretion is therefore limited to linear trends that form narrow ridges; these ridges are a dominant feature of the sea floor topography of outer shelves and upper slopes in the eastern Caribbean¹⁰.

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Climatic signals in tree-ring chronologies for British oaks

HUGHES *et al.*¹ claim that analyses of the ring-widths of British oaks should make it 'possible to produce maps of annual or even seasonal climatic anomalies' and that these 'would provide a significantly more detailed picture of climate in the past 250 years than is now available'. This claim is based on what they regard as clear climatic signals from regression analysis applied to monthly means of temperature and precipitation and to mean ring-widths of 10–35 oaks for periods of 44–60 yr on three sites around the Irish Sea.

In fact, as their Fig. 1 shows, few of the responses to the various monthly means have 95% confidence limits that are significantly different from zero, and they are also inconsistent from one site to another. Thus the effect of temperature is positive in April and negative in May at Rostrevor but the reverse at Raehills. They do not mention any test for reproducibility on a site either by using different sets of trees or successive periods of time.

Our criticism of the work does not relate to the statistical method with its use of principal components² that Hughes *et al.* use: this has proved successful where the growth of trees is primarily controlled by one climatic factor. We believe the weakness of the claim comes from two limitations.

The first is the unsuitability of the data used. Chronologies of mean ring-widths were developed for dating by dendrochronology and are not necessarily appropriate to dendroclimatology. Monthly meteorological means are merely convenient summaries of climate: their use with oak is suspect because in different years the date of bud-burst varies by several weeks and therefore frost damage to young leaves can apply in a different calendar month. Other climatic anomalies and extremes are also obscured by the use of monthly means.

When regression analysis is used in an exploratory role, the result will be reliable

only if the major factors which affect the tree growth are included. The second limitation concerns the failure to determine the extent to which the oaks suffered damage from defoliating caterpillars, and to include this factor, if appreciable, in the analysis. Measurements of the considerable reduction of the annual growth of oak as a result of damage by caterpillars and other insects have been made in Russia³, Germany⁴, Denmark, (K. Christensen, unpublished data), and by ourselves at Oxford⁵, and warnings of the implications of this factor on dendroclimatology expressed. In the light of these considerations, the exploratory work reported by Hughes *et al.* requires evidence for reproducibility before their claim to have found clear climatic responses can be accepted. Our studies on oaks show that monthly correlations for the past 45 yr of growth with monthly climatic data are not maintained when the past 90 yr are examined.

One successful application in Europe of the Fritts method has been the outcome of preliminary studies to select a suitable parameter for use in the analysis in conjunction with a climatic stress that is strong, reproducible and not vitiated by the limitations of monthly meteorological data. Schweingruber, Bräker and Schär⁶ found that damage by insects, such as the larch-bud moth on conifers in the Alps, could be avoided by using the maximum annual density (derived by X-ray radiography) in place of ring-widths. In this way, the growth of alpine conifers on many sites in the Alps were found to be consistently related to the summer temperatures in July, August and September. We believe that the future of dendroclimatology in the British Isles needs a similar experimental approach to achieve success.

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HUGHES ET AL. REPLY—Varley *et al.*'s¹ comments are based largely on misunderstandings of dendroclimatological techniques²⁻⁷. We agree that it is not appropriate to use mean ring-width chronologies. We did not do so but rather used standardised indices of ring-width² for well-documented reasons^{5,7,8}. We did not use regression analysis of ring-widths on monthly meteorological figures as suggested¹. We stated clearly² that ring-width indices were regressed on the eigenvectors of climatic data. Consequently our results should not be compared with those referred to by Varley *et al.*^{1,9} where no account has been taken of the non-orthogonality of the data. We have maximised tree-ring variance attributable to the eigenvectors of climate, not the number of significant response function elements. The two are not simply related⁵. We discussed differences between the reported response functions. Such 'inconsistency' was ecologically expected and dendroclimatologically welcome^{5,10}. The choice of meteorological variables related to climatic reconstruction, not tree physiology⁵.

In their reference to defoliators Varley *et al.* miss the fundamental point of dendroclimatology. The material selected for site chronologies conforms strongly to a common pattern of ring-width variation². This could only be produced by factors acting similarly and synchronously on all the trees selected. No sound evidence for caterpillars doing this on British oaks is produced¹. The European examples^{11,12} concern aggregate effects on stand samples, not dendrochronologically selected material. The English example^{9,13} rests on a regression analysis of % mean latewood increment and % mean caterpillar numbers (5 trees, 20 yr) where no test of difference of the gradient from zero is given. As Varley¹³ uses the intercept to estimate effect of caterpillars it is surprising that no confidence limits are given. Incomplete statistics are given for an ecologically incomplete analysis¹⁴.

Our results are inevitably reproducible within sites as only strongly cross-dated trees are used. Chronologies from subsets of 13 and 10 trees (S. J. Milsom, unpublished data) at Maentwrog are extremely similar ($t = 15.4$, 261 yr overlap)¹⁵ as is the Rostrevor chronology¹⁶ and one from 11 nearby trees ($t = 17.4$, 223 yr overlap). Consequently they produce very similar response functions except for two differences in precipitation elements between the Rostrevor sites.

We will not use response functions to reconstruct climate. For this, we will use transfer functions between time-series of spatial anomalies of tree-rings and climatic variables³⁻⁵. Recent work (B.G., unpublished data) has shown there to be spatially coherent, temporally consistent patterns of ring-width variability. Very similar patterns for the first five eigenvectors accounted for 65% (1825-94) and

70% (1895-1964) of variance in a 15-chronology tree-ring grid between 50°-60° N and 10° W-10° E. Patterns of UK mean monthly temperature (B. Gray, unpublished data) and annual rainfall (R. Tabony, unpublished data) share similar properties. It is such coherent, consistent large-scale patterns of tree-ring variation that will be calibrated for use in reconstructing past climate. These new results further support our expectation that tree-ring chronologies from the British Isles will provide valuable proxy climatic records² and bring the generation of testable reconstructions closer.

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Cell density is determined by a diffusion-limited process

FROM the culture of 3T3 cells in media with various viscosities, Whittenberger and Glaser¹ have concluded that the saturation density of these cells is not dependent on a diffusion-limited factor. A flaw exists in the design of their experiments. In their culture conditions the cell

Table 1 Effect of viscosity on cell density

Treatment	Cell density ($\times 10^{-4}$ cm ⁻²)
1% Serum	9.5 $\pm$ 1.7
1% Serum plus 1% Methocel	3.8 $\pm$ 0.6
1% Serum plus 1.5% Methocel	2.5 $\pm$ 0.7
1% Serum plus 2% Methocel	1.3 $\pm$ 1.1

Swiss 3T3 (clone 4A) cells were plated on 14-mm glass coverslips in Dulbecco modified Eagle's medium containing 10% serum. After 1 d single coverslips, with cells at an initial density of 3×10^3 cells cm⁻², were transferred, one coverslip each, to 15-cm plastic culture dishes. To each dish 50 ml of medium was added with or without methylcellulose (Methocel A4M, Dow) as indicated above. Treatments were in triplicate. After a further 5 d incubation in a CO₂ incubator, the coverslips were removed, washed gently with Tris-saline buffer, and the cells were fixed in formal-saline. The cells were stained and 10 fields per coverslip were counted under a microscope with an eyepiece grid (Means and s.d. are given for the 30 fields counted per treatment). Observations under the microscope indicated that there was no loss of cells during the washing and fixing procedures. The cell densities at 5 d approach equilibrium densities². Approximately the same variation of cell density with viscosity was observed when the coverslips were placed individually in 100-ml glass beakers containing 50 ml of medium with the same Methocel concentrations listed above, indicating that the geometry of the culture vessel is not critical. The low cell density observed in medium with 2% Methocel was also found when the cells, on a coverslip, were beneath a 3-mm layer of viscous medium (2% Methocel) in the bottom of a beaker, and 50 ml of medium without Methocel was placed above the viscous layer. Therefore, it is not the absence of convection currents in the bulk of the medium that leads to the lower cell density. The Methocel used was shown to be non-toxic by culturing the cells in a small volume of medium (0.2 ml cm⁻² of cells) with 1% serum, with and without Methocel, in normal culture conditions. As was observed by Whittenberger and Glaser¹, Methocel had little effect on the cell density attained. As Dextran and Ficoll give lower than normal cell densities at 5 d in normal culture conditions (attributed by the authors to 'toxicity' of these agents), these materials were not investigated.

density of 3T3 cells is limited primarily by the total supply of serum growth factors in the medium, not simply by the concentration of serum. As high viscosity does not alter the total supply of medium constituents, it is not to be expected that the cell density attained should be affected by viscosity in their conditions.

In the culture conditions that were used, with the usual small volume of medium (0.2 ml cm⁻² of cells), quiescent 3T3 cells destroy serum growth factors quickly after each medium change and before the cells have been exposed long enough for many cells to initiate DNA synthesis. Any increase in cell number after a medium change is balanced by cell loss when the concentrations of serum growth factors reach their minimum levels before the next medium change. High viscosity, if it has any effect, will prolong the period of destruction of serum factors in these conditions, and this prolongation will lengthen the exposure of the cells to a serum concentration high enough to initiate DNA synthesis. This might even increase the cell density. Therefore, the experiments of Whittenberger and Glaser

are inconclusive. Fortunately, there is a simple approach that avoids these problems.

This approach involves culturing 3T3 cells with a sufficiently large volume of medium (20 ml cm⁻² of cells) to maintain effectively a steady-state concentration of serum. Culturing 3T3 cells on a small coverslip in a large volume of medium² results in much higher cell densities and demonstrates the importance of the total supply of serum factors. For example, with a large volume of medium containing 1% serum, 3T3 cells grow to a 'saturation density' expected in 10% serum, that is, to approximately 10 times the cell density attained in normal culture conditions with a small volume of medium². Therefore, if the supply of medium is adequate, a 1% serum concentration will support the growth of high density 3T3 cells. A conclusion from the 1971 experiments, still not generally appreciated, is that the saturation density of 3T3 cells observed in normal culture conditions in 10% serum is not the cell density that is in equilibrium with 10% serum; the cell density in equilibrium with 10% serum is much higher². Instead, the saturation density observed with 10% serum is the cell density that is in equilibrium with the depleted level of serum growth factors present in conditioned medium. This is the cell density that is re-established in a 'wound'. The earlier experiments make clear that, with 3T3 cells, equilibrium conditions require a large volume of medium relative to the cell area.

As high viscosity can lower the effective local concentrations of diffusible factors in the medium but not their total supply, a meaningful test of the effect of high viscosity on cell density must be carried out in conditions that provide a large excess of medium. We have carried out such an experiment and the results are summarised in Table 1. The cell density is clearly affected by the viscosity of the medium. In the absence of methylcellulose, a high cell density is attained. As the viscosity is increased, the cell density decreases. These results demonstrate that the saturation density of 3T3 cells is in fact determined by a diffusion-limited process when the culture conditions are appropriate to test the importance of diffusion.

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WHITTENBERGER AND GLASER
REPLY—Holley and Baldwin disagree with our conclusion¹ that the growth of 3T3 cells in 10% serum is not limited by the rate of diffusion of mitogens to the cell surface. They have carried out experiments similar to ours in 1% serum in a large volume of medium and they observe an effect of Methocel on the cell directly after 5 d of growth. Thus in their conditions they conclude that growth is limited by diffusion and then extrapolate from this to our previous experiments.

While the lack of an effect of a viscous polymer on growth indicates that viscosity has no effect on growth in the specified conditions, the observation of such an effect is not unequivocal, as it is possible to consider that at low serum concentrations the polymer (in this case Methocel) actually binds mitogenic factors. Also we do not know whether the results of Holley and Baldwin represent a change in the rate of growth or in the limiting cell density. In addition, we need to consider the possibility that as the concentration of serum components is decreased a new situation arises in which the rate or extent of growth does become diffusion limited.

In 1971 Holley and Kiernan² reported that incubation of confluent 3T3 cells with growth medium containing 10% serum depleted the medium of mitogenic factors, although the data were not presented. We believe that Table 1 represents a confirmation of their original data. This experiment shows that incubation of medium with confluent cells for 24 or 72 h reduced their mitogenic effect on 'sparse' growth-arrested cells by about 40%, which, because of the shape of the saturation curve, is equivalent to that of 1.5% serum. Thus serum must contain a rapidly utilised mitogen(s) and more stable mitogens. However, these experiments suggest that daily addition of fresh medium to confluent 3T3 cells should result in continued growth (at 60% of maximum) unless at high density other factors become limiting. This is essentially the conclusion of the Dulbecco wounding experiments³. The two factors that have been proposed are an effect of cell contact or a limitation by diffusion. Our experiments seem to rule out diffusion and therefore support the notion that cell to cell contact is important in this phenomenon. This is not meant to imply that in different conditions the rate of growth may not be influenced by the rate of mitogen diffusion to cells. Previous experiments by Thrash and Cunningham also seem to rule out mitogen depletion as the primary reason that cells cease to grow at high density⁵.

The implication by Holley and Baldwin that when diffusion is limited the cells are exposed to mitogen for longer periods of time is correct, but as the steady state concentration of mitogen that they see is lower, it seems unlikely that this will result in an increased cell density. While there is

Table 1 Ability of confluent 3T3 cells to deplete medium of mitogenic factors

Medium	DNA Synthesis	
	Rate per 4 h (relative amount)	% Labelled nuclei (0-24 h)
0.2%	1	0.7
2%	19	69
5%	24	71
10%	24	72
Depleted media		
24 h	14	48
72 h	16	44

Swiss 3T3 cells were plated in 2 cm² Linbro multi-well trays at 1.5×10^3 cells ml⁻¹, 1.0 ml per well, in Dulbecco modified Eagle's medium (DMEM) containing 10% calf serum. Cells to be pulsed always received L-³⁵S-methionine to 1 μ Ci ml⁻¹ to label cellular protein. After 72 h plating the medium was changed to DMEM containing 0.2% serum in 0.6 ml per well in order to render the cells quiescent. One day later the medium was changed to DMEM containing 0.2% dialysed calf serum. Three days later the medium was changed again to DMEM containing either 0.2%, 2.0%, 5.0%, or 10% dialysed calf serum (0.6 ml per well). For chronic labelling ³H-thymidine was also added to 1 μ Ci ml⁻¹, 6.7 Ci mmol⁻¹. Some dishes received medium from confluent cultures (in 35-mm dishes, 2.0 ml per 8 cm²) of 3T3 cells. This medium had been added to the cells either 24 or 72 h previously as DMEM per 10% dialysed calf serum. At the time of removal of the medium from the monolayers the cells had DNA synthesis rates of 0.6% (72-h₃ medium) or 12.8% (24-h medium) as determined by a 2-h pulse with ³H-thymidine and compared with the rate of sparse cultures. Twenty hours after the last medium change some wells were pulsed with ³H-thymidine (3 μ Ci ml⁻¹, 6.7 Ci mmol⁻¹) for 4 h and processed as described.

some loss of cells at high density this cannot be greater than the total rate of cell division of confluent cells which is generally less than 5% that of sparse cells.

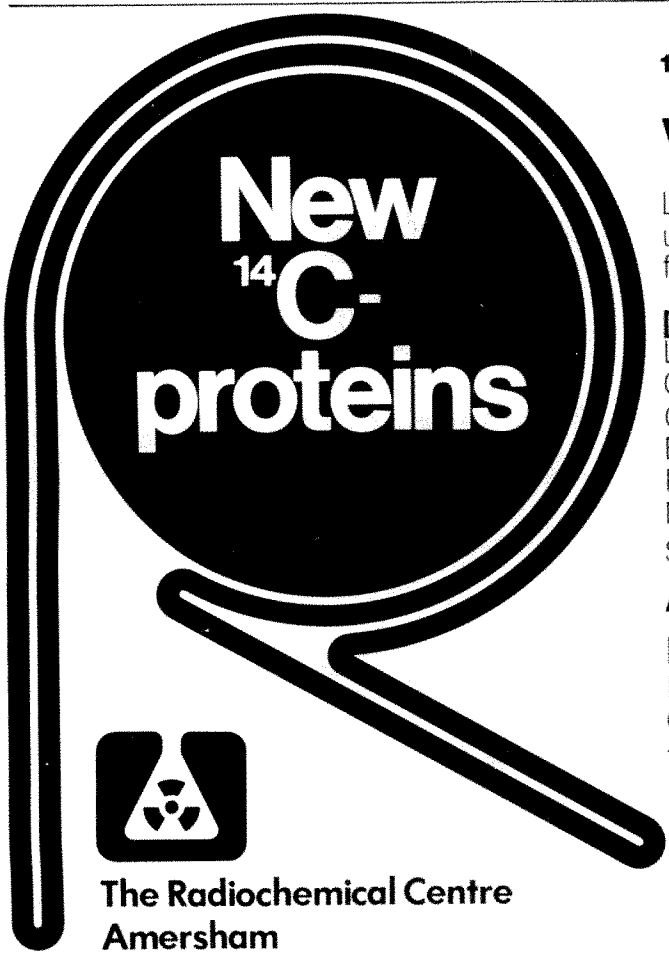
Thus while we believe that Methocel affects the growth of sparse cells in 1% serum in the conditions described by Holley and Baldwin, we do not believe that this observation affects our original conclusion. If growth were limited by diffusion of mitogens in our conditions, we would have observed an effect both on the rate of growth and on the final cell density, which we did not observe.

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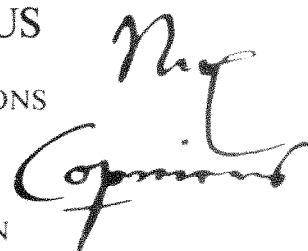
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reviews

Szilard and his bomb

Edward Bullard

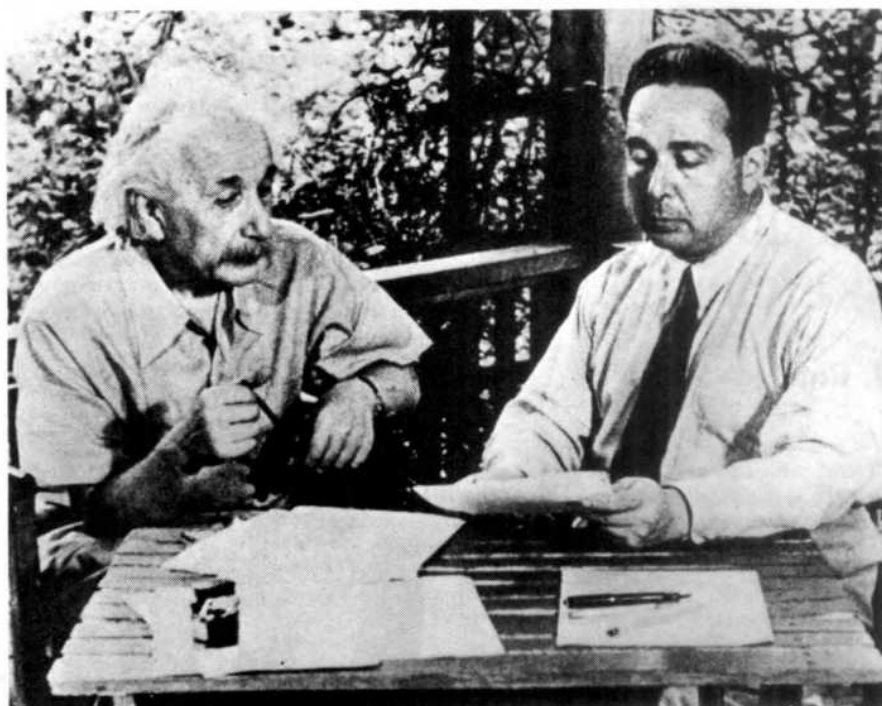
Leo Szilard: His Version of the Facts. Selected Letters and Correspondence. Edited by S. R. Weart and Gertrud Weiss. Szilard. Pp.244. (MIT Press: Cambridge, Massachusetts, and London, 1978.) \$17.50; £12.25.

SZILARD'S scientific and technical papers were published in 1972 as Vol. 1 of his *Collected Works*. The present volume deals with political and organisational questions concerning the development of nuclear weapons before and during World War II.

Unfortunately Szilard did not write an autobiography; he did, however, record his thoughts on tape and preserved copies of letters and memoranda. The book is compiled from this material. It consists of seven chapters arranged chronologically; each has a few pages of introductory narrative skilfully patched together from Szilard's tapes and notes; and these are followed by related letters and memoranda.

The first chapter gives an account of Szilard's life from his birth in Budapest in 1898 to the end of 1938. It includes a plan by the 32 year old Szilard for a very elitist organisation intended to train a group of intelligent young men who were to form and guide public and official opinion. With hindsight one can easily see such a body turning into the Hitler Jugend or the Young Communist League. It is a little surprising that anyone as far sighted as Szilard should have taken the scheme seriously. How far sighted he was is clear from his remark, made at the age of 16, that he did not know how World War I would end but he knew that it ought to end by the defeat of Germany and Russia. By the end of chapter 1 he has passed through Berlin, London and Oxford, has taken out patents on a nuclear reactor and bomb and moved to New York.

Szilard's patents of 1934 presupposed the existence of a reaction in which the impact of a neutron on a nucleus released more than one neutron from the target. No such reaction was known, but in 1939 Hahn discovered neutron-induced fission. Szilard at once saw that neutrons would be emitted from the fragments and that there was a good chance of his reactor and his bomb becoming a reality.



The rest of the book has three themes; first, his efforts to show that a reactor and a bomb are possible; second, his attempts to restrict the spread of the knowledge of the possibility; and third, his efforts to persuade the government that nuclear bombs should not be used on Japanese cities.

The first part of the programme was entirely successful. In August 1939 Szilard persuaded Einstein to write to President Roosevelt saying that it seemed likely that a nuclear bomb could be made. This was the start of interest by the US Government in the matter. The illustration shows a reenactment of the meeting between Szilard and Einstein (no photograph was taken at the time.) A little later Szilard and Fermi invented the graphite-moderated uranium reactor which was in operation in Chicago at the end of 1942. Similar reactors at Hanford produced the plutonium used in the bomb dropped on Nagasaki. The second part was clearly hopeless; after the discovery of fission the possibility of a chain reaction was bound to occur, and did occur, to many people. The use of the bomb was also inevitable, though it is greatly to the credit of Szilard and those who supported him that they did

attempt to prevent it. Use of the bombs was inevitable in the face of the gloomy prognostications of the casualties likely to occur in the landings in Japan, which were to take place early in November 1945.

Szilard was one of the group of Hungarian emigrés who made such a deep impression on American science, particularly on theoretical physics in the 1930s and 1940s. On the whole they got on well together (Szilard tells Einstein that Edward Teller is 'besonders nett' and has letters from Wigner signed 'Wigwam'). Relationships with the management of the Chicago laboratory and of the Manhattan project were something quite different. Szilard seems to have had little confidence in any of those concerned. In fact, arguments about Szilard's patents became so acrimonious that in October, 1942, two months before his reactor first went critical, Arthur Compton, the Director of the Chicago laboratory, ordered Szilard to leave (he later relented). Relations would have been even worse had Szilard known that General Groves had drafted an order for his internment; this information has only very recently been unearthed (see Bernstein, B. G., *Inquiry*, 8 January, 1979, p27).

Why did things go so badly for Szilard?

lard? Was it just that he felt he had a mission to save the world? Was it that he and his friends were almost all foreigners? Was it perhaps that the Chicago lab had no charismatic leader, such as Oppenheimer was at Los Alamos. Oppenheimer seems to have been able to cope with General Groves and his entourage even when doing things much more open to misconstruction than anything Szilard ever did.

The book is 'Szilard's version of the facts' and of events that have had an overwhelming influence on the relationships of science and government

and on the position of science in the modern world. It is not the whole picture, there are all kinds of cross links and loose ends that need to be traced out. It is, however, a book that must be read by anyone interested in World War II or in the roots of our present scientific establishment. It is also a story about the impact of America on a very clever and deeply humane man, coming in a time of crisis from a quite different tradition. □

Sir Edward Bullard is an Emeritus Professor at the Scripps Institution of Oceanography, La Jolla, California.

Infeld's sketches

J. Rotblat

Why I Left Canada: Reflections on Science and Politics. By Leopold Infeld. Pp. 212. (McGill-Queen's University Press: Montreal and London, 1978.) \$16.95.

LEOPOLD INFELD was internationally renowned for his important contributions to theoretical physics, as well as for his gift to write on science for the layman. But he was also an extraordinary personality and had an unusually interesting life. He was a legend in his native Poland even in the pre-War years. I happened to do my stint of teaching in the grammar school in Warsaw where Infeld had been teaching twelve years earlier, and his exploits, mainly of a romantic nature, were still a major topic of conversation in the common room. It was many years later that I met him and got to know him.

Infeld described the first 42 years of his life in a remarkable autobiography *The Quest*, published in 1941; the book concluded with his decision to settle in Canada. But nine years later he returned to Poland for good, and there, in 1964, he continued his autobiography in a book entitled *Sketches from the Past*, which consisted of a number of essays. It is these essays, together with one written just before his death in January, 1968, that constitute the book *Why I left Canada*, published in Canada.

The long interval between the writing and publication in English has not detracted from its value. Although I had read *The Sketches from the Past* soon after their publication in Poland, I was still fascinated on re-reading them in English. The perfect translation (by Infeld's widow) has succeeded

in retaining the characteristic flavour, and the introduction and notes added to the English edition have enhanced the book, although in my opinion it would be more appreciated if it were read together with *The Quest*.

The title of the book *Why I left Canada* is taken from the title of one of the essays. In it Infeld describes the 'atom spy' hysteria in Canada in the post-War years, and which resulted in

those who obstructed his academic career in pre-War Poland for anti-semitic reasons. But above all his decision must have been influenced by the terrible devastation of Polish science brought about by the War.

The events after his return are described in the essay "Poland". Infeld did not suffer from modesty, but the essay only hints at the great heights he reached when he came back to Poland. To the public he was a hero, and officialdom treated him with reverence. As far as science was concerned his word was command, and he used his prestige and influence to foster the reconstruction of science in Poland. Naturally, his own subject did best, and in a very short time he set up the Institute of Theoretical Physics, with fifty scientists working in it.

Not all was sugar and spice. Although he was in sympathy with the regime, he was too much of an independent character to accept its rigidity. In particular, he was galled by the idolatry of Stalin, which was normal practice in Polish science in the early 1950s. At one time he even re-



Einstein and Infeld

the hounding of Infeld and his family after he had made a short visit to Poland in 1949. He was planning to spend the following year in Poland on a sabbatical, but when his Canadian passport was withdrawn he decided to remain in Poland for the rest of his life. The Canadian Government re-ven-ge-d itself in the subsequent cancellation of Canadian citizenship of his two children, who were born in Canada.

Although the harassment in Canada was the direct reason for Infeld's return to Poland, it is very likely that he would have done it in any case. There were several reasons for this. Politically, he had always been on the left (he considered himself a Marxist); the new regime in Poland therefore appealed to him much more than the 'flesh pots' of Canadian life. There may also have been an element of personal satisfaction in getting the upper hand over

gretted having returned to Poland. But a few years after Stalin's death the atmosphere became more relaxed, and later he was even able to write about it in the book published in communist Poland.

The essay "Einstein" describes the contacts with him in the last years of Einstein's life. Infeld had a very successful collaboration with Einstein in the 1930s, and he resumed it after the War, first directly, when Infeld was still in Canada, and later by correspondence. Although Infeld did not agree with Einstein about the unified field theory, they worked in harmony, as is evident from the exchange of letters. Some of Einstein's letters were published in the book for the first time in English, and this is particularly welcome in the year of Einstein's centenary.

Throughout the book Infeld portrays many scientists, living and dead, whom

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Perspectives in Grassland Ecology

Results and Applications of the US/IBP Grassland Biome Study

Editor: N. R. French

1979. 58 figures. XVII, 686 pages.

(Ecological Studies, Volume 32)

ISBN 3-540-90384-4

In preparation

Contents: Introduction. – Grassland Primary Production: North American Grasslands in Perspective. – Processes Controlling Blue Grama Production on the Shortgrass Prairie. – Analysis of the Response of a Grassland Ecosystem to Stress. – Grassland Biomass Trophic Pyramids. – Patterns of Consumption in Grasslands. – An Ecosystem-Level Trophic Group Arthropod and Nematode Bioenergetics Model. – Factors Influencing Input and Output of Nitrogen in Grasslands. – Simulated Impact of Management Practices upon the Tallgrass Prairie. – Data-Based, Empirical, Dynamic Matrix Modeling of Rangeland Grazing Systems. – Prinzipal Subsystem Interactions in Grasslands. – Index.

Perspectives in Grassland Ecology examines the biomass structure of grasslands in North America and other areas of the world. It compares trophic structure, and describes structure modifications occurring when water and nutrients are artificially supplied. The most important driving variables, processes, and the major functional aspects of production are analyzed and modeled mathematically. Unique in its detailed comparative analysis of the major grassland types, this book will be appreciated by researchers in range science, ecology, agronomy and botany.

Grassland Simulation Model

Editor: G. S. Innis

1978. 87 figures. XXVI, 298 pages

(Ecological Studies, Volume 26)

Cloth DM 49,60; US \$ 27.30

ISBN 3-540-90269-4

Contents: Objectives and Structure for a Grassland Simulation Model. – Abiotic Section of ELM. – A Simulation Model for Grassland Primary Producer Phenology and Biomass Dynamics. – A Mammalian Consumer Model for Grasslands. – Simulation of Grasshopper Populations in a Grassland Ecosystem. – A Simulation Model for Decomposition in Grasslands. – A Grassland Nitrogen Flow Simulation Model. – Simulation of Phosphorous Cycling in Semiarid Grasslands. – Sensitivity Analyses in the ELM Model. – Critiques and Analysis of the Grassland Ecosystem Model ELM.

This model for grassland ecosystems covers abiotic, producer, consumer, decomposer and nutrient cycling aspects. The result of several years' work by a team of scientists, the model was conducted under the auspices of the US/IBP Grassland Biome Study. An extensive field data acquisition program complemented the modeling effort, as did a simulation compiler development effort. The completed model is analyzed for sensitivity, and a critique section evaluates the success of the effort, suggesting ways in which subsequent efforts might be improved.

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he met, and in general he speaks about them with kindness. But he has no kind word for Oppenheimer, in an essay written in the last year of Infeld's life and published after his death. The cause of his wrath was a lecture on Einstein given by Oppenheimer in 1965, and which—in the version available to Infeld—was an amazing denigration of Einstein. Point by point, Infeld proved Oppenheimer's distortions, and in the process he lashed out

at him with all the might of his pen. Even the news of Oppenheimer's death, which reached Infeld while he was writing the essay, did not mollify him, because he believed that the saying that one should speak only good of the dead does not apply when referring to a figure belonging to history.

I adhered to this view in writing this review of Infeld's book. □

J. Rotblat is Emeritus Professor of Physics in the University of London, UK.

Journey through relativity

Joe Schwartz

Einstein's Universe. Written by Nigel Calder. Narrated by Peter Ustinov. Produced by Martin Freeth. (BBC-TV: London; WGBH: Boston, 1979.) First UK transmission: 14 March, 1979, BBC-2, 1900 h. Repeated 18 March.

I HAD watched one of Nigel Calder's previous BBC science extravaganzas—*Key to the Universe*—with friends: an immunologist, a developmental biologist and a psychotherapist. The developmental biologist went upstairs, the psychotherapist fell asleep (we didn't wake her), and the immunologist and I discussed immunology. Calder's approach to physics is characterised by a "gosh, isn't science grand" school-boy attitude and in *Key to the Universe*, his taste could be given free rein. Quarks (with colour and flavour), strangeness, hypercharge, charm, were right up his alley. Calder is captivated by the terminology of high energy physics and obviously enjoys the mystery and incomprehensibility of it all. This view is not uncommon among science journalists. James Gorman, a New York writer, confessed last year: "I value physics, not for how well it explains the world, but much as the irreligious loved the Latin mass, for how it sounds."

So I waited for *Einstein's Universe* with some apprehension. Einstein hated obfuscation and mystification. "Since the mathematicians have attacked relativity, I myself no longer understand it", he wrote sarcastically in 1909. The core of his work has long been acclaimed for its simplicity. Special relativity (1905) is based on two simple physical propositions; all steady motion is relative and there are no instantaneous interactions in nature.

General relativity (1916) is built on the simple physical fact that all objects fall at the same rate in a gravitational field.

Einstein is legendary for having been able to extract far-reaching conclusions from these slim physical clues. In the special theory he replaced Newton's metaphysical absolutes of space and time with a material absolute; there are no instantaneous interactions in nature, no signal can propagate faster than the speed of light. In the general theory, Einstein put geometry on a material basis. The validity of Euclidean geometry became a problem for physical investigation, not for abstract contemplation. Experiment would decide whether we live in a Euclidean world where two parallel lines meet at infinity or whether we do not. (We do not.)

Calder's programme was far better than I expected. *Einstein's Universe* is a major improvement over *Key to the Universe* with numerous innovations that need to be developed further. The use of Peter Ustinov as a foil avoids the phenomenon of authoritative talking heads speaking directly to the camera. Moving the set out of the studio and into a live observatory (McDonald) gives more immediacy. The models and animations are quite effective, particularly the curved billiard table. Roger Penrose projects an intensity and seriousness that is a welcome contrast to the usual bland pronouncements from the scientific orthodoxy. And the informal discussion at the end of the programme expressed some of the tone and quality of research work at the frontiers of physical theory.

But it is not enough. I will be greatly surprised if many viewers found their interest sustained for the entire show. The problem lies in the script. Calder concludes the show with an Einstein quote: "The most beautiful thing that we can experience is the mysterious." But Einstein, for whom clarity was paramount, did not mean mystery at the hands of his fellow human beings. He meant the concrete mysteries of nature. The mystery the programme leaves us is a lack of

physical understanding of the astounding results of relativity; black holes, the expanding universe, clocks running slow, $E=mc^2$. We do not know how the theory produces these results or what were the problems for which these results are the solutions. It is Einstein turned on his head: the results of the investigations are seen to be mysterious, not the problems that gave rise to them in the first place.

Wheeler's summary of general relativity is excellent. "Space tells matter how to move and matter tells space how to curve." But how many people will be able to say that they understand what Wheeler means. It is irritating to hear the constancy of the speed of light, the physical basis of the special theory, repeatedly referred to as Einstein's "obsession". The physics has got lost in the whizzing shots of complex machinery, cables, flashing lights and intricate experimental arrangements. If the point is to explain and not to mystify a way has to be found to make the equipment more than a mere spectacle. Einstein actually was quite knowledgeable about experimental work. In 1908 he and a friend Paul Hahich patented a design for a sensitive voltmeter accurate to half a millivolt which was in advance of the instruments of the time.

The striking results of the theory get

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us in the front door. But once we are there nothing much is shown. Where are the workers in the field, the graduate students, the post-docs. Where are the arguments. Some experimentalists and theorists do not believe in black holes. Where are they. The Ustinov character is an advance but he is too passive. We need a character who can force the explainer to acknowledge that some of the ideas are poorly understood and insufficiently tested. We should see some actual data instead of simply being told that an experiment satisfies Einstein's theory to 1%. The human substance is missing and the presentation of shocks and thrills wears thin half-way through.

Journalists have to dig harder for their stories. They need to use the same

critical sense they reserve for other subjects and apply it to science reporting. A more critical less idolised approach is needed. Einstein recognised this problem himself: "In my view, there is but one way to bring a great scientist to the attention of a larger public; it is to discuss and explain the problems and solutions which have characterised his life work. Otherwise the result is a banal hero-worship based on emotions and not on insight." [Emphasis added.] Science is not more objective and mysterious than any other human social activity. Everyone in science knows this. It is time to start showing it to the public. □

Joe Schwartz is a freelance journalist on leave from the City University of New York.

Foundations of relativity

P. C. W. Davies

General Relativity from A to B. By Robert Geroch. Pp.225. (University of Chicago Press: Chicago and London, 1978.) £8.40; \$11.95.

TEN years ago introductory books on general relativity were a rarity. Since then, advances in the subject and a greater awareness among students that gravity is an intriguing, even bizarre, branch of physics, have stimulated a stream of works appealing to the bottom end of the market. An enormous number of people want an appetite-wetter that will "get them into" gravity without the traditional years of investment in advanced mathematics, and not only so that they can bore dinner guests with virtuoso expositions about black holes. Often more formal treatments do not succeed in communicating the sense of awe and iconoclasm that stems from topics such as total gravitational collapse, event horizons and singularities in spacetime. In addition, mathematically based texts tend to ignore the reader's struggle for sound physical intuition, and depend upon a sort of intellectual osmosis in which feeling for the physics is supposed to slowly and spontaneously filter through the formulae.

Robert Geroch is a man of outstanding international reputation, whose personal contributions to the subject of general relativity are great. He is also a skilful expositor, and has taken enormous trouble to write a book in which the reader starts out from basics. The book is a product of a lecture course given to non-science under-

graduates at the University of Chicago, but it can be read with profit by science specialists who want to acquire a historical and philosophical perspective, to scrutinise the fundamental concepts that underpin the theory of relativity, and to perceive what is a rather specialised branch of physics, with a human face. The text is liberally punctuated by line drawings, some of which might, but should not, frighten the potential reader browsing through the pages.

Part A of the book dwells, perhaps a little too laboriously, on the image of our world as a spacetime threaded by worldlines of matter. To communicate the crucial and fundamental concept of the relativity of motion, much space is devoted to the old and discredited Aristotelian and Galilean concepts of space and time. These sections certainly help to focus the reader's attention on the basic issues at stake, but carry the danger of making Aristotle seem more reasonable than Einstein. There is a sense in which relativity is equally as elegant and natural as the everyday, intuitive view of space and time, but a historical approach to the subject does not always do it justice.

The greater part of the book (Part B) gets to grips with general relativity in its full splendour. Professor Geroch adroitly guides the reader through the geometrical basis for gravity without burdening him with elaborate formalism or needless jargon. Every new concept is strongly rooted in concrete physics. Meter sticks, clocks, light signals, records—people—inhabit the author's models, observing and measuring all sorts of phenomena. Great emphasis is laid on physical meaning and consistency, on asking and answering exactly what is meant by distance, interval, and so forth. It is quite the most elaborate discussion of the funda-

mental aspects of relativity that I have encountered in a non-specialist book.

The price paid for this admirable but extended devotion to "getting it straight" is that Einstein's actual theory does not surface until three-quarters of the way through the book, and when it does, perhaps inevitably for a book at this level, it is largely in the form of analogy. This leaves very little room for applying the theory to anything exciting, but a mandatory chapter on black holes is included. Readers who have taken the effort to get this far will find that the weird and wonderful properties of these entities can in fact be understood in a straightforward way now that the spacetime geometry concept has been so thoroughly well explained. Professor Geroch explicitly excuses himself from



Einstein expounding to Ramsay MacDonald

what he calls "gee-whizzes", but still manages to convey the immensely engaging nature of the black holes subject. I was a little disappointed not to see an extensive discussion of singularities, and also the big bang and cosmology, but there is always the possibility of a *C to D* sequel.

In summary, *General Relativity A to B* is one of the few books that treats the foundations of the theory of relativity seriously and in elaborate detail, at the non-specialist level. It cannot be read, however, without effort and commitment. The index is rather sparse and the price a shade too high, but otherwise it is a nicely produced book that in spite of its narrow scope illuminates many grey areas of spacetime physics. □

P. C. W. Davies is Lecturer in the Department of Mathematics at King's College, University of London, UK.

obituary

Margaret Mead, 1901–1978

MARGARET MEAD died on 15 November 1978 just a month before her 77th birthday. Thus ended a unique career. Boundlessly energetic and phenomenally productive (her bibliography runs to nearly 4,000 items), alert to every new development in the humane sciences, sensitive to urgent social issues and with a talent for working with and through others, she achieved a world-wide fame.

Her place in the history of American anthropology is assured; for she played a decisive part in its transformation from a narrow academic specialism into a modern humane science with world-wide interests. But she will also have a place in the social history of the United States for her catalytic influence on the development of educational and moral ideals and values after World War II. Field work, she often proclaimed, was the 'living stuff' of anthropology and she made over 20 field trips between 1925 and 1975. But in between spells of writing, teaching and organising research, she also travelled indefatigably in America and abroad, to lecture, to preside at conferences, to advise academic bodies or governmental agencies, and so on. But whatever the occasion might be, she always took her stand as an anthropologist, drawing attention to the many alternatives mankind had invented for dealing with the common problems of personal and social life.

Margaret Mead was born on 16 December 1901 in Philadelphia. As her father was a Professor of Economics and her mother an early student of sociology, she could say with pride, in her autobiography *Blackberry Winter* (1972), that she grew up in a 'social science' family. To their influence was added that of her grandmother, whose example inspired the ideals which made Dr Mead essentially an educator.

Dr Mead came into anthropology by chance. After a miserable year at De Pauw University she moved to Barnard College in New York in 1920. While completing her degree in psychology she was drawn into Franz Boas's circle. What most influenced her, however, was her friendship with Ruth Benedict, Boas's assistant, which was to last a lifetime of close collaboration. Anthropology was in a ferment just then,



Functionalism, with its emphasis on fieldwork and the analysis of the internal integration of primitive societies, had been launched in England. In America the complementary 'configurationalist' movement, later to come to brilliant fruition in Benedict's *Patterns of Culture* (1934) was under way. And in the background loomed the figures of Freud, Jung and later Piaget. Mead took something from each to form her guiding principle that culture shapes character, is learned and is lived by individuals.

Mead's fieldwork began in 1925 with her study of a group of adolescent girls in Samoa. She found that unlike American girls, they suffered no emotional crises in the transition to adulthood, and she attributed this to the benign sexual mores and family system of Samoans. Addressing herself to the general public in her deliberately 'novelistic' best-seller *Coming of Age in Samoa* (1928) she drew the moral. A new image emerged of anthropology as a science of public enlightenment. Later, reacting to straight-laced anthropological criticism, in a conventional monograph, and journal articles, she presented the technical anthropological and psychological data on which she based her book; and she did likewise with all her subsequent fieldwork.

Dr Mead's next field study, shared

with her second husband Reo Fortune (her first was the archaeologist Luther Cressman) took her to the lagoon-dwelling Manus of the Admiralty Islands, New Guinea. Here, supplementing ethnographic observation with ingenious psychological tests, she found that contrary to some psychologists' theories children were not naturally animistic. A puritanical, competitive culture made fearful animistic adults of them. Her second popular book, *Growing Up in New Guinea* (1931) was another best-seller.

In 1931, after a depressing interlude in a disintegrating Omaha community (described in Mead's *The Changing Culture of an Indian Tribe*, 1931) they returned to New Guinea. Three strikingly different tribes were visited. There they were joined by Gregory Bateson, fresh from his Iatmul field work. Discussions with him crystallised an hypothesis that cultural patterns incorporated different psychologically identified temperamental types. Mead's *Sex and Temperament in Three Primitive Societies* (1935) developed this hypothesis to explain the startling contrasts in the norms of sexual identity and behaviour in these three tribes. The book, like its predecessors, was severely criticised by some anthropologists. But her argument that what are now called 'gender' differences are culturally inculcated not biologically determined, brilliantly anticipated the position vigorously maintained by women anthropologists today.

Mead elaborated this theme in lectures and articles in both popular and learned journals. Fourteen years later she refashioned it in *Male and Female* (1949), her most influential book. Comparing observations in seven non-Western cultures with the American scene, she examined the cultural variations in the roles of men and women in the reproductive process and in the socialisation of children, and related this to the way the oedipal conflict is resolved, thus drawing attention to the significance of the father in character formation. Here again she anticipated what has become a topic of major research activity among psychologists, anthropologists and some behaviour biologists.

Breaking with Fortune in 1936, she

married Bateson; and this partnership led to the field study in Bali (1936–1939) which they claimed, with some justice, to have been a major advance in anthropology.

In Bali they were faced with a complex oriental civilisation very different from primitive New Guinea, though, as before, their concern was primarily with culture-learning, the ways as they put it 'living persons embody their culture'. Their main innovation was the huge and minutely detailed photographic documentation that foreshadowed the post-war rise to prominence of the ethnographic film. Their fieldwork concentrated on interpersonal relationships and interactions, particularly of parents and children in the context of child-rearing and socialisation processes, that were central to Dr Mead's interests. Her imaginative psychological commentary on the photographic record in their book *Balinese Character* (1942) and in other publications, provoked controversy but also stimulated new approaches in ethnological psychology. The 'anthropology of the body' is a new field of research concerned with problems of the cultural specification of postures, gestures, and body images and processes, and it owes much to the pioneering work of Bateson and Mead.

World War II brought unexpected challenges to American anthropologists. Dr Mead worked with various governmental agencies concerned with the war effort. But most important was the leading part she played in the international team of specialists assembled by Ruth Benedict to compile reports on the 'national character' of the belligerents. Field work being ruled out, they resorted to studying 'culture at a distance', through interviews with immigrants and analysis of literary, film and archival sources. As the publications of the team showed—notably Benedict's celebrated study of the Japanese, *The Chrysanthemum and the Sword* (1946) and Mead's passionate morale-booster *And keep your Powder Dry* (1943)—the method was remarkably effective, so much so that the project was continued until 1951 (with 120 participants!) under the auspices of Columbia University.

The war and especially, by Mead's own account, the shock of Hiroshima, brought home to her the urgent need for a new social and moral vision in western, especially American society. The war had broken down the cultural isolationism which she had helped to expose in her popular books. It had also, she learned, produced unprecedented changes in New Guinea. This led her, in 1953, to return to Manus, accompanied by a student couple.

She found the community completely transformed. Partly owing to the war and to Australian government agencies, but mainly to the charismatic leadership of one local man, the people had abandoned their entire traditional way of life and were rapidly adopting western institutions and ideals. Examining these changes in her *New Lives for Old* (1956) Dr Mead concluded that a unanimous primitive community, once convinced of the desirability of western cultural ideals and institutions, can, given inspired leadership, radically change its way of life in a generation. Thenceforth she advocated the wholehearted support by anthropologists of speedy westernisation in primitive communities with the right leadership and aspirations. She visited the Manus thrice again thus keeping benevolently critical track of their progress in self government, in family life, and in personality development, until 1975.

Watching Manus inspired in Dr Mead an abiding concern with the problems of changing society. Thus orientated, between 1953 and 1975 she also visited the other groups in which she had earlier worked, as well as the growing urban centres in New Guinea. Preoccupied now with the problems of reconciling continuity and change in cultural development (as in her book *Continuities in Cultural Evolution*, 1964) she travelled widely in America, the Caribbean, Europe and the Southern Hemisphere, often enough to participate in academic or policy-making activities, and so to enlarge her understanding of urgent world issues. The key to a more enlightened and humane future, she believed, lies in the way the culturally specified relations of successive generations of grandparents, parents and children, where character is shaped and culture is transmitted, are managed. She stressed in particular the models of the alternatives anthropology has revealed for handling the universal problems of intergenerational conflict.

Never hiding her light under a bushel, Margaret Mead yet disdained pretensions to originality. On the contrary she constantly stressed her indebtedness to others, friends, famous scholars, students and predecessors. But as the long list of visiting professorships, honorary degrees, other academic distinctions, and awards and prizes conferred on her testifies, the pre-eminence of her scientific achievements, her devotion to America and her dedication to the ideals of human betterment were widely esteemed. A phenomenon like Margaret Mead could perhaps not have emerged in any other country than modern America. From her base in the American Museum of

Natural History, where she worked, and taught from the time of her appointment as an assistant curator in 1925 to the end of her days, she exercised a decisive influence both on the development of the social and human sciences in America and elsewhere, and on American educational, and moral values and cultural ideals. But she will undoubtedly be best remembered, as she would herself have wished, as one of the most creative anthropologists of this century.

Meyer Fortes

Bernard Halpern

BERNARD N. HALPERN, one of the first workers on antihistamines, died in Paris on 23 September 1978, after suffering for several years from an incurable and painful illness.

Born in 1904 in Russia, Bernard Halpern came to France after some very difficult childhood years, to complete his advanced studies for a degree of Doctor of Medicine (1936). In order to study, he worked for several years as a technical assistant in the physiological laboratory at the Ecole pratique des hautes Etudes, under the direction of J. Gautrelet.

In 1937, Halpern took over as director of the pharmacodynamic research laboratory of the Société Rhône-Poulenc. He left this position in 1945 to join, as a research worker, the Centre National de la Recherche Scientifique and to direct the Pasteur-Vallery-Radot laboratory at the Broussais hospital. In 1949, he was nominated Director of Research at the CNRS; ten years later, he added to these functions those of Director of Laboratories at the Ecole pratique des hautes Etudes. In 1961, Halpern was awarded the Chair of Experimental Medicine at the Collège de France, a chair formerly held with distinction by Claude Bernard, and was elected a member of the Académie des Sciences in 1964.

After early work with J. Gautrelet on the action of snake venoms, Halpern devoted himself from 1942 onwards to the study of synthetic compounds behaving as antihistamines, at the Rhône-Poulenc laboratories.

This research topic was, it must be said, initiated some years earlier (1937) by G. Ungar, J.-L. Parrot, and D. Bovet, who used in their experiments compounds belonging to the sympathomimetic and sympatholytic groups obtained from E. Fournau's laboratory at the Institut Pasteur. In the same year (1937), D. Bovet and A. M.

Staub had detected the same antihistamine properties in thymoxyethyl-diethylamine (929 F) and, some years later, the study of some derivatives of phenylethylenediamine and aminopyridine showed equally favourable properties. As a result of other work by A. M. Staub (1939), Halpern proved in 1942 the relatively high antihistamine effect of N-(2-dimethyl aminoethyl)-N-benzylaniline or Antergan on spasms in unstripped muscles and pointed out the possible use of this compound in human therapy.

In 1944, another compound appeared, Neoantergan, equally suitable for human application which, while chemically similar to Antergan, was more active (Bovet *et al.*, 1944). Still more important appeared to be the N-alkylamine derivatives of phenothiazine, among which Halpern noted the efficacy of Phenergan (1946). It is well known that, apart from its antihistamine effects, this compound was found by doctors to be a psychodepressant and formed the starting point for many neuroleptic derivatives currently used in psychiatry.

From 1950, following research by Jancsó, the work of Halpern and his collaborators took another direction: the functional study of the reticulo-endothelial system (RES). Profiting from the granulopoietic activity of this tissue, they investigated the removal from the blood of various colloids as a function of time. These data once established, the authors found that stimulation of the RES increases the level of immunity of the organism against various infections.

In 1959, Bernard Halpern showed, for the first time, that administration of BCG increases the resistance of the organism to malignant tumours.

In 1964, he described the outstanding immuno-stimulatory properties of *Corynebacterium parvum* on the reticulo-endothelial system. This effect is shown by the increase in the phagocytic index of macrophages and by the increase in liver volume and of splenic cells. Later, Halpern established that the administration of *Corynebacterium parvum* inhibits the development of grafted malignant tumours and their propensity to metastasis. In May 1974, he chaired the first international conference on *Corynebacterium parvum* and its use in oncology, a conference which has led us to revise our ideas of the relations between the host and the malignant tumour. *Corynebacterium parvum* has been employed for several years in human therapy in the treatment of cancers in combination with antimetabolic chemotherapy and the results of this method appear promising.

G. Valette

W. O. James

PROFESSOR WILLIAM OWEN JAMES, FRS, who died on 15 September 1978 will be remembered as one of the leading British plant physiologists of this century.

The major part of his academic life as a scholar, teacher and author (from 1929 to 1958) was spent as Demonstrator and then Reader in the Department of Botany at Oxford University. He was elected to the Royal Society in 1952. Latterly from 1958 until his retirement in 1967 he was an innovative Head of Botany at Imperial College, London at a time when the college was rapidly expanding.

During his professional life of over 50 years, W. O. James made many distinguished contributions to several different fields of plant physiology including photosynthesis, mineral nutrition, anaerobic and aerobic respiratory metabolism, alkaloid metabolism and to the understanding of the function of subcellular organelles. His endeavour was always to discover and understand the biochemical events responsible for the physiological functions of plants and to account for these responses quantitatively. He wrote with exceptional clarity and his many scientific papers and several books reflect his scholarly analysis of plant physiological phenomena and his imaginative application of contemporary experimental techniques as aids in understanding them. He retained an enthusiasm for plant biology to the end of his life and was always particularly delighted to hear of progress in the several areas of research in which he himself had been involved.

W. O. James brought to the study of the many-faceted problems of the physiology of plants incisive thought and great care in the design and execution of experiments. These qualities were already manifest in his first paper on the external factors affecting photosynthesis reporting his studies as a graduate student under F. F. Blackmann in Cambridge in the mid 1920s. James demonstrated departures from the limiting factor form of the curve for photosynthesis and explained why they were to be expected. During his postdoctoral years, spent from 1925 in V. H. Blackmann's unit of Plant Physiology at Rothamsted, his interest in mineral nutrition of plants, particularly of potassium, developed, and on his move to Oxford in 1927, the effects of potassium deficit on extractable enzyme activity and respiration rate were among the first problems he tackled. He soon realised that useful interpretation of the experimental effects of mineral nutrition on plants was severely limited by lack of under-

standing of the respiratory processes occurring in normal plants and he and his associates turned their attention to the study of plant respiration.

It is for his contributions to our understanding of plant respiration that James will probably be best remembered. His group in Oxford carried out some of the first definitive work on glycolytic systems in plants and in the early 1940s demonstrated unequivocally that pyruvate was produced from fructose diphosphate in extracts of barley. Later they examined the enzyme systems involved using the phosphorylated intermediates and cofactors which had recently become available commercially, and showed the key enzyme enolase was present in barley sap.

His group published detailed descriptions of the respiratory characteristics of several different plant tissues including leaves, roots and embryos and sought to explain their *in vivo* respiration quantitatively in terms of the activity of enzymes which could be isolated from them. It was demonstrated, for example, that sugar lost from carrot tissue during a period in N_2 could be accounted for entirely by alcohol and CO_2 production. In attempts to explain the terminal oxidation processes in plants whose respiration was differentially inhibited by specific respiratory inhibitors, James and his associates also devoted considerable attention to the examination of soluble and insoluble plant oxidases. Much useful detailed information about the distribution and activity of the variety of plant oxidases was assembled, although no convincing evidence that any oxidase other than cytochrome oxidase plays a major role in plant respiration has yet emerged. H. Beevers in James' laboratory in the late 1940s first examined the exceedingly rapid cyanide-insensitive respiration of the *Arum spadix* and later the Oxford group were the first to show the location of this activity in isolated mitochondria. James' years of study of the respiratory processes in plants culminated in the publication of his book *Plant Respiration* in 1953, hailed as a scholarly summary of a whole epoch of plant respiratory studies.

During his years in Oxford, in addition to his research activities, James also taught plant physiology to several generations of undergraduates and, during the second World War, was a prime mover in the Oxford Medicinal Plants Scheme. The objective of the scheme was to supply medicinal drugs by extracting them from natural plant sources. Many of these drugs were alkaloids and in addition to improving methods for their extraction and

analysis, his group obtained much fundamental information about the synthesis and internal concentration of alkaloids in cells.

Shortly before his move to London, James' group had been amongst the first in the world to isolate active mitochondria from plants and study their oxidative and phosphorylative capacities. The group had also begun to use the new techniques becoming available for the examination of the ultrastructure of cell organelles for James had the vision to see the potential of structural studies in understanding intracellular metabolic processes. At Imperial College he set up a Unit of Analytical Cytology where electron microscopists and biochemists worked together to isolate, purify and characterise subcellular plant organelles. New electron microscopical techniques were developed to monitor the purity and integrity of the isolated organelles used in a continuation of the studies of mitochondria to localise cellular respiratory activities. Later plant nuclei, ribosomes and chloroplasts were examined and the group were the first to publish methods for the isolation of structurally intact chloroplasts and also plastoglobuli and grana from them.

In addition to introducing the study and teaching of analytical cytology of plant cells, James also initiated and fostered the development of new sections of the department at Imperial College to study plant taxonomy, palaeobotany and nitrogen fixation. He guided and encouraged those who shared his enthusiasm for all aspects of plant biology and by example and positive criticism showed the high standard of scientific expertise required to gain a true insight into plant function.

In addition to publishing numerous papers reporting his own research findings, for many years James was a co-editor of *New Phytologist* and a member of the editorial panel of *Endeavour*. His uncluttered literary style also contributed to his skill as a good writer of school and university texts and semi-popular articles and books. All his publications were characterised by the high standard of the beautifully presented and appropriate illustrations. For almost 50 years his now classical text *Introduction to Plant Physiology* (first edition 1931, seventh edition 1972) has been the introduction for successive generations of schoolchildren and undergraduates to this field. In the preface to the fourth edition he looks forward to the time when the reader of his book will take part in finding some of the answers to unsolved physiological problems: indeed there have been

many whose initial interest was aroused by his text and who have gone on to make their own contributions as professional plant physiologists. His *Introduction to Plant Biology* is still used as 'the bible' for school and university courses. His amusing book *Background to Gardening* became a best-seller. After his retirement he published *Cell Respiration* (1971) which gives a comprehensive account of the respiration of the whole range of living cells.

James' distinguished contributions as a scientist were made despite recurrent ill-health throughout his life. All who knew W. O. James and his wife recognise the magnificent supportive role she played throughout his career. His many former colleagues now working in all parts of the world have cause to be grateful to W. O. James for his scholarly example.

R. M. Leech

Francis Aylward

PROFESSOR FRANCIS AYLWARD who was Professor of Food Science in the University of Reading from 1968 to 1976 died on 28 September 1978, aged 67. A memorial service for him was held in Westminster Cathedral on Thursday, 7 December.

After graduating at Liverpool University with first class honours in chemistry, he remained at Liverpool studying lipid chemistry under Hilditch (whose school was of international repute) and obtained his PhD. His subsequent contribution to lipid chemistry and biochemistry was recognised by the award of a DSc in 1958.

Francis began his scientific career by holding research fellowships at the Hannah Dairy Research Institute and Johns Hopkins University. The war years were spent with ICI Ltd. In 1947 he was appointed Head of the Department of Chemistry and Food Technology at the Borough Polytechnic. It was at this time that it began to be recognised, largely as a result of food research carried out in USA and UK, that a substantial and systematic body of knowledge had accumulated, and the time had arrived for food technology to be taught as a discipline particularly to provide the food industry (which was rapidly expanding and moving from a craft based to a scientifically oriented industry) with its scientific manpower. The Borough Polytechnic was one such teaching institution that recognised this need, and under Francis's able and progressive leadership the food technology element of his department grew and flourished.

Though still retaining his interest in lipids he began to take a greater interest in the role of food technology in relation to nutrition and food supplies.

This led naturally to his appointment in 1960 to serve for two years as FAO Adviser on Food and Nutrition to the Government of Ghana, and for the period 1961-62 as Head of the newly created Department of Biochemistry, Nutrition and Food Science at the University of Ghana. From that time he developed a major interest in nutrition in relation to national and international food problems. From Ghana he moved to Poland and during 1962-65 he was Director of the Polish Food and Nutrition Project supported by the UN special fund.

In 1965 he returned to Britain as Director of the Fruit and Vegetable Preservation Research Association, Chipping Campden. Then in 1968, following the retirement of Professor Crossley as Professor of Dairying and the decision of Reading University to broaden the interests of the department to cover food science, Francis was appointed its first professor. In many ways this was an apt and fortunate appointment. Francis believed that institutes of higher education have a duty to assist developing countries, and this view agreed well with the tradition of Reading's Faculty of Agriculture. He obtained a substantial grant from the Leverhulme Foundation which permitted him to support two Senior Fellows and with their help he built up a large postgraduate school of food science—the largest in Europe—drawing most of its students from developing countries.

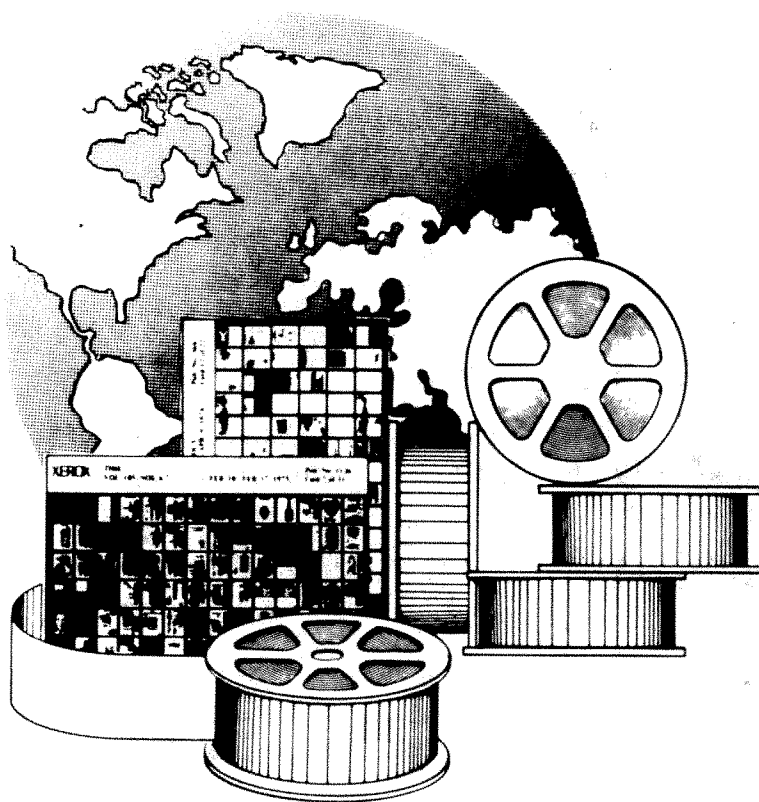
While at Reading he continued to act as consultant on behalf of organisations of the UN. This work was complementary to his service as Chairman of the University's Overseas Service Committee. Within the UK he was an active member of the Food Group of the Society of Chemical Industry. He was a member of the Food Additives and Contaminants Committee of the Ministry of Agriculture, Fisheries and Food. After retiring from his University post he continued to put great effort into helping with nutrition programmes overseas. He was appointed a member of the UN Advisory Group on Nutrition and was engaged in co-operating with UNEDO in regional services of food industries in the developing countries.

The heavy demands his career made on his time were not allowed to prevent him from playing an active role in the Roman Catholic Church, and recognition was given in 1951 when he was appointed a Knight of St Gergory.

With the untimely death of Francis Aylward, we in food science and technology have lost not only a highly respected colleague, but a friend of great warmth and personal kindness.

E. Rolfe

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APPOINTMENTS VACANT

UNIVERSITY COLLEGE OF NORTH WALES

Applications are invited for the post of

LECTURER IN AGRICULTURE

Applicants should have a degree in Agriculture, Agricultural Science or a related discipline, together with appropriate postgraduate experience in grass production and utilisation.

The appointment, to commence on September 1, 1979, or any earlier date by agreement, will be to the University's Lecturer Scale: £3,883 to £7,754 p.a. (under review).

Applications (two copies), giving details of qualifications and experience, together with the names and addresses of three referees should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, from whom further particulars may be obtained.

Closing date for applications. April 1979 24(A)

UNIVERSITY OF READING

LECTURESHIP AT THE NATIONAL COLLEGE OF FOOD TECHNOLOGY

Applications are invited for a Lectureship, tenable at the National College of Food Technology, Weybridge, from persons with qualifications and experience in applied microbiology preferably of particular relevance to the food industry. The successful candidate will be expected to contribute to teaching at undergraduate and postgraduate levels and develop an experimental research programme. Opportunities for research are available in all aspects of food microbiology.

The appointment will date from October 1, 1979.

Further information may be obtained from the Registrar (Room 214, Whiteknights House), The University, Whiteknights, Reading RG6 2AH, by whom applications should be received not later than April 30, 1979. 49(A)

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF BIOCHEMISTRY SCHOOL OF BIOCHEMICAL AND PHYSIOLOGICAL SCIENCES RESEARCH ASSISTANT/ FELLOW—SYNTHETIC ORGANIC CHEMISTRY

Synthesis of Novel Antibiotics

Applications are invited for a research assistant/fellow to participate in a programme of work directed towards the synthesis of novel antibiotics. The vacancy will suit an organic chemist with a Ph.D. degree but chemistry graduates wishing to move into the area of bio-organic chemistry are encouraged to apply.

The position, which in the first instance is for a ONE YEAR appointment, is available immediately.

Starting salary up to £4,382 per annum, depending on qualifications and experience. USS benefits.

Applications giving date of birth, curriculum vitae and the names and addresses of two referees should be sent as soon as possible to Mrs P. Vaughan-Smith, The University, Southampton SO9 5NH, quoting reference 1057/R/N 48(A)

UNIVERSITY OF LEICESTER

LECTURESHIP IN BIOCHEMISTRY

Applications are invited for a Lectureship in the Department of Biochemistry. It is likely that preference will be given to candidates with a strong background and current interest in chemical and physical aspects of Biochemistry. Excellent research facilities are available. Candidates should be prepared to take up their duties from October 1, 1979.

Salary according to qualifications and experience on the scale £3,883 to £7,754 (under review) with superannuation benefits.

Further particulars from the Registrar, University of Leicester, to whom applications on the form provided should be sent by April 27, 1979 56(A)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London) POSTDOCTORAL RESEARCH ASSISTANT

required for an M.R.C. three-year project working with Dr. J. A. Firth in the Department of Structural Biology on the mechanisms of trans-placental transport of hydrophilic molecules. Applicants should have a background in Mammalian Physiology and be interested in the application of ultra-structural tracer methods, experience in electron microscopy is desirable but not essential. Salary scale £3,883: £4,133: £4,382 (under review) plus London allowance £450 p.a. Further particulars and application forms available from the Establishment Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. 99(A)

IMMUNOCHEMISTRY UNIT DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD TECHNICIAN GRADE 3

A vacancy exists for a technician with experience in general biochemical techniques to work in the field of protein chemistry. Education to O.N.C. level or equivalent is required. Salary on the scale £2,688 to £3,060 p.a. Applications as soon as possible, including the names and addresses of two referees, should be sent to:

The Administrator,
Dept. of Biochemistry,
South Parks Road,
Oxford OX1 3QU 34(A)

INSTITUTE OF CANCER RESEARCH There is a vacancy for a **TECHNICIAN** to work in the Radiotherapy Research Laboratories at Sutton in a research programme on *in vitro* tests for the sensitivity of human tumour cells to cancer chemotherapeutic agents. The work involves specialised tissue culture techniques as well as some laboratory animal work with xenografted human tumours. Experience in pharmacology would be an advantage. The appointment will be on the Technician scale which starts at £3,261 per annum plus London Allowance of £354 per annum. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 14 Sumner Place, London SW7 3NU, quoting ref 301/B/62. 19(A)

POSTDOCTORAL POSITION

available immediately for research in the biosynthesis and assembly of basement membranes. Previous research experience with proteins and/or carbohydrates desirable. Experimental techniques include cell culture and somatic cell hybridization. Salary \$12,000, renewable. Send curriculum vitae and two letters of recommendation to Dr Albert E. Chung, Department of Biological Sciences, University of Pittsburgh, Pa., 15260, U.S.A. An Equal Opportunity/Affirmative Action Employer 54(A)

Laboratory SCIENTIFIC OFFICER required as soon as possible for work on a three-year research project, supported by the Medical Research Council, examining the effect of steroids on experimental and human immune complex disease. Techniques used will include basic immunological methods, analytical ultracentrifugation, tests of macrophage function and electron microscopy. Salary for qualified Laboratory Scientific Officer not less than £3,261 plus £354 London Weighting. Apply in writing, stating age and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref R.U.1 47(A)

INSTITUTE OF CANCER RESEARCH TECHNICIAN or JUNIOR TECHNICIAN required for the electron microscope department at the Chester Beatty Research Institute, Fulham Road, London, SW3. Experience in a biological electron microscope laboratory is desirable but candidates with experience in biochemical techniques or histopathological methods will be considered. The salary will accord with M.R.C. scales in the range £1,905 to £4,680 per annum plus £354 per annum London Allowance. Apply in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 14 Sumner Place, London SW7 3NU, quoting ref 301/B/61 18(A)

The Gambia

Assistant Director (Research and Specialist Services)

Department of Agriculture Up to £9,980

Candidates aged between 30 and 50 should hold a degree in Agriculture or related subject and have at least ten years experience of agricultural research work on sub-tropical crops. They should also have some knowledge of administrative procedure in a Government Department of a developing country. Duties will involve co-ordinating the planning and implementation of research and specialist services programmes.

Salary includes a substantial tax-free allowance paid under Britain's overseas aid programme. Basic salary attracts 25% tax-free gratuity.

Benefits include free passages, paid leave, children's holiday visit passages and education allowances, appointment grant and interest-free car loan.

The terms on which civil and public servants may be released if selected for appointment will be subject to agreement with their present employers.

For full details and application form write quoting MD/214/NA

Crown Agents

The Crown Agents for Overseas Governments and Administrations, Recruitment Division, 4 Millbank, London SW1P 3JD 6(A)

UNIVERSITY OF
NEWCASTLE UPON TYNE
DEPARTMENTS OF PATHOLOGY
AND MEDICAL STATISTICS
JUNIOR
RESEARCH ASSOCIATE

Applications are invited from honours graduates, or prospective honours graduates, in the biological sciences for a post as Junior Research Associate to assist in a research project investigating cell proliferation in the gastrointestinal tract. The work, which is sponsored by the Cancer Research Campaign, will involve cell kinetic and other experiments on hyperplastic and neoplastic gut in rodents. The post is available for three years starting on August 1, 1979 or as soon thereafter as can be arranged. Commencing salary £3,384 per annum on the National Range 1B scale. The successful candidate will be expected to enrol for a higher degree.

Further particulars may be obtained from Dr A. J. Watson, Department of Pathology, University of Newcastle upon Tyne, NE1 7RU, (0632 25131 Ext 445) to whom applications giving full curriculum vitae and the names of two referees should be sent by April 30, 1979. 22(A)

QUEENSLAND INSTITUTE
OF MEDICAL RESEARCH
MOLECULAR
PARASITOLOGIST

Scientists with substantial post-doctoral experience in immunology or molecular biology of parasites are invited to apply for the post of head of a parasitology unit. Current interests include laboratory studies in helminths and protozoa of medical importance, together with the application of modern technology in an epidemiological context. The Institute as a whole has commitments in the fields of infectious disease, oncology, molecular biology and genetics. There are currently some collaborative programmes in the Asian and Pacific regions and participation therein of the appointee will be encouraged. Appointment is initially for five years, renewable, with salary in the range \$A16,322 to \$A20,405 per annum. Applications stating full personal and professional details plus the names of at least two professional referees should be forwarded to Dr C. Kidson, Director, Queensland Institute of Medical Research, Bramston Terrace, Brisbane, 4006, Australia by April 20 59(A)

The Research Organization of an internationally recognized European Pharmaceutical Group located in Paris requires a

SENIOR CARDIOVASCULAR
PHARMACOLOGIST

To join its Department of Biology. The high success rate and rapid growth of this pharmaceutical group is related to the quality of people they employ and their continued commitment to research and development.

For this key position in the Cardiovascular Group we require a professional and highly motivated scientist who will be responsible for various projects within the group and should also be able to conduct independent research programmes.

Applicants can be either male or female and should have relevant post-doctoral experience (a maximum of five years). It is essential that the candidate should have experience of the pharmaceutical industry.

A realistic salary can be expected and relocation assistance will be available with other financial benefits.

All applications will be treated in the strictest confidence and should be sent directly to "Nature", c/o Box No 58(A), 3 Dymers Buildings, Holborn, London EC1N 2NR. 58(A)

GRADUATE OR POSTDOCTORAL Research Assistant required for 1½ years in the Department of Microbiology for an M.R.C. research project on cytomegalovirus immunovirology. Laboratory experience in virology essential. Applications, with full curriculum vitae and the names of two referees, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. M.C.2 9(A)

Research Archivist

As one of the largest pharmaceutical companies, Hoechst operates on a world-wide scale and the UK Research Laboratories play an integral part in the company's activities. At our new Research Laboratories at Milton Keynes, Bucks, we have a challenging vacancy for a Research Archivist.

This involves responsibility for maintaining a central archive for all laboratory data and providing information services to a group of multi-disciplinary Scientists. It offers an ideal opportunity for an Information Scientist or Graduate Scientist with information services experience to start a career.

The ideal candidate will be familiar with handling questions in organic chemistry.

This appointment carries a good salary, with at least four weeks holiday a year. Benefits include flexible working hours, free private health scheme, subsidised restaurant meals, excellent sports and social facilities. Generous relocation assistance will be provided if applicable.

Please write or telephone for an application form to:
Mr. A. Forrest, Personnel Department, Hoechst UK Limited,
Walton Manor, Walton, Milton Keynes, Bucks.
Tel: Pineham 5088.

2(A)

Hoechst



INSTITUTE OF CANCER RESEARCH: JUNIOR TECHNICIAN required for the Department of Molecular Biology, Chester Beatty Research Institute, Fulham Road, London SW3, in the general field of protein chemistry. The successful candidate will assist in the isolation and characterization of proteins and peptides. Applicants should have at least 2 'A' levels in Mathematics, Physics or Chemistry. Salary in scale £2,370 to £3,015 p.a., plus London Allowance of £354 p.a. Applications in duplicate, with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting Ref 301/B/63. 66(A)

MEDICAL
RESEARCH COUNCIL
RESEARCH ASSISTANT

(Technician/Research Officer)
Salary scale £3,261 to £4,902

The M.R.C. Clinical Psychiatry Unit requires a Psychologist or Social Science Graduate for 3 years to join a small team working on clinical and psycho-physiological studies which include suicide, depression, schizophrenia and expressive movements in psychiatric disorders. The job entails psychological testing and previous clinical experience is necessary. There will be opportunities for training in research methods, and the person appointed will be encouraged to accept individual responsibility for specific aspects of the research projects. Starting salary will depend on previous research experience. The post becomes vacant on June 1, 1979.

Applications to the Director, M.R.C. Clinical Psychiatry Unit, Graylingwell Hospital, Chichester, Sussex PO19 4PQ, enclosing curriculum vitae and the names of two referees. Closing date for applications: April 20 78(A)

INSTITUTE OF CANCER RESEARCH: TECHNICIAN required at the Sutton, Surrey laboratories for work with mammalian cell culture applied to radiobiology. Applicants should have a degree, H.N.C. or equivalent in a relevant subject. Experience in cell culture methods would be an advantage but is not essential. Salary in range £3,261 to £4,680 per annum plus London Allowance of £354 per annum (M.R.C. Technician scales). Applications in duplicate with the names of two referees should be sent to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref. 301/B/59. 21(A)

UNIVERSITY OF DURHAM
DEPARTMENT OF CHEMISTRY

Applications are invited for the post of

SENIOR DEMONSTRATOR

In the Department of Chemistry from October 1, 1979. The main duties will be to help with the practical teaching of ORGANIC CHEMISTRY, with tutorials for second and third year students, and to carry out research. An interest in some branch of organofluorine chemistry or polymer chemistry (preparative or structure and bonding by means of photoelectron spectroscopy) and a willingness to join existing groups working in these fields would be an advantage. There will be opportunities for the person appointed to give some lectures.

The appointment will be for three years. Initial salary in the range £3,384 to £4,631 per annum (under review) on Scale 1B or 1A, depending on qualifications, with membership of USS.

Applications (3 copies) naming three referees should be sent by April 18, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. 25(A)

THE OPEN
UNIVERSITY

Faculty of
Science

Biophysics Research
Fellow—Oxford

Applications are invited for the post of Research Fellow to assist in the study of the structure and transparency of the corneal stroma by a variety of biochemical and biophysical methods working with Professor G. F. Elliott at the Biophysics Research Group, Oxford Research Unit. Techniques used in the laboratory include X-ray diffraction, microelectrode measurements and gel electrophoresis and computer experience would be useful. The post would suit a physicist or physical chemist interested in biology or possibly a physically-minded physiologist.

The post, funded by a grant from the U.S. National Eye Institute is available for three years from April 1, 1979. Salary within the range £3,883 to £5,387 p.a. on the Research Fellow Grade 1A scale according to experience.

Application forms and further particulars are available, by postcard request please, from The Recruitment Office (JD/3796/1), The Open University, P.O. Box 75, Walton Hall, Milton Keynes MK7 8AL or by telephone from Milton Keynes 83868 (24 hour answering service). Closing date for applications: March 28. 3(A)



Open to both
men and women

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Canada
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Canada

PLANT PATHOLOGIST — CEREAL CROPS

(109-031-003)

Salary up to \$34,045 depending on experience

Ref. No: 78-RES-73 (N)

Agriculture Canada

Charlottetown Research Station — Charlottetown, P.E.I.

The Public Service of Canada has an immediate requirement for a scientist to conduct a research program in plant pathology with an emphasis on the epidemiology and control of major foliar diseases of barley and wheat in the Atlantic provinces.

Qualifications

Applicants must possess a Ph.D. or have a lesser degree with research experience and productivity equivalent to a doctorate, in plant pathology. It is also essential, for applicants to receive further consideration, that they have experience in conducting research related to cereal pathology. Knowledge of English is essential.

How to Apply

Send your application form and/or résumé to:

D.E. Fraser

Public Service Commission of Canada

National Capital Region Staffing Office

Ottawa, Ontario K1A 0M7

Closing Date: April 30, 1979

Please quote the applicable reference number at all times.

UNIVERSITY OF OXFORD

SIBTHORPIAN PROFESSORSHIP OF RURAL ECONOMY

The electors intend to proceed to a selection to the Sibthorpian Professorship of Rural Economy which will fall vacant on September 1, 1979. On appointment the professor will be required to assume the duties of Head of the Department of Agricultural Science and subsequently, for a period of five years, of the Departments of Agricultural and Forest Sciences which are to be amalgamated. Thereafter the Iebdomadal Council will assign the leadership of the amalgamated departments to either the Sibthorpian Professor of Rural Economy or the Professor of Forest Science for specified periods of not less than five years at a time; the professor who for the time being is not head of department will act as deputy head.

The stipend of the professor is at present £10,828 a year plus pensionable allowance as head or deputy head of department.

Applications (nine copies) naming three referees but without testimonials, should be received not later than May 8, 1979 by the Registrar, University Offices, Wellington Square, Oxford OX1 2JD, from whom further particulars may be obtained. 46(A)

MEDICAL RESEARCH COUNCIL RESEARCH ASSISTANT (Technician/Research Officer Salary scale £3,261 to £4,902)

The M.R.C. Clinical Psychiatry Unit requires a Psychologist or Social Science Graduate, with some training in Statistics, to join a small team working on clinical and epidemiological studies of psychiatric illness. The applicant will be expected to assist with the analysis and interpretation of data derived from clinical studies and a psychiatric case register; familiarity with or an interest in the use of an advanced desk top calculator would be an advantage. The main interests of the Unit are suicide and attempted suicide, depression and the neurophysiology of mental illness.

The person appointed will receive training in research methods and be encouraged to develop an individual interest in specific aspects of the work. Salary will depend on experience. The post becomes vacant on June 1, 1979, and is for the duration of 3 years.

Applications to the Director, M.R.C. Clinical Psychiatry Unit, Graylingwell Hospital, Chichester, Sussex PO19 4PQ, enclosing curriculum vitae and the names of two referees. Closing date for applications: April 20, 77(A)

COMMONWEALTH AGRICULTURAL BUREAUX Vacancy for SCIENTIFIC INFORMATION OFFICER

at the
COMMONWEALTH
FORESTRY BUREAU

Commonwealth Forestry Institute, South Parks Road, Oxford OX1 3RD
Duties of post: The scrutiny of technical literature, selection and preparation of abstracts in many subjects related to forestry or forest products (this may involve visits to outside libraries), indexing, preparing for the press and dealing with technical enquiries.

Flexible working hours are based on 37 hours per week. Applicants wishing to work 18½ hours per week, with appropriate adjustments of salary, etc., will be considered.

Qualifications: Applicants should be graduates in forestry or in a biological science or in a relevant branch of physical or engineering science. Strong preference will be given to candidates with a good reading knowledge of Russian.

Salary: In the range £2,998 to £5,448 per annum and a compensatory allowance (taxable but not superannuable) of 3.3 per cent to offset personal contribution to Superannuation. Starting salary according to qualifications, experience and age. Promotion to higher scales on merit.

Application forms and full particulars from:

The Executive Director,
Commonwealth Agricultural
Bureaux,

Farnham House, Farnham Royal,
Slough SL2 3BN.

Closing date for applications: April 30, 1979. 33(A)

LINCOLN COLLEGE (University College of Agriculture)

New Zealand

ASSISTANT LECTURER/ LECTURER IN SOIL SCIENCE

(DEPARTMENT OF
SOIL SCIENCE)

The Council of Lincoln College invites applications for appointment to a Lectureship in Soil Science. Applications will be considered from graduates in Agricultural Science or in any other discipline covering the general field of soil-plant relations, but preferably with a sound background in Chemistry.

The successful applicant will be appointed either as an Assistant Lecturer or Lecturer at a salary commensurate with his qualifications and experience. Current academic scales are: Assistant Lecturer NZ\$8,682 to NZ\$10,659; Lecturer NZ\$11,894 to NZ\$14,615. New Zealand Government Superannuation is available. Removal and travel expenses reimbursed up to specified limits.

Further details and Conditions of Appointment are obtainable from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the College.

Applications close on April 30, 1979. 41(A)

UNIVERSITY OF GLASGOW INSTITUTE OF NEUROLOGICAL SCIENCES

RESEARCH FELLOW (IMMUNOLOGY)

Work involves study of senile dementia and other diseases of the brain. Previous experience of cell culture and immunofluorescence techniques desirable.

Salary in scale £4,000 to £6,000 according to experience and qualifications.

Apply with curriculum vitae to:

Dr P. O. Behan,

Senior University Lecturer in

Neurology,

Institute of Neurological Sciences,

Southern General Hospital,

Glasgow G51 4TF. 90(A)

UNIVERSITY OF MALAYA

Applications are invited for the following CHAIRS in the University of Malaya.

Faculty of Dentistry:

CHAIR OF CHILDREN'S DENTISTRY

Candidates should have high academic qualifications and wide experience in teaching and research. They are also required to undertake research and to have publications of academic standing.

Faculty of Science:

CHAIR OF INORGANIC CHEMISTRY

CHAIR OF PHILOSOPHY OF SCIENCE

Candidates should possess the following: (1) Ph.D. in the required field with—(a) 3 years experience as Senior Lecturer/Reader/Associate Professor; or (b) 5 years experience as Lecturer; or (2) A Master's degree in the required field with—(a) 5 years experience as Senior Lecturer/Reader/Associate Professor; or (b) 8 years experience as Lecturer. Candidates are also required to undertake research and to have publications of academic standing.

Salary Scales (All inclusive) [approx. sig. equiv.]: £8,313 by 420 to £8,733/ Review Point £9,014 by 420 to £9,434.

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF.

The closing date for the receipt of applications is April 15, 1979. 60(A)

FACULTY POSITION IN NATURAL PRODUCTS CHEMISTRY

DEPARTMENT OF
ENTOMOLOGICAL SCIENCES,
COLLEGE OF NATURAL

RESOURCES
UNIVERSITY OF

CALIFORNIA, BERKELEY

Full-time, 11-month research (80%) and teaching (20%) position available in Natural Products Chemistry at the Assistant Professor level. Ph.D. in organic chemistry or other field(s) relevant to natural products required with evidence of interest in chemicals active in insects.

Responsibilities will be in basic and applied research involving bioactive chemicals from insects, plants, and other biological materials that influence insect growth, development, reproduction, and behaviour. Research will include identification and synthesis of active components, and studies on their potential use in managing insect populations. Teaching duties may include developing a course in chemical ecology, environmental chemistry, insect biochemistry, or chemical pest control.

Send full curriculum vitae, official transcripts, copies of significant publications or manuscripts, and names of at least three references by April 1, 1979 to Evert I. Schlinger, Acting Chairman, Department of Entomological Sciences, 137 Giannini Hall, University of California, Berkeley, California 94720. The University of California is an equal opportunity, affirmative action employer. 39(A)

UNIVERSITY OF ESSEX FELLOW IN THE

DEPARTMENT OF PHYSICS

Applications are invited for the SRC-supported post of Fellow (Range 1A) in the above department, tenable for three years starting as soon as possible (salary scale £3,883 to £6,555, under review). The person appointed will be engaged in experimental studies of wave-wave interactions in laser-generated plasmas and will participate generally in the work of the Laser Plasma Interaction Group at the SRC Rutherford Laboratory Laser Facility.

Applications (three copies), including a curriculum vitae and the names and addresses of two referees, should reach the Registrar (AG/70/N), University of Essex, Wivenhoe Park, Colchester CO4 3SQ, from whom further particulars may be obtained, by April 5, 1979. 4(A)

**FACULTY POSITION IN
INSECT PATHOLOGY
DEPARTMENT OF
ENTOMOLOGICAL SCIENCES
COLLEGE OF
NATURAL RESOURCES
UNIVERSITY OF
CALIFORNIA, BERKELEY**

Full-time, 11-month research (75%) and teaching (25%) position available in insect pathology at the Assistant Professor level available July 1, 1979. Expertise mandatory in one of the following areas: bacteriology, comparative virology, mycology, or protozoology. Ph.D. and background in pathology required and postdoctoral experience desirable.

Responsibilities will be in basic and applied research and teaching in insect pathology and pest management. Experience in pathogen-insect relationships, pathogenicity, and the manipulation of pathogens for insect control will be weighing factors when judging applicants.

Send full curriculum vitae, official transcripts, copies of significant publications or manuscripts, and names of three references by April 1, 1979, to Evert I. Schlinger, Acting Chairman, Department of Entomological Sciences, 137 Giannini Hall, University of California, Berkeley, California 94720. The University of California is an equal opportunity, affirmative action employer. 40(A)

**CHELSEA COLLEGE
University of London
BIOLOGICAL SCIENCES GROUP
LECTURESHIP IN
APPLIED MYCOLOGY**

Applications are invited for the vacant post of Lecturer in the Department of Applied Biology, Biological Sciences Group. Candidates should preferably hold a Ph.D. degree or equivalent and have had some postdoctoral research experience in the area of applied mycology and/or plant pathology. Additional interests in the chemical control of plant disease or the genetical aspects of plant pathogen control would be an advantage.

Salary scale: £3,883 to £7,754 per annum (under review) plus £450 London Allowance.

Application forms and further particulars may be obtained from the Personnel Officer, Chelsea College, Friese Greene House, Chelsea Manor Street, London SW3 3TW. Closing date: April 6, 1979. 91(A)

**MOLECULAR BIOLOGY OF
ONCOGENIC VIRUSES
ASSISTANT OR
ASSOCIATE MEMBER**

The Division of Virology, St Jude Children's Research Hospital, invites applications from individuals with promise (assistant) or evidence (associate) of outstanding research abilities. We favour candidates with interests in nuclear DNA viruses (papova, adeno, herpes) and experience in recombinant DNA technology. However, exceptional candidates with other research interests will be considered. This is a full-time research position with no teaching or service responsibilities. St Jude Hospital is an equal opportunity employer. Please submit curriculum vitae, summary of research plans, and names of three referees to: Dr Allan Granoff, Chairman, Division of Virology, St Jude Children's Research Hospital, Memphis, TN 38101. 92(A)

**BLOOD GROUP REFERENCE
LABORATORY
SCIENTIFIC OFFICER**

A graduate is required to assist Dr C. M. Giles in reference work related to blood transfusion. This laboratory is recognised by the World Health Organisation as a Collaborating Laboratory for Reference and Research in Blood Grouping and many varied problems are investigated. Opportunities are available for study for a higher degree. Experience in the field of blood transfusion desirable.

Applications in writing, with curriculum vitae to Miss K. Lomas, Blood Group Reference Laboratory, Gatliff Road, off Ebury Bridge Road, London SW1W 8QJ. 9972(A)

**Notice of Vacancy
DIRECTOR**

**University of Minnesota
ST PAUL CAMPUS LIBRARIES**

Under the general direction of the Director of University of Minnesota Libraries, the Director of the St Paul Campus Libraries is responsible for the administration of this Department, which includes a Central Library, which is undergoing major expansion, and six physically separate subject libraries. The collections total over 200,000 catalogued volumes as well as substantial holdings in government publications and other materials, which support programmes in agriculture, biological sciences, and veterinary medicine. There is an operating budget of \$770,000, including \$275,000 for acquisition and binding, and a staff of 41 FTE, including 12 professionals. As director of one of the seven major departments in the University Libraries, the individual participates fully in University Libraries-wide planning and programme development.

Applicants must have demonstrated exceptional skills in leadership, administration, planning, and interpersonal relations. They must possess the M.L.S. or an appropriate equivalent, as well as significant applicable experience in a major university library. An educational background in the agricultural, biological sciences, or supporting science is desirable. Applicants should be able to meet the requirements of a universities libraries faculty appointment with tenure at the rank of associate professor or professor.

The minimum starting salary is \$25,000; this includes an administrative stipend.

Closing date for receipt of applications: May 15, 1979.

The University of Minnesota is an equal opportunity educator and employer.

Send application and detailed resume and names of three references to: Clarence Carter, Personnel Officer, 499 Wilson Library, University of Minnesota, Minneapolis, Minnesota 55455. 93(A)

**UNIVERSITY OF
NEWCASTLE UPON TYNE
READERSHIP IN
PHYSIOLOGY**

Applications are invited for an appointment from September 1, 1979 to a vacancy in an established Readership in Physiology. The Department of Physiology is responsible for teaching and research in Physiology and Physiological Biochemistry.

Salary will be at a point according to age, qualifications and experience on either the pre-Clinical Reader's scale £8,508 to £9,263 (under review) for a medically qualified appointee or the non-medical Reader's scale £7,506 to £9,263 (under review) with membership of the appropriate University superannuation scheme.

It may be possible to negotiate a clinical sessional commitment of Consultant status for a medically qualified appointee in acknowledgement of defined clinical duties and responsibilities.

Further particulars may be obtained from the Deputy Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU, with whom applications (15 copies) giving the names of not more than three referees, must be lodged not later than April 14, 1979. (Applicants from outside the British Isles may submit one copy only). 82(A)

**Queen's University at Kingston
POSTDOCTORAL
APPOINTMENTS**

in Solid State Physics, Nuclear Physics and Astronomy.

Specific fields include:

- Stellar Structure and Evolution
- Fast Ion Transport in Solids
- Experimental Nuclear Physics
- Theoretical Nuclear Physics
- Positron Studies of Metals
- Low Temperature Studies
- Physics of Dielectrics

Salary: ca. \$13,000 with N.S.E.R.C. approval. Submit applications with three references to Dr M. Sayer, Head, Department of Physics, Queen's University, Kingston, Ontario, Canada K7L 3N6. 94(A)

Occupational Hygiene

in newly formed units at Devonport, Portsmouth, Rosyth and Chatham

The Occupational Health Organisation of the MOD (Navy) provides a comprehensive medical, nursing and hygiene service to HM naval bases and regional shore establishments in the UK. These posts, in the regional hygiene units, offer experienced and newly qualified hygienists the opportunity to develop and apply their specialist knowledge in an important and expanding field. Each Unit will be staffed by two officers working on the identification, measurement and evaluation of a variety of chemical and physical hazards and advising on suitable methods of control. This will involve close liaison with the safety organisation, MOD Laboratories, the Institute of Naval Medicine and regional establishments.

Candidates, normally aged under 32, should have a good honours degree or equivalent in an appropriate discipline. The Diploma of Occupational Hygiene, Membership of the Institute of Occupational Hygienists or equivalent is essential for some posts, which are at senior scientific officer level.

Appointment as Senior Scientific Officer (£5150-£6900), Higher Scientific Officer (£4100-£5450) or Scientific Officer (£2840-£4410) according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 6 April 1979) write to Ministry of Defence, CM(S)rb3, Room 210, Savoy Hill House, Strand, London WC2E 0BX. Please quote ref: SA/2/E.

Ministry of Defence

Ministry of Agriculture, Fisheries and Food, Fisheries Laboratory, Burnham on Crouch

Protection of the Marine Environment

This post is in the Aquatic Protection Division of the Laboratory, where the scientist appointed will be responsible for advising on the effect of effluent discharges on the marine environment, particularly tidal and estuarine waters. This work will require the use of a variety of data sources related to land based discharges and petroleum hydrocarbons and calls for an ability to assess the significance and extent of pollutants in the marine and other aquatic environments.

Candidates, normally aged under 32, should have a good honours degree, or equivalent, in mathematics, physics, engineering, chemistry or closely related discipline, coupled with at least 4 years relevant experience. A knowledge of effluent control is highly desirable.

Appointment as Senior Scientific Officer (£5150-£6900) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 6 April 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote ref: SB/44/AD.

ALL THESE POSTS
ARE OPEN TO MEN AND WOMEN.

**Science
Group**
CIVIL SERVICE



Wellcome

**WELLCOME
REAGENTS
LIMITED**

Production Manager

Wellcome Reagents Limited, part of The Wellcome Foundation Limited, a major British-owned international pharmaceutical company, manufactures a wide range of *in vitro* diagnostic reagents covering Immunology, Bacteriology, Serology and Clinical Chemistry.

We currently seek a Production Manager who, reporting to the General Manager, will be responsible for all Reagents production and distribution operations located on our Beckenham and Hither Green sites, employing around 130 staff.

Candidates, men or women, with a degree or equivalent qualification in life sciences or laboratory medicine, should have at least 5 years' successful experience in the production of biologically based products. Good administrative skills are essential.

Salary will attract candidates currently earning around £9,000+. Excellent conditions of employment include pension scheme, 4 weeks' holiday and generous relocation expenses where appropriate.

Please write, giving brief relevant details of background and experience, to the Personnel Manager, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.



THE OPEN UNIVERSITY FACULTY OF SCIENCE



Assistant Cartographer (EARTH SCIENCES)

The successful applicant would be expected to prepare geological maps and diagrams for research publications, undergraduate courses, displays, meetings and symposia.

Applicants should have four years' practical cartographic experience, period of training inclusive. A recognised cartographic qualification would be desirable but proven ability to produce high quality artwork for reproduction (black and white and colour) is essential and short-listed candidates would be expected to present a portfolio of their work at interview.

Salary on the scale £2,955 to £3,402 p.a. (under review)

Ref: 3791

Research Technician (BIOLOGY)

Dr Richard Holmes requires a technician to assist his Research Group investigating Biochemical and Physiological variations in fish and amphibians. The post would suit someone able to work on their own initiative and wishing to be actively involved in an expanding research project.

Applicants should possess an H.N.C. or equivalent and at least six years' experience in Biochemical or Biology Research.

Salary on the scale £3,186 to £3,720 p.a. (under review).

Ref: 1983

Application forms and further particulars are available from the Recruitment Office (JD3780/3), The Open University, P.O. Box 75, Walton Hall, Milton Keynes MK7 6AL or telephone Milton Keynes 63404: there is a 24 hour answering service on 63868.

PLEASE QUOTE THE REFERENCE NUMBER OF THE POST IN WHICH YOU ARE INTERESTED.

Closing date for applications: March 29

57(A)

45(A)

UNIVERSITY OF NEWCASTLE New South Wales FACULTY OF MEDICINE SENIOR LECTURESHIPS IN PATHOLOGY (3) ANATOMICAL PATHOLOGY MICROBIOLOGY IMMUNOLOGY

The Faculty of Medicine is developing a fully integrated curriculum, based on clinical problem-solving in small groups, within a non-departmental organisational structure. Research interests currently represented in the area of Pathology include mucosal immunology and host defences, molecular, cellular and epidemiological studies of virus infections, tissue localisation of antigens. The appointees will be expected to contribute to the teaching programme and to develop research relevant to their own interests; they will be encouraged to participate in laboratory and/or clinical aspects of their specialty in the teaching hospitals of the Hunter region (e.g. as an anatomical pathologist, infectious diseases physician or clinical immunologist). A medical qualification is desirable but not essential.

Commencing salary is within the range \$A21,180 to \$A24,667 per annum depending upon qualifications and experience. A clinical loading of up to \$A5,000 per annum depending on the degree of clinical responsibility may be paid to medically qualified appointees. There is a limited right of private practice.

For further information regarding academic matters and teaching duties contact Professor R. Clancy at the Royal Newcastle Hospital, New South Wales.

Applications close May 4, 1979. GENERAL INFORMATION: Conditions of employment including method of application and other particulars may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PP.

72(A)

THE AUSTRALIAN NATIONAL UNIVERSITY RESEARCH SCHOOL OF PHYSICAL SCIENCES DEPARTMENT OF THEORETICAL PHYSICS POSTDOCTORAL FELLOW/ RESEARCH FELLOW/ SENIOR RESEARCH FELLOW

Applications are invited for appointment as Postdoctoral Fellow, Research Fellow or Senior Research Fellow in the Department of Theoretical Physics, Research School of Physical Sciences (one appointment). The Department would prefer to make an appointment in the research areas of elementary particle physics or field theory, but applications from theoretical physicists with research interests in other areas will be considered. The Department's main current interests are in fundamental aspects of particles and fields, statistical mechanics, solid-state physics, plasma physics and nuclear physics. A substantial part of the work is related to the experimental interests of the Research School.

CLOSING DATE: MAY 18, 1979.

SALARIES will be in accordance with qualifications and experience within the range: Postdoctoral Fellow, a fixed point between \$A15,786 and \$A20,606 per annum; Research Fellow \$A15,786 to \$A20,606 per annum; Senior Research Fellow \$A22,049 to \$A26,301 per annum. Current exchange rates: \$A1=56p, \$US1.13.

TERMS OF APPOINTMENT: Postdoctoral Fellow—between one year and two years; Research Fellow/Senior Research Fellow—three years in the first instance with the possibility of extension to five years.

OTHER CONDITIONS: Reasonable appointment expenses are paid. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with housing is provided for an appointee from outside Canberra.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain the further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PP. 73(A).

UNIVERSITY OF LEICESTER DEPARTMENT OF PHYSICS RESEARCH ASSOCIATE IN CONDENSED MATTER PHYSICS

Applications are invited for a postdoctoral research associate to undertake a study of liquid semiconducting alloys using photoelectron spectroscopy. The project is part of a wider investigation of the properties of disordered systems being carried out in the Physics Department and supported by the Science Research Council.

The post is tenable initially until September 1981 and the salary is from £3,843 per annum. Applications with a curriculum vitae and the names of two referees should be sent to Dr C. Norris, Department of Physics, University of Leicester, LE1 7RH. 9999(A).

FRESHWATER BIOLOGICAL ASSOCIATION WINDERMERE LABORATORY RESEARCH ASSISTANT

Applications are invited for the post of research assistant to work on the physiology of teleost fish. Experience with chromatography, radio isotopic techniques and electron microscopy useful but not essential.

The salary scale, according to age, qualifications and experience, will be either £2,188 to £3,303 (Assistant Scientific Officer) or £2,839 to £4,415 (Scientific Officer). Persons expecting to graduate in July 1979 may be considered for this post.

Further details and application forms from: The Secretary, Freshwater Biological Association, The Ferry House, Ambleside, Cumbria LA22 0LP. 31(A).

**AGRICULTURAL
RESEARCH COUNCIL
INSTITUTE OF
ANIMAL PHYSIOLOGY
BABRAHAM
CAMBRIDGE CB2 4AT
Neurobiological Research**

As part of an expanded programme of work in Neurobiology at Babraham the following three new research appointments are available at Senior Scientific Officer level.

N.B.1. Peptide chemist for research in Neuroendocrine Group on peptidergic neurones. Candidates should have experience in isolation and characterisation of neuropeptides and an interest in collaboration with neurophysiologists.

N.B.2. Behavioural physiologist to investigate brain mechanisms in motivational behaviour of farm animals.

N.B.3. Neurophysiologist for research involving single unit neuronal recording from conscious animals to study neural mechanisms in ingestive behaviour of farm animals.

Candidates should possess a first or upper second class honours degree together with at least four years relevant postgraduate experience, with preferably a Ph.D. degree and a good record of published work.

Salary £5,154 to £6,856 p.a. Non-contributory pension scheme.

Application forms and further details may be obtained from the Secretary of the Institute by quoting the appropriate reference number. Closing date for applications: April 2, 1979.

76(A)

**PUBLIC HEALTH
LABORATORY SERVICE
BOARD
MICROBIOLOGIST
—BASIC GRADE**

Graduate Electron Microscopist

Applications are invited for the post of electron microscopist in the Public Health Laboratory and Department of Microbiology, Central Middlesex Hospital, London NW10. This post offers opportunities to develop electron-microscopy diagnostic techniques in clinical virology and other aspects of microbiology. In addition to a service commitment, the person appointed will be expected to undertake research work and to teach electronmicroscopy to members of the laboratory staff and others as required. Participation in other aspects of virology may be required on occasion. Applicants should possess a first-class (or upper second class) honours degree or higher qualification in microbiology.

N.H.S. terms and conditions of service. Salary scale £2,991 to £4,899 plus £354 London Weighting.

Closing date, April 5, 1979.

The laboratory may be visited by arrangement with the Director, Dr D. A. McSwiggan, 01-965 1603, to whom an application (giving curriculum vitae together with the names and addresses of three referees) should be sent.

64(A)

**INSTITUTE OF
BASIC MEDICAL SCIENCES
(University of London
and
Royal College of Surgeons
of England)**

**DEPARTMENT OF
PHARMACOLOGY**

Applications are invited from medical or science graduates for the post of

LECTURER

in the Department of Pharmacology. Candidates will be expected to have completed several years of postgraduate research in a subject related to the main research work of the department.

Salary scale £3,883 to £7,754 p.a., plus £450 London Allowance. (Award pending.)

Applications naming two referees to: Mr W. F. Davis, Secretary, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN, by Friday, April 20, 1979, from whom further particulars may be obtained.

67(A)

**KUWAIT
UNIVERSITY OF KUWAIT
FACULTY OF MEDICINE**

Medical Science and Clinical Appointments

Applications are invited for the following appointments in the Faculty of Medicine.

ANATOMY—Associate Professor. Candidates should have areas of specialisation in electron microscopy. The successful applicant responsibilities will include directorship of the E.M. Unit, which is equipped with scanning and transmission microscopes. Assistant or Associate Professor. Preference will be given to candidates who have experience of teaching the anatomical discipline to allied health students. The successful candidate will be responsible for co-ordinating allied health teaching in the Department of Anatomy.

COMMUNITY MEDICINE—Professor or Associate Professor in Epidemiology. Professor or Associate Professor in Public Health.

MEDICINE—Associate Professor. Candidates should be general physicians with a special interest in renal medicine and experience in haemodialysis, peritoneal dialysis and renal transplantation. Experience of renal morphology by light microscope would be an advantage.

MICROBIOLOGY—Professor or Associate Professor in Medical Bacteriology. Professor or Associate Professor in Clinical Bacteriology.

PATHOLOGY—Associate and Assistant Professors. Candidates should have experience in academic pathology, particularly in anatomic and histopathology. The successful candidates will have responsibilities in diagnostic pathology at the teaching hospitals.

PHYSIOLOGY—Professor or Associate Professor in Neurophysiology. Professor or Associate Professor in Endocrinology.

Professor or Associate Professor with specialisation in one of the following areas: Haematology, Gastrointestinal, Muscle & Cardiovascular.

SURGERY—Professor or Associate Professor in Urological Surgery. Associate Professor in Traumatology and Burns.

Assistant Professor in Orthopaedics. Applicants should possess a Ph.D. or equivalent higher medical qualification, and have conducted and published research in the field. Professors should have 14 years' experience, 4 as an associate professor or its equivalent, i.e. senior lecturer or reader. Associate professors should have 9 years' experience, 4 as an assistant professor or its equivalent, i.e. lecturer.

CONDITIONS OF APPOINTMENT

Salaries: Total monthly salaries will be within the following scales according to qualifications and experience (1KD=£1.80, US\$3 approx.).

Professors with clinical appointments: KD 755-860 (6 increments).

Professors medically or non-medically qualified with medical science appointments: KD 701-797 (6 increments).

Associate Professors with clinical appointments: KD 650-755 (6 increments).

Associate Professors medically qualified with medical science appointments: KD 605-701 (6 increments).

Associate Professors non-medically qualified: KD 575-665 (6 increments).

Assistant Professors with clinical appointments: KD 520-625 (6 increments).

Assistant Professors medically qualified with medical science appointments: KD 484-560 (6 increments).

Assistant Professors non-medically qualified: KD 460-550 (6 increments).

Clinical supplements: In addition to the above university salaries there will be a monthly clinical supplement paid by the Ministry of Public Health for medical school staff with clinical service commitments. These will be within the following scales:

Professor and Chairman: KD 200-250 (5 increments).

Professor: KD 150-200 (5 increments).

Associate Professor: KD 100-150 (5 increments).

Assistant Professor: KD 75.

Gratuity: There is a gratuity of one month's basic salary for each year employed which is payable on termination of contract.

Housing: Suitably furnished, air-conditioned accommodation, electricity and water are provided free of charge.

Medical Care: Free, comprehensive treatment is available in Kuwait under the State Health Service.

Travel: Airline tickets are provided from the country of recruitment for the appointee, spouse and up to 3 dependent children under 20 years. Thereafter return tickets are issued annually to the country of origin. On termination of contract, tickets are provided to the country of recruitment. A baggage and freight allowance is also provided. In addition, travel expenses for attending one scientific meeting a year are paid by the University.

Vacation: Sixty days paid annual leave as well as various official holidays.

Education: This is provided free in state schools where the instruction is in Arabic. Staff who have to send their children to non-Arabic schools in Kuwait will have the cost of up to a maximum of three met by the University.

Taxation: There is no income tax in Kuwait. Currency is freely transferable without restrictions.

Further details about these appointments and the method of application can be obtained from: The Dean, Faculty of Medicine, University of Kuwait, P.O. Box 5969, Kuwait.

Completed applications must be received by no later than April 25, 1979.

9993(A)

**UNIVERSITY OF GLASGOW
DEPARTMENT OF ZOOLOGY
RESEARCH ASSISTANT**

Applications are invited from graduates with a training in neurobiology to work on a project concerned with the function of interneurons in the connectives of the locust. The project is funded by the SRC and is supervised by Dr M. D. Burns.

The position will be for three years, with starting salary within £3,384 to £3,883 (Range 1B, Research and Analogous Staff Scales). It may be possible to register for a higher degree.

Applications, enclosing curriculum vitae and the names of two referees, should be sent to:

Dr M. D. Burns,
Zoology Department,
Glasgow University,
Glasgow G12 8QQ.

In reply please quote Ref No 4392M

28(A)

**UNIVERSITÄT HEIDELBERG
Am Institut für Theoretische Astro-
physik ist die Stelle eines
PROFESSORS (C 3)**

zu besetzen.
Arbeitsgebiete am Institut (auch im Rahmen des Sonderforschungsbereichs 132 "Theoretische und praktische Stellarastrophysik"): Sternatmosphären, ausgedehnte Hüllen und Nebel, interstellare Materie, Spektroskopie, Transportprozesse und Hydrodynamik.

Mitarbeit im Sonderforschungsbereich ist erwünscht. Angemessene Beteiligung an den Lehraufgaben der Fakultät für Physik und Astronomie wird erwartet.

Bewerbungen mit Lebenslauf und Schriftenverzeichnis bis 1. Mai 1979 an das Dekanat der Fakultät für Physik und Astronomie, Albert-Überle-Str. 11, 6900 Heidelberg.

37(A)

**THE NATIONAL INSTITUTE FOR HIGHER
EDUCATION, LIMERICK, IRELAND**

Further expansion of the Institute is taking place at undergraduate and postgraduate levels within the extensive complex of world bank financed facilities which have been commissioned as part of the first phase of development of the riverside campus.

APPLIED MATHEMATICS-BUSINESS

Lecturer/Assistant Lecturer

Applicants should have particular expertise in the application of Mathematics to the solution of business problems.

APPLIED MATHEMATICS-CONTROL

Lecturer/Assistant Lecturer

Applicants should have particular expertise in the mathematical theory of control.

Applicants for the above posts should have experience in the application of Mathematics and should hold an appropriate Postgraduate qualification.

SALARY: Lecturer £6,901 to £8,766

Assistant Lecturer £5,294 to £6,592

Application material available from the Personnel Office, The National Institute for Higher Education, Limerick, Ireland, to be completed and returned by Friday, April 20, 1979.

80(A)

SENIOR ENGINEER

Signal Analysis
& Pattern Recognition

Newcastle-upon-Tyne up to £6,774

The British Gas On Line Inspection Centre has achieved an international reputation for the development of improved methods for inspecting high pressure pipelines. This has involved making use of advanced technology in several fields, namely electronics, computing and mechanical engineering. Due to rapid expansion, the Centre is moving to Cramlington, Northumberland, but is currently at Killingworth, Newcastle-upon-Tyne.

Computer analysis of the inspection data is a key aspect of the development programme. Signal analysis, pattern recognition and mathematical modelling expertise is required to develop and refine data processing techniques.

Applicants should be numerate graduates with a proven record of success in the above field. Previous experience with multi-sensor systems in seismology, medical inspection, speech recognition, ultrasonics or electromagnetics would be a distinct advantage as would a higher degree in a relevant subject. Extensive computing facilities are available.

Salary within the range £5,253 to £6,774 plus current self-financing productivity payment.

Excellent conditions of service are offered—these include pension and sick pay schemes.

Please write for an application form quoting reference OLI/331/N to Margaret Harrison, Personnel Assistant, On Line Inspection, Engineering Research Station, Killingworth, Newcastle-upon-Tyne.

Tel: (0632) 684828, ext. 380.

BRITISH GAS



98(A)

Scottish Institute of Agricultural Engineering

Head of Soils Section

Senior Scientific Officer/ Principal Scientific Officer

(Ref. AG/18/B)

Re-advertisement

Applications are invited for the post of Senior Scientific Officer/Principal Scientific Officer to lead a team investigating the effects of agricultural machinery on the physical properties of field soils with particular reference to cultivations, zero-tillage and compaction.

Qualifications

A first or upper second class honours degree, and preferably a higher degree, or equivalent qualifications in a relevant branch of science or engineering. The applicant should have had considerable experience of research in soil physics or soil mechanics.

Salary Scales

Appointment in the grade of Senior Scientific Officer (£5,154–£6,898) or Principal Scientific Officer (£6,609–£8,461) according to qualifications and experience. Non-contributory superannuation.

Application forms and further information from the Secretary, Scottish Institute of Agricultural Engineering, Bush Estate, Penicuik, Midlothian, EH26 0PH, to whom applications should be submitted by April 2, 1979. 9917(A)

POSITION DESCRIPTION PROFESSOR OF BIOCHEMICAL PHARMACOLOGY AND CHAIRPERSON DEPARTMENT OF PHYSIOLOGY, BIOCHEMISTRY AND PHARMACOLOGY

The New York State College of Veterinary Medicine at Cornell University invites nominations and applications for the position of Chairperson of the Department of Physiology, Biochemistry and Pharmacology. The speciality of the applicant should be within the discipline of biochemical pharmacology. Credentials should include evidence of outstanding research achievements and potential, administrative ability and considerable teaching experience, and there should be a demonstrated commitment to excellence in teaching and research.

The department is multidisciplinary with responsibility for teaching, research and service. The 17,000 square feet of teaching and research laboratory space and 6,000 square feet of specialised animal-holding space are well-equipped for research and instruction on a wide range of subjects and species. The department is also engaged in interdisciplinary research projects and training programs in toxicology and comparative gastroenterology.

Applicants should submit their curriculum vitae, and request that three letters of recommendation be sent directly to the Search Committee. Applications accepted through June 1, 1979.

Please address all correspondence to: Professor R.H. Wasserman, Chairman Search Committee, Department of Physical Biology, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853.

Affirmative Action/Equal Opportunity Employer. 30(A)

FACULTY POSITION AVAILABLE IN RETINAL HEMODYNAMICS AND BLOOD FLOW STUDIES

University of Illinois

Eye and Ear Infirmary

and
Abraham Lincoln School
of Medicine

The University of Illinois
at the

Medical Center, Chicago

Equal Opportunity/Affirmative
Action Employer

Applications are invited for a faculty position at the assistant or associate professor level. The major responsibility will be supervision of research of vascular flow in the retina, utilizing a unique non-invasive computerised ophthalmoscopy system. Active interchange with engineers and clinical ophthalmologists will be fostered. An advanced degree (Ph.D. or M.D.) is a prerequisite.

Those interested should direct inquiries together with a curriculum vitae and bibliography to

Morton F. Goldberg, M.D.
Professor and Head
Department of Ophthalmology
University of Illinois Eye and
Ear Infirmary
1855 West Taylor Street
Chicago, Illinois 60612
312 996-6590. 95(A)

LABORATORY SCIENTIFIC OFFICER with HNC or equivalent qualification (or Junior Laboratory Scientific Officer of high quality) required as soon as possible for one year to complete an M.R.C. supported study on cytomegalovirus infections in Department of Microbiology. Experience in virology or cell culture essential. Salary for qualified Laboratory Scientific Officer not less than £3,261 plus £354 London Weighting. Applications with full details and names of two referees to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. M.C. 10(A)

UMIST DEPARTMENT OF MATHEMATICS LECTURER IN MATHEMATICS (OPERATIONAL RESEARCH)

Applications are invited from candidates of either sex for the above post. Applicants may have research interests in any branch of Operational Research (stochastic and/or deterministic).

Salary will be in the range £3,883 to £7,754 (under review) and the appointee will be expected to commence duties by September 1 or as soon as possible thereafter by arrangement.

Write for application forms and further particulars quoting reference MAT/34/A1 to the Registrar UMIST, P.O. Box 88, Manchester M60 1QD. 70(A)

WELSH NATIONAL SCHOOL OF MEDICINE DEPARTMENT OF MEDICINE and UNIVERSITY COLLEGE, CARDIFF

DEPARTMENT OF
BIOCHEMISTRY

TWO POSTDOCTORAL RESEARCH OFFICERS

Applications are invited for two Research Officer positions, one work with Dr John Kay at University College, Cardiff, and the other with Dr R. Marks, Reader and Consultant in Dermatology, Welsh National School of Medicine, on a collaborative project to identify serum component of possible importance in wound healing. The work in the first post will include large scale fractionation of human serum while the second will be involved with assay systems, org culture and histometric techniques.

Candidates for the first post should have, or expect to obtain shortly, a Ph.D. degree in Biochemistry or a related subject and research experience in protein purification systems, especially affinity chromatography and ultrafiltration procedures would be an advantage. For the second post, a Ph.D. in Biochemistry or other biological discipline would be acceptable and experience with histologic procedures and tissue culture system would be advantageous.

The appointments, which will be for one year in the first instance (renewable for a second year) are financed by a grant from the Medical Research Council. Starting salaries up to £3,800 per annum (under review).

Applications in the form of a curriculum vitae, with the names and addresses of two referees, should be sent to the Registrar and Secretary, Welsh National School of Medicine, Health Park, Cardiff, 3 weeks from the date of appearance of this advertisement (quoting Ref No M6/53). 65(A)

UNIVERSITY COLLEGE OF NORTH WALES, BANGOR DEMONSTRATOR IN MARINE BIOLOGY

Applications are invited for the above appointment, to commence as soon as possible.

The successful applicant will be required to assist the preparation of teaching of practical classes, the courses and tutorial classes in marine zoology, particularly in its ecological aspects. Facilities for individual research will be available.

The appointment will be to the Oth Related IB Scale £3,384 to £5,604 p annum, and will be for one year in the first instance.

Applications (two copies), giving details of age, education and experience, together with the names and addresses of two referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DX. Closing date for applications: March 26, 1979. 83(A)

UNIVERSITY OF LONDON CHAIR OF CLINICAL IMMUNOLOGY AT THE CARDIOTHORACIC INSTITUTE

The Senate invites applications for the above Chair.

The Cardiothoracic Institute is associated with the Brompton, the National Heart and London Chest Hospitals, but the holder of the Chair will head the Department of Clinical Immunology at the Fulham Road Branch, linked to the Brompton Hospital. Within the Institute there is a number of other units with complementary interests, including pharmacology, microbiology, pathology, oncology and occupational lung disease, as well as adult and paediatric clinical departments with whom collaboration would be encouraged. Appropriate clinical facilities are available at the Brompton Hospital and the opportunity exists to develop further links with the other Hospitals within the Group.

Applications (11 copies) must be received not later than April 27, 1979 by the Academic Registrar (N), University of London, Malet Street, London WC1E 7HU, from whom further particulars and the salary scale should first be obtained. 42(A)

THE HANNAH RESEARCH INSTITUTE FOR STUDIES RELATING TO THE PRODUCTION AND UTILISATION OF MILK METABOLIC PHYSIOLOGIST

A vacancy exists for a Higher Scientific Officer in the Physiology Department to study metabolism in goats and cows in relation to the control of milk secretion and to nutritional and environmental factors.

Applicants should possess a First or Upper Second Class Honours Degree in a relevant discipline with, preferably, a Ph.D.

Salary will be in the range £4,101 to £5,448. At least two years' relevant postgraduate experience is required for appointment to HSO. Non-contributory pension scheme in operation.

Further particulars may be obtained from:

**The Secretary
The Hannah Research Institute
AYR KA6 5HL**

to whom applications, which should include a curriculum vitae, list of publications, and the names and addresses of three referees, should be sent by: April 12, 1979. 87(A)

WESTMINSTER MEDICAL SCHOOL RESEARCH ASSISTANT

Organic Chemist or Biochemist, required to join a small team working on pigment changes in disease. Work will include the isolation and assay of pigment intermediates from the body fluids of patients undergoing treatment. Post available for 1 year, initially.

Salary on the Whitley Council Scale for Hospital Biochemists.

Applications, with curriculum vitae and the names of two referees, to Professor J. R. Hobbs, Department of Chemical Pathology, Westminster Medical School, 17 Page Street, London SW1P 2AR, by March 30, 1979. 79(A)

RESEARCH CHEMIST

A Research Chemist to work in the areas of co-ordination and bio-inorganic chemistry is required for research, in the first instance, into the chemistry of molybdenum iron-sulphur cluster systems and related species.

The post is in the Higher Scientific Officer grade, on a salary scale increasing from £4,101 to £5,448 per annum, by eight yearly increments.

Qualifications: 1st or upper 2nd class honours degree with at least two years relevant postgraduate experience.

Superannuation under a non-contributory scheme.

Applications in writing to the Secretary, Agricultural Research Council, Unit of Nitrogen Fixation, University of Sussex, Brighton, Sussex BN1 9RQ, with curriculum vitae and the names of three referees. Closing date: March 30, 1979. 74(A)

SENIOR/ASSOCIATE SCIENTISTS IN EXPERIMENTAL CELL MORPHOLOGY AND CYTOGENETICS

The W. Alton Jones Cell Science Center is interested in expanding its research program by the recruitment of two new staff members at the level of SENIOR or ASSOCIATE SCIENTIST (FULL OR ASSOCIATE PROFESSOR EQUIVALENT). The Center is an operational unit of the Tissue Culture Association and has an active program of research on a variety of topics. It is concerned, as well, with advanced training and education involving cell tissue and organ cultures. The laboratories are modern, spacious, and well equipped. The Center has affiliations with the University of Vermont, SUNY at Plattsburgh, and the University of Rhode Island.

Suitable candidates will have a Ph.D. or M.D. and have made significant contributions to their chosen field. They should currently hold positions of not less than Assistant Professor or equivalent. It is expected that the candidates' research will involve the use of cell tissue or organ culture. It is expected as well that the candidates will have an established track record in obtaining research support from the funding agencies.

For further information on the Center and its programs, contact the Office of the Director, W. Alton Jones Cell Science Center. Resumes and names of four references to the Chairperson, Search Committee, In Care of W. Alton Jones Cell Science Center, Old Barn Road, Lake Placid, New York 12946.

The W. Alton Jones Cell Science Center is an Affirmative Action/Equal Opportunity Employer. Applications from women and minority groups are encouraged. 9922(A)

AGRICULTURAL RESEARCH COUNCIL INSTITUTE OF ANIMAL PHYSIOLOGY, BABRAHAM, CAMBRIDGE CB2 4AT

A vacancy exists in the Immunology Department for a research scientist who has made a significant contribution to the field of cellular physiology of the immune response. The candidate should be experienced in the analysis of lymphocyte and macrophage kinetics *in vivo*, with particular reference to cells engaged in the immune response, and in the induction and maintenance of tolerance. Applicants should have a first or upper second class honours degree and preferably a Ph.D. in a relevant subject. Appointment as Principal Scientific Officer—£6,609 to £8,461 p.a. Non-contributory superannuation scheme. Applications should be sent to the Secretary of the Institute by April 2, 1979, quoting Ref. I.D.4. 35(A)

UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF ZOOLOGY The University invites applications for the post of DEMONSTRATOR

in the Department of Zoology. Preference will be given to candidates with experience in comparative physiology or experimental zoology. Applicants should possess a higher degree or have equivalent postgraduate research experience. The appointment will be for a period of three years tenable from September 1, 1979.

Salary will be at an appropriate point on the Grade IB (bar) scale £3,884 to £4,882 per annum according to age, qualifications and experience. Membership of the appropriate university superannuation scheme will be required.

Further particulars may be obtained from the Senior Assistant Registrar, The University, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU, with whom applications (three copies), together with the names and addresses of three referees, should be lodged not later than April 9, 1979. Please quote reference N. 20(A)

Plant Virologist

Senior Scientific Officer/ Higher Scientific Officer/ Scientific Officer

£2,839 to £6,898

Applications are invited for a pensionable post in the Plant Pathology Division of the Department of Agriculture.

The successful candidate will be expected to conduct research and provide specialist advice on plant virus diseases with particular reference to diseases of grass, forage, cereal and horticultural crops.

The person appointed may also be required to undertake teaching duties in the Faculty of Agriculture and Food Science, Queens University, Belfast.

SENIOR SCIENTIFIC OFFICER

Over 25 and under 32 years of age on December 31, 1979 with a first or second class honours degree in a scientific or agricultural discipline pertinent to Plant Pathology with at least 4 years' postgraduate experience in Plant Virology.

HIGHER SCIENTIFIC OFFICER

Under 30 years of age on December 31, 1979 with an honours degree as above and at least 2 years' appropriate postgraduate experience.

SCIENTIFIC OFFICER

Under 27 years of age on December 31, 1979 with an honours degree as above. Exceptionally applications may be considered from candidates over the age limits who have specialised experience.

The Civil Service Commissioners may decide to interview only those applicants who appear from the information available (including the level of academic attainment and relevant experience) to be best qualified.

SALARY SCALES

Senior Scientific Officer	£5,154 to £6,898
Higher Scientific Officer	£4,101 to £5,448
Scientific Officer	£2,839 to £4,415

(The salary scale will be subject to review from April 1, 1979).

Grading and starting salary will be related to qualifications and experience. There are prospects of promotion to Principal Scientific Officer (£6,609 to £8,461).

Please write or telephone for an application form quoting job reference SB 137/79/T to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585 ext 256). Completed forms must be returned to arrive not later than March 29, 1979. (27A)



NORTHERN IRELAND CIVIL SERVICE

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY DEPARTMENT OF BIOCHEMISTRY POSTDOCTORAL RESEARCH ASSISTANT

required to join a group involved in genetic manipulation of industrial microorganisms for new fermentation processes. Experience in some aspects of recombinant DNA technology would be an advantage. The appointment is available from October 1, 1979 and would be for two years in the first instance on the scale £3,883 to £6,555 plus London Allowance, £450 per annum.

Applications including a curriculum vitae together with an indication of relevant experience and future aspirations for research should be addressed to Professor B. S. Hartley, F.R.S., Department of Biochemistry, Imperial College, London SW7 2AZ. 44(A)

UNIVERSITY OF GLASGOW TEMPORARY LECTURESHIP IN GEOLOGY

Applications are invited for a temporary lectureship in the Department of Geology during the session 1979-80 in marine ecology and sedimentology. Research experience in palaeontology and interaction of organisms and sediments would be valuable.

Salary will be within the range £3,975 to £5,830 of the Lecturers' scale £3,975 to £8,250, with placement according to age, qualifications and experience.

Applications (8 copies), giving the names and addresses of three referees, should be received by the Secretary of the University Court (Room 18), University of Glasgow, Glasgow G12 8QQ by April 13, 1979. Further particulars may be obtained from Professor B. E. Leuke, Department of Geology (Tel 041-339 8855, ext. 7435).

In reply please quote Ref. No. 4393 97(A)

Firmenich



Leading international company engaged in the manufacture and supply to industry of synthetic perfumes and flavours has an opening for a

SENIOR TOXICOLOGIST

at its corporate headquarters in Geneva, Switzerland.

Reporting directly to the corporate Management, the person appointed will assume the following major responsibilities:

- to represent FIRMENICH worldwide in matters of product safety;
- to provide a comprehensive advisory service to customers and government agencies, as well as to other companies in Firmenich's international Group;
- and, in general, to help formulate and implement Group policy for product safety, including the organization, supervision and interpretation of toxicity and dermatology tests carried out by independent laboratories.

The successful candidate will already have made a substantial impact in this field.

Ability to speak and write fluently in English as well as a good working knowledge of French are necessary, other languages being a further asset.

For more information please write in confidence to

Mr. ERNEST K. WEBER, Director of Personnel

FIRMENICH SA - Département du Personnel
Case postale 239 - 1211 Genève 8

9925(A)

THE UNIVERSITY OF LANCASTER DEPARTMENT OF PHYSICS RESEARCH ASSOCIATE

Applications are invited for the post of postdoctoral Research Associate in the Low Temperature Physics group. The post is financed by S.R.C. for three years on the R.A. 1A scale (£3,883 to £6,555, under review). The research associate will work with Drs Pickett and Guénault on experiments below 1mK on superfluid helium-3. A nuclear demagnetisation refrigerator constructed for this research programme is now nearing completion.

Further particulars may be obtained (quoting reference L.123/D) from the Establishment Officer, University House, Bailrigg, Lancaster LA1 4YW, to whom applications (5 copies) naming two referees, should be sent not later than March 30, 1979. 96(A)

UMIST DEMONSTRATOR IN BIOCHEMISTRY

Applications are invited for a Demonstratorship, initially until September 30, 1980, but with the possibility of renewal. Duties include assistance with the teaching of undergraduates mostly in the laboratory and postgraduate research. Qualifications: recent Ph.D. or good degree in Biochemistry or related discipline. Laboratory experience in Microbial or Protein Biochemistry desirable but not essential. Salary up to £3,883 per annum.

Applications by letter, quoting reference BIO/35/AT, with curriculum vitae and the names of two referees should be addressed to Professor A. A. Eddy, Department of Biochemistry, UMIST, P.O. Box 88, Manchester M60 1QD. 69(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF EARTH SCIENCES Applications are invited for the post of LECTURER

in the Department of Earth Sciences. Duties will consist mainly of the teaching of structural geology at undergraduate level plus appropriate research according to the particular interests of the appointee.

Salary on the scale for lecturers: £3,883 to £7,754 per annum (under review), according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 49/11/D. Closing date for applications: April 19, 1979.

Applicants from overseas may apply by telegram in the first instance, naming three referees. 11(A)

UNIVERSITY OF EDINBURGH FORBES CHAIR OF ORGANIC CHEMISTRY

Applications are invited for the Forbes Chair of Organic Chemistry which will become vacant on August 1, 1979, on the resignation of Professor J. I. G. Cadogan, F.R.S.

Further particulars may be obtained from the undersigned, with whom applications (12 copies) giving the names of three referees, should be lodged by May 9, 1979. Overseas candidates need submit only one copy of their application. Please quote Reference 3/79. 68(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF CHEMICAL PATHOLOGY Applications are invited for the post of LECTURER

in the above Department. Candidates should have a higher degree and some postdoctoral research experience.

An interest in planning, organising and taking major responsibility for the Neonatal, Paediatric and Obstetric aspects of the work of the Department would be an advantage. These activities will be sited in Phase I of the Leeds General Infirmary complex when this is opened in 1982. There will be excellent opportunities for research and the successful candidate will contribute to the teaching role of the Department.

Salary on the scale for lecturers: £3,883 to £7,754 (under review), according to age, qualifications and experience.

Those wishing to discuss the post in more detail or visit the Department should contact Professor D. B. Morgan (Telephone (0532) 32799, ext. 416).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 84/9/D. Closing date for applications: April 11, 1979. 71(A)

UNIVERSITE DE LAUSANNE

L'Université de Lausanne ouvre une inscription en vue de pourvoir le poste de

PROFESSEUR ORDINAIRE DE BIOLOGIE VEGETALE

enseignement: cours et travaux pratiques à des étudiants de 1er cycle (1ère année) et de 2e cycle (4e année)

orientation des recherches: contrôle biochimique et biophysique de la croissance des cellules végétales, avec préférence pour des problèmes de phytoenzymologie.

entrée en fonction: 1er septembre 1979.

Les candidatures (curriculum, plan de recherches et publications) doivent parvenir avant le 20 avril 1979 à M. le Doyen de la Faculté des Sciences, Collège propédeutique, 1015 Lausanne-Suisse. 84(A)

UNIVERSITY OF EDINBURGH DEPARTMENT OF ZOOLOGY LECTURER

(Sub-Department of Parasitology). Applications are invited for the post of Lecturer in the Department of Zoology (Sub-Department of Parasitology). Candidates must be prepared to teach helminthology.

Salary scale £3,883 to £7,754 per annum (under review), with placement according to qualifications and age, and with superannuation benefit.

The successful candidate will be expected to take up duty on October 1, 1979.

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, with whom applications (six copies), giving the name of two referees, should be lodged not later than April 10, 1979. Please quote Reference 1028. 9998(A)

The Edinburgh School of Agriculture UNIVERSITY OF EDINBURGH Department of Agricultural Biochemistry

RESEARCH ASSISTANTS

Two Research Assistants are required to work on

- (1) The biochemical aspects of the nitrogenous changes during the ensilage of grass. (Ref. ARC/18N).
- (2) Nitrogen metabolism in young chicks consuming microbial protein and nucleic acids. (Ref. ARC/19N).

Candidates should have a good Honours degree in Biochemistry, Biological Sciences, Agricultural Science or equivalent.

Salary initially £3,384 per annum.

Application forms and further particulars can be obtained from the Secretary, Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG.

Closing date for receipt of completed applications—April 7, 1979. 7(A)

UNIVERSITY OF STRATHCLYDE

Applications are invited for a

LECTURESHIP

in the DEPARTMENT OF MATHEMATICS

Preference will be given to those candidates who have a research interest in applied analysis. Applicants with other interests will, however, be considered.

Salary scale: £3,883 to £7,754 (under review) with placing according to qualifications and experience. Superannuation benefit.

Application forms and further particulars (quoting 13/79) and enclosing a self-addressed envelope (9in x 4in), may be obtained from the Academic Appointments Officer, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW, with whom applications should be lodged by March 21, 1979. 15(A)

UNIVERSITY OF STRATHCLYDE DEPARTMENT OF APPLIED MICROBIOLOGY PROFESSOR IN APPLIED MICROBIOLOGY

Applications are invited from suitably qualified persons for full-time appointment to the Professorship in Applied Microbiology which will become vacant on September 30, 1979 on the retirement of Professor E. O. Morris.

The post will be remunerated within the Professorial range for universities with U.S.S. benefits.

Application forms and further particulars (quoting 12/79) may be obtained from the Registrar, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW, with whom applications must be lodged by April 6, 1979. 16(A)

GERMAN CANCER RESEARCH CENTER HEIDELBERG

Institute of Virus Research
(Prof Dr H. C. Kaerner)

A position is vacant for one year starting June 1, 1979 for a research assistant. Applicants are invited with experience on protein biochemistry and virology. This appointment will carry a salary on scale of BAT. For further details please contact German Cancer Research Center, Administration, Im Neuenheimer Feld 280, 6900 Heidelberg. 53(A)

POSTDOCTORAL POSITION

Biochemist, Bio-inorganic Chemist or Physical Chemist with interests in quantum biology. Research on spin-spin magnetic interactions in reactive coenzyme B₁₂. Experimental experience with metallo-enzymes or coenzymes preferred, and interests in quantum chemical methods will be helpful. Salary \$12,000. Renewable. Write: Dr R. E. Coffman, Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, U.S.A. The University of Iowa is an equal opportunity and affirmative action employer.

51(A)

UNIVERSITY OF KENT AT CANTERBURY RESEARCH ASSISTANT IN MICROBIOLOGY

Applications are invited for the post of Research Assistant to work on a project supported by the Agricultural Research Council concerning the breakdown of cellulose in soil, particularly as it applies to the spatial relationships between microorganisms, enzymes, substrates and soil particulates, under the supervision of Dr R. G. Burns. The appointment is tenable for three years and a suitably-qualified applicant would have the opportunity of registering for a Ph.D. degree. Applicants must have, or expect to obtain, a first or upper second class honours degree in microbiology, biochemistry or a closely-related subject. Salary range £3,384 to £3,883. Particulars and application forms may be obtained from the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH, to whom completed applications should be returned by April 6, 1979. Please quote ref. A20/79. 8(A)

INSTITUTE OF CANCER RESEARCH: TECHNICIAN required at the Chester Beatty Research Institute, Fulham Road, London, SW3 to assist in studies on the development of systems for the delivery of toxic agents to specific targets. Candidates should possess an H.N.C., a university degree or equivalent in biochemistry. Experience in column chromatography and electrophoresis would be an advantage. The salary will accord with M.R.C. scales in the range £3,261 to £4,680 per annum plus £354 per annum London Allowance. Apply in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref. 301/B/60. 17(A)

New Zealand UNIVERSITY OF CANTERBURY Christchurch

Applications are invited for the following position:

LECTURER IN ENVIRONMENTAL SCIENCES (RESOURCE MANAGEMENT)

Preference will be given to applicants who hold a higher degree in resource economics, have varied and practical experience in resource management and have a commitment to teaching.

Applications close on **April 30, 1979**. The salary for Lecturers is on a scale from NZ\$11,894 to NZ\$14,615 per annum.

Particulars, including information on travel and removal allowances, study leave, housing and superannuation, may be obtained from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. 63(A)

Department of Biochemistry TWO POSTDOCTORAL RESEARCH ASSISTANTSHIPS

Applications are invited for two S.R.C. supported Postdoctoral Research Assistantships (Grade 1A) to participate in research programmes concerned with NMR studies in biological systems.

1. **One person appointed** will join a group under the direction of Dr I. D. Campbell and Sir Rex Richards and will be involved in developing the application of a 470 MHz NMR instrument to a variety of biochemical problems of interest to the Oxford Enzyme Group. This position is for two years and preference will be given to a candidate with a background in physics or physical chemistry with some interest in NMR techniques.

2. **The second appointment** is for three years. The person appointed will join the group under the direction of Drs G. K. Radda and D. G. Gadian concerned with studies of perfused organs (heart, kidney and muscle) by NMR and biochemical techniques. Persons with a background in physiology, biochemistry or chemistry will all be considered.

Both positions are available from October 1, 1979 and are on a salary scale of £3,883 to £4,882 per annum, dependent on age and qualifications, with U.S.S. superannuation benefits.

Applications should be sent to the Administrator, Department of Biochemistry, South Parks Road, Oxford OX1 3QU before March 31, 1979 from whom further particulars may be obtained. 36(P)

UNIVERSITY OF AUCKLAND DEPARTMENT OF CELL BIOLOGY POSTDOCTORAL FELLOWSHIP

The Molecular Genetics group in the Department of Cell Biology is studying plasmid DNA replication mutants, and has a position available for studies on plasmid replication proteins. Experience in enzyme purification and polyacrylamide gel electrophoresis techniques would be an advantage but consideration will be given to applicants with other, relevant, backgrounds. The position is for one year, starting on or after May 1, 1979. The salary will be \$8,400, and a return air fare is provided. Further particulars are available from Professor P. L. Bergquist or Dr H. E. D. Lane, Department of Cell Biology, University of Auckland, Private Bag, Auckland, New Zealand, to whom applications, together with a curriculum vitae and the names of two referees, should be addressed. 32(E)

QUEEN MARY COLLEGE University of London CHEMISTRY DEPARTMENT Applications are invited for a POSTDOCTORAL RESEARCH FELLOWSHIP

for work with Professor R. Bonnett (in collaborations with St Mary's Hospital Medical School) on the development of tetrapyrrole photo-sensitisers. The project would suit an organic chemist with experience in heterocyclic synthesis, but an interest in biochemistry and photochemistry would be an advantage. Starting date as soon as possible.

Salary in range £4,359 to £4,858 per annum (under review, including London Allowance).

Please apply by letter giving age, qualifications, experience and names of two referees to The Registrar (N), Queen Mary College, Mile End Road, London E1 4NS. 62(E)

UNIVERSITY OF ALBERTA, Department of Biochemistry. Postdoctoral Fellowship for recent graduate at a salary of \$12,200 (first year), \$13,350 (second year) to do research on the biological control of glycogen metabolism and/or structure-function relationships in phosphorylase. Write Dr N. B. Madsen, Department of Biochemistry, University of Alberta, Edmonton, Canada T6G 2H7. 38(E)

HOWE SENIOR RESEARCH FELLOWSHIP

Applications are invited by the Council of the Royal Society for the Howe Senior Research Fellowship, established from the Mr and Mrs R. L. Howe Bequest for the promotion and encouragement of scientific research. On this occasion applications are restricted to those working in the fields of fundamental or applied biological science. The appointment will be tenable in any appropriate university department or research institution in the United Kingdom approved by the Council of the Royal Society. Candidates, who must be citizens of the United Kingdom or the Commonwealth, must supply the usual personal details and give two referees. Testimonials will not be considered. The subject of the proposed research and where it would be done, together with the name of the head of the department, whose consent should first be obtained, must be stated.

The appointment, which will be subject to the Society's general regulations governing research appointments, will be tenable for five years from October 1, 1979 (or another date to be arranged) and may be renewable for successive five-year periods until the end of the academic year in which the holder becomes 65. Applicants should preferably be under 45 on October 1, 1979. The stipend scale will be £8,031 to £252 to £8,787 to £249 to £9,036 per annum, and the point of entry will be determined by Council. Superannuation benefits will be provided. Some provision for research expenses will be available.

Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1W 5AG, and returned by April 30, 1979. 26(E)

UNIVERSITY OF DURHAM DEPARTMENT OF PHYSICS

Applications are invited from graduates, preferably with Ph.D. in the field of High Energy Astrophysics, for a

POSTDOCTORAL SENIOR RESEARCH ASSISTANTSHIP

tenable for three years from as soon as possible. The successful candidate will join a research group working in experimental GAMMA RAY ASTRONOMY and should have some experience with balloon borne experiments. Salary on National Research Range 1A £3,883 to £6,555 (under review) plus superannuation.

Applications (three copies) naming three referees should be sent by March 26, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. 9956(P)

ST PETER'S COLLEGE OXFORD JUNIOR RESEARCH FELLOWSHIP IN MEDICAL SCIENCES

St Peter's College, Oxford, proposes to make an appointment from October 1, 1979 to a Sidney Perry Junior Research Fellowship in Medical Science. The Fellowship, which is for research in clinical or non-clinical medical science, is open to non-medical as well as to medical graduates, and will be tenable for a period of two years and renewable for a third.

Salary £3,000 per annum. Further particulars available from the Master, St Peter's College, Oxford. Closing date for applications April 27, 1979. 89(E)

RESEARCH POSTS AT CAMBRIDGE

Applications are invited for two postdoctoral research positions in the Department of Applied Mathematics and Theoretical Physics, University of Cambridge, Silver Street, Cambridge (Tel: 0223 51645): (1) C.E.G.B. Fellowship in Fluid Mechanics, for theoretical or experimental research in an area of interest to C.E.G.B., tenure 3 to 5 years from October 1; (2) Research Associateship, for work on ocean acoustics with Dr B. Uscinski, tenure 3 years, in the first instance, from an early date. Further information available from Head of Department. 75(E)

FELLOWSHIPS & ASSISTANTSHIPS

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF ANATOMY POSTDOCTORAL RESEARCH FELLOWSHIP

This post is for a study of the expression of myosin genes during early development and under pathological conditions, using monoclonal antisera. It is supported for three years by the M.R.C. and we would like to appoint as soon as possible. This is a chance to be in on the development of a unique combination of experimental techniques and to have the satisfaction of using them to tackle problems of fundamental scientific and clinical importance. The team is multidisciplinary and includes excellent technical backup. Salary is on the Research Fellow IA scale (£3,883 to £6,555) plus superannuation.

Your application should include a curriculum vitae, details of research experience and the names of two referees. Send it to the Assistant Registrar, Faculty of Medicine, University of Birmingham, Birmingham B15 2TJ by April 13, 1979 quoting reference RF/ANT/179 or talk to Dr S. Salmon on (021) 472 1301 ext. 2093. 81(E)

JUNIOR RESEARCH FELLOWSHIP IN CHEMISTRY

The College proposes, if a suitable candidate presents himself or herself, to elect a Junior Research Fellow in Chemistry for a period of three years as from October 1, 1979. The Fellowship is open to men and women pursuing research in any branch of Chemistry, and the Fellow elected may, with his or her consent, be assigned some share in the tutorial work of the College (provided that the total teaching involved shall not exceed six hours per week). Candidates are normally required to be under the age of 30 on January 1 in the year of election (ie 1979).

The stipend of a Junior Research Fellow is currently £3,325 per annum with superannuation, but this is now under review. In addition, the Fellow will be assigned rooms in College free of charge, and be provided with free meals. If the Fellow is married, a housing allowance of £350 may be payable in lieu of rooms.

Applications should reach the College Secretary, from whom further details may be obtained, not later than April 10, 1979.

Balliol College, Oxford OX1 3BJ. Oxford 49601. 85(E)

**BRITISH DIABETIC ASSOCIATION
LAWRENCE RESEARCH FELLOWSHIP**

SUBJECT: DIABETES MELLITUS

ELIGIBILITY: Open only to UK residents. Candidates must be appropriately qualified in medicine or science. **VALUE:** Appropriate point on NHS or university scale up to registrar (or equivalent university) grade. **TENABLE:** In any approved institution in UK, for 2 years. **OTHER INFORMATION:** Awarded for independent research. **NUMBER:** Two offered, normally every two years. (Offered for 1979/80.) **PARTICULARS:** Available from Secretary-General, British Diabetic Association, 10 Queen Anne Street, London W1M 0BD. Tel: 01-323 1531.

CLOSING DATE: April 18, 1979.

29(E)

**THE OPEN
UNIVERSITY**

Faculty of
Science



**Research Assistantships
in Chemistry**

Applications are invited for full-time Research Assistantships in Chemistry, tenable from 1st October 1979 or before for three years, to work in the following subject areas:

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Applicants should possess a good honours degree in chemistry or an equivalent qualification. Salary will be within the range £3,384 to £3,883 p.a. on the Research Assistant 1B scale.

Application forms and further particulars are available from The Recruitment Office (JD3813/4), The Open University, P.O. Box 75, Walton Hall, Milton Keynes, MK7 6AL or telephone Milton Keynes 63404; there is a 24 hour answering service on 63868

Closing date for applications:

April. 20 1(P).

**UNIVERSITY OF HULL
DEPARTMENT OF
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Applications are invited for three research fellowships, which are immediately available, for a period of up to three years.

Department of Applied Physics

1. A research fellow is required to measure the frequency characteristics of the current generation of pulsed O₂ lasers and to devise ways of improving their frequency stability using techniques such as hybridisation and injection.

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Department of Applied Physics/Physics
3. A research fellow is required to investigate the use of ion implantation for fabricating fast, photo-voltaic, infrared detectors in materials such as HgCdTe.

These fellowships are suitable for graduates in Physics, Applied Physics, Electronic Engineering with at least three years' research experience in relevant fields.

Salary: The starting point will be within the scale £3,883 to £4,382 per annum (under review).

Applications should give full details of education, qualifications and research experience, together with the names of two referees, and should be sent by March 31, 1979 to Dr E. L. Thomas, Department of Applied Physics, The University of Hull, Hull HU6 7RX from whom further details may be obtained. 23(E)

**UNIVERSITY OF
STRATHCLYDE
PROJECT MASS—TEAM
RESEARCH IN MARINE
TECHNOLOGY**

Applications are invited from suitably-qualified graduates in Metallurgy (with an interest in Welding) or from Postgraduates in Welding Technology for a RESEARCH ASSISTANTSHIP in the DEPARTMENT OF METALLURGY to join an existing multi-disciplinary research team involving six departments in the University to work on the challenging problems associated with underwater maintenance activities. The appointee will be required to research in Underwater Welding problems in relation to offshore maintenance activities.

Project MASS has been initiated by the University of Strathclyde as a positive contribution to underwater maintenance research and is supported by a major grant from the Science Research Council. The work has the active backing of the Marine Industry.

Appointment to commence as soon as possible and initially until September 30, 1980 with the exception of extension subject to the agreement of both parties.

Commencing salary on Range 1A (£3,883 to £6,555) of the national salary structure for research and analogous staff, depending on age, qualifications and experience. Superannuation benefit.

Application forms and further particulars (quoting R10/79) may be obtained from the Academic Appointments Officer, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW, with whom applications must be lodged. 14(P)

**UNIVERSITY OF
STRATHCLYDE**

Applications are invited for the following research posts in the DEPARTMENT OF PURE AND APPLIED CHEMISTRY, available in October, 1979 in biologically oriented ORGANIC CHEMISTRY.

1. A POSTDOCTORAL RESEARCH FELLOWSHIP financed by S.R.C. for a period of two years to research on the Scope and Mechanism of Selective Hydroxylation by Alkyl-substituted Chelates of Iron.

2. A POSTDOCTORAL RESEARCH ASSISTANT financed by M.R.C. for a period of three years to research on the Synthesis and Evaluation of Potential GABA-mimetic Substances.

Appointments on Range 1A of the national salary structure for research and analogous staff, with a commencing salary on the scale £3,883 to £4,382 per annum with placing according to qualifications and experience. Superannuation benefit. Appointments will commence from October 1, 1979 or as soon as possible thereafter.

Written applications (quoting R9/79) with names and addresses of two referees should be submitted to Dr C. J. Suckling, Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow G1 1XL, from whom further information can be obtained. 13(E)

STUDENTSHIPS

**UNIVERSITY OF
STRATHCLYDE**

DEPARTMENT OF
PURE AND APPLIED
CHEMISTRY

**RESEARCH IN
ORGANIC CHEMISTRY**

Applications are invited for the following research post in biologically oriented organic chemistry available in October 1979.

Potential Chemotherapeutic Agents based upon Latent Irreversible Inhibitors or Dehydrogenases. S.R.C. C.A.S.E. Studentship in collaboration with Wellcome Research Laboratories.

Applications with names and addresses of two referees should be submitted to Dr C. J. Suckling, Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow G1 1XL, from whom further information may be obtained. 12(F)

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NATURE

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**Pliocene footprint
in Tanzania**

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Cover picture

A dual trail of hominid footprints from the Pliocene. These prints, from the Laetoli Beds in Tanzania suggest a fully-upright, bipedal and free-standing gait. Leakey and Hay report a large variety of Pliocene footprints on page 317.

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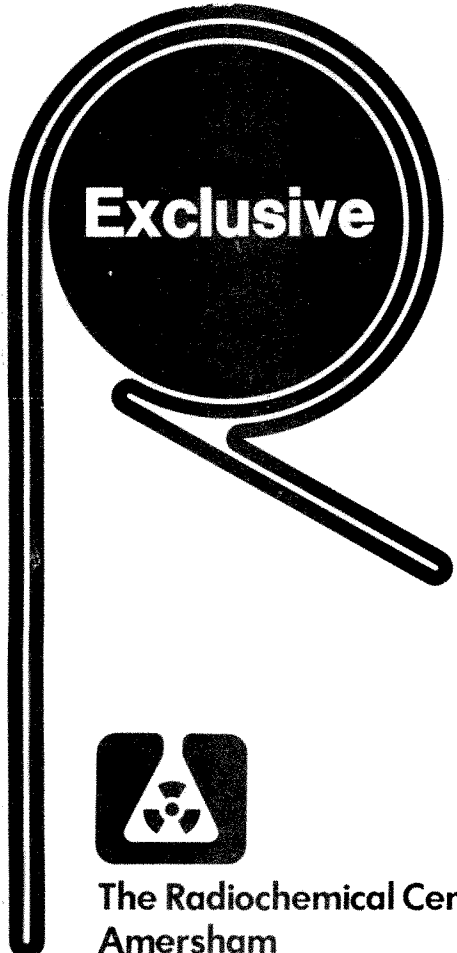
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Further information concerning editorial policy can be obtained from the Editorial Office: Stazione Zoologica, Villa Comunale, 80121 Naples, Italy. Publisher: Leo S. Olschki, P.O.B. 66, 50100 Florence, Italy, to whom also requests concerning back issues should be addressed.

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nature

22 March 1979

Chemical warfare: rearmament or disarmament?

THIS past week a party of diplomats from a wide range of countries has been flying around Britain looking at the demolition of a chemical weapons factory in Cornwall and inspecting the Birmingham premises of a manufacturer who handles phosphorus compounds. This somewhat unusual exercise is part of an Anglo-German effort to put the problems of chemical disarmament in clearer context. But despite occasional flurries of activity, indeed despite draft treaties tabled with the Conference of the Committee on Disarmament, there is as yet nothing like unanimity amongst nations on what sort of agreement would be acceptable, and there are substantial pressures, most notably in the United States, for chemical rearmament.

Biological weapons were effectively banned by a 1972 convention in which all major states agreed 'never in any circumstances to develop, produce, stockpile or otherwise acquire or retain microbial or other biological agents, or toxins . . .'. Additionally the Geneva Protocol of 1925, also widely adhered to now, prohibits the use in war of 'asphyxiating, poisonous or other gases' although this protocol does not close the door on research and development, production or stockpiling of chemical weapons against possible first-use by another nation. It is this lack of prohibition on 'defensive' chemical weaponry which has allowed the development of mustard gas, hydrogen cyanide and nerve agents, particularly sarin, soman and VX, together with the means of delivery. It is known that the Soviet Union, France and the United States maintain stocks of chemical weapons; whether any other nation, particularly in the Middle East, possesses them is unclear. However, material captured during the 1973 Middle-East War showed that the Soviet Union was prepared to supply allies with the wherewithal to fight in a toxic environment.

Since then the United States has been taking serious stock of its ability to cope with a chemical warfare attack and, if necessary, to retaliate in kind. Binary weapons, comprised of two harmless constituents which mix in the missile only after launch, are being discussed seriously (*Nature*, June 15, 1978, page 481). And this past year two important documents have been published: a plea for the US government to give chemical warfare much more attention ('The Neglected Threat of Chemical Warfare, by A. M. Hoeber and J. D. Douglass, in *International Security*, Vol. 3, No. 1) and the proceedings of a conference which looked at the wide spectrum of possibilities for responding to Soviet activity, in particular by arms control measures (*Chemical Weapons and Chemical Arms Control*, Matthew Meselson, ed., Carnegie Endowment, 11 Dupont Circle, Washington, DC).

The most likely venue for any chemical attack would be central Europe, and the most likely target would be small pockets of soldiers, or military installations. A chemical strike against an airfield might be particularly effective. There are clear similarities between chemical and tactical nuclear weapons, but, of course, an obvious distinction is

that once the nuclear road had been taken in a war the possibilities of escalation would be almost infinite.

This poses a dilemma: if the Soviet Union were to lead with a chemical attack, would NATO respond in kind or would it use tactical nuclear weapons? There is a fair amount of disagreement about the answer. The United States maintains large stockpiles (almost all back home at present) and is busy equipping its soldiers with new protective clothing; its army would undoubtedly like to have the capability of responding in kind. On the other hand West Germany does not store chemical weapons and does not train personnel to use them. Britain too does not maintain retaliatory capability, relying on nuclear deterrence, but also stresses the value of anti-chemical defences (having been successful in selling protective clothing to her allies).

There is even disagreement about the size of the Soviet threat. Hoeber and Douglass speak of Soviet superiority over the United States being of 'two or three orders of magnitude', making chemical warfare, even uncoupled from nuclear attack, 'a major contingency for which the Soviet Union plans'. On the other James Leonard, an arms controller, speaks of a *de facto* chemical disarmament in Europe and says he has never been satisfied that there was a significant Soviet capability to wage a chemical war, whilst Julian Perry Robinson, of Sussex, asserts that the threat of Soviet chemical weapons 'can at most be marginal'. Faced with a diversity of assessments (based, it must be said, on desperately little hard data) and faced with an alleged aversion, even by military men, to contemplating the use of such unchivalrous means of warfare, is it possible to neutralise the chemical weapons threat, not by pressing on with bigger and better stockpiles, or by improving protective clothing, but by arms control measures?

The military man will say: yes, but first have a strong retaliation capability as a bargaining chip; on the other hand the arms controller might even propose unilateral measures of disarmament to build confidence. Between nations, however, the sticking point is the difficulty in achieving any satisfactory degree of verification. The Soviet Union's favoured brand of monitoring is self-monitoring. Western proposals are much tougher on international safeguards, such as on-site inspection. A resolution of these differences does not seem imminent.

In the meantime, what does Britain do? At present her initiatives seem to be in promoting chemical defence and arms control. But this supposes that retaliation by nuclear weapons will be effective and acceptable. Could Britain start work on chemical offensive weapons again? Not under a Labour government, but might a Conservative government sanction modest activities? We simply don't know. Public discussion of chemical warfare is just about non-existent in Britain; presumably in the restricted circles of ministers, generals and top civil servants some people are thinking hard about options. It is time the debate was widened. □

Einstein: disagreement delays publication of collected works

David Dickson reports on the circumstances that have delayed the publication of Albert Einstein's collected writings, which fill 28 file drawers at Princeton University

PLANS to produce a multi-volume edition of Albert Einstein's published and unpublished writings are virtually at a standstill following the failure of the trustees of Einstein's estate, the Princeton University Press, and the project's editor to agree on mutually acceptable terms under which the editorial work can proceed.

Already a project funded by the National Science Foundation, whose initial purpose was to prepare the papers for publication, has had to be revised to the more modest goal of indexing and preparing duplicate copies of the papers. These are kept at the Institute for Advanced Studies in Princeton, where Einstein worked after coming to the USA.

There are no plans at present for the NSF to provide further support for the project after the current contract of the editor of the collected works, Professor John Stachel, expires on 14 July. Nor has the university press announced how it plans to pursue the project after this date.

The trustees of Einstein's estate, who have never refused access to the papers by "reputable scholars", have taken great care to ensure that the editorial work is carried out in a way that they feel would have met his approval. (Dr Otto Nathan, one of the two trustees, has expressed strong disapproval of the National Academy of Science's plan to erect a statue of Einstein in Washington, since he claims this is explicitly against the physicist's wishes).

However a number of physicists and historians of science, many of whom are reluctant to be quoted for fear of exacerbating the current situation, are concerned that the trustees have interpreted their responsibilities so rigidly as to restrict a full appreciation of Einstein's contribution to twentieth century science and thought.

Dr Paul Foreman, for example, organiser of a current exhibition on Einstein at the Smithsonian Museum in Washington, has expressed his regret that the trustees have refused, as a matter he claims of "unbending policy", to loan the exhibition letters and photographs from the estate.

Albert Einstein was a prolific writer on both scientific and non-scientific issues. During his lifetime he published 274 scientific papers and 332 papers of

a general content; these, and a large quantity of unpublished material, form the archives which currently fill 28 file drawers at Princeton University.

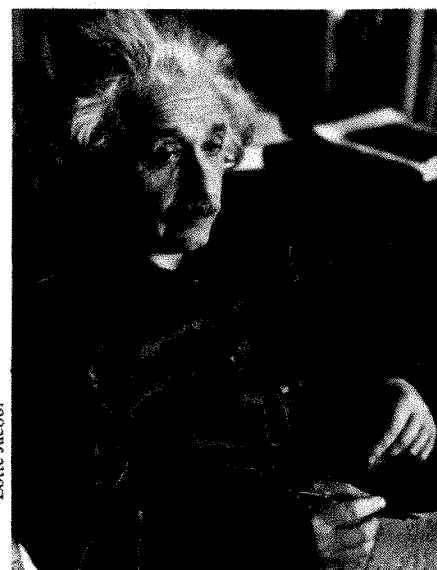
His correspondents range from scientists such as Erwin Schrodinger, Max Planck, Wolfgang Pauli and Marie Curie, to contemporaries in other fields, such as Sigmund Freud, Bertrand Russell, Franklin Roosevelt and George Bernard Shaw. And some of the letters, particularly to physicists, suggest further avenues of research which Einstein himself was unable to follow.

On his death in 1955, the literary rights to his papers passed to a trust, of which the two trustees are Helen Dukas, his secretary from 1928 to 1955, and Dr Otto Nathan, an economist and close friend who, like Einstein, came to the US as a refugee from Europe. (Under the terms of the will, the beneficiaries of the trust are Miss Dugas and Margot Einstein, his stepdaughter; eventually the estate will pass to the Hebrew University in Jerusalem).

Negotiations over the publication of the collected works began within weeks of Einstein's death. In 1971, the trustees reached an agreement with the Princeton University Press, which had expressed great interest in the project from the very start. And in 1976 the trustees and the press appointed Professor John Stachel, a physicist on leave from Boston University who has recently co-edited selected papers of Leon Rosenfeld, to be the editor.

In the same year, the university press submitted a grant proposal to the National Science Foundation, requesting \$787,300 over a five-year period to support editorial preparation for the multivolume project. This, they suggested, would be a "fitting monument" to Einstein, and would include all his published and unpublished writings in the original languages, with English translations of some of the more important works.

The foundation agreed to provide initial support to allow Professor Stachel to plan the editorial work, and to establish the general principles of editing the whole project. The foundation awarded \$80,000 towards this in 1977 and 1978. But difficulties soon arose in agreeing on a work plan that would be acceptable both to the



Prolific writer: 606 papers published

trustees and to Princeton University press.

Dr Nathan insisted, for example, that only the editor be allowed to handle the original documents, thus making it necessary for Dr Stachel to do much of the work that would otherwise have been done by research assistants. And other areas of disagreement arose over how the editorial work on the documents should proceed.

It soon became clear to the participants that it would be impossible to proceed with the preliminary editorial work plan that had been developed under the NSF grant, or to complete negotiations with the foundation on the initial five-year grant proposal (even though the plan had been endorsed by an editorial advisory board which met to discuss the situation early in 1978).

At the prompting of NSF's review panel, the university press and Professor Stachel approached the foundation again to support the completion of work on a duplicate archive and an index, to make sure that "when the time comes, the editing of the Einstein papers can proceed without the past year-and-a-half being wasted".

The trustees and the press wrote in a joint statement that a duplicate archive was "absolutely necessary for the continuation of the editorial work if we are able to proceed after 14 July 1979," but added that "if the work of editing *The Writings of Albert Einstein* should cease, one copy of the entire duplicate archive will be kept intact and unmarked so that a new editor, when appointed, could use it with confidence".

Work is now almost completed on the material for the duplicate archive being carried out under an NSF grant which runs out on 30 June; so, too, is the preparation of an index to all the

papers in the archives, the total number of which may be as high as 60,000. And Professor Stachel has also been able to contact many of those who were in correspondence with Einstein, asking for their comments on copies of letters which have been sent to them.

But there the matter rests. Mr Herbert Bailey, head of the university press, admits that the project has had "problems", and does not accept that it has gone into abeyance. "We have a lot of reasons to think that the project, on which we are as keen as we have always been, will go forward" he said last week; however he declined to reveal how the project will proceed after the NSF grant money, and Professor Stachel's contract, run out in the summer.

Dr Nathan is equally adamant that claims that the trustees have placed unworkable restrictions on how the editing work should proceed are "nonsense", and that the trustees "are being maligned" if such charges are being made.

He says that since a few days after Einstein's death the estate has been attempting to get a complete edition of the physicist's writings published, but that he has been "blocked again and again", and that he feels that "at the moment we do not know where we are going".

Historians of science are hoping that the stalemate can be resolved by some means (there are rumours of possible litigation, but these cannot be confirmed). No one disputes that the

collected works, when they are eventually published, will be a major contribution to twentieth century scholarship, helping to provide a far greater understanding of Einstein's role in the development of both modern science and the modern world; but few do not express frustration at the current impasse.

"When completed it will be—or should be—what historians and philosophers of science, and others, have been waiting for since Einstein died", Dr Jeremy Bernstein, professor of physics at Stevens Institute of Technology, and author of a biography of Einstein, wrote recently. "Not all this material is of great interest . . . but what matters is that it should be available." □

FDA scientists dispute ethics of testing laetrile

A SCIENTIST with the Food and Drug Administration has filed a "citizen's petition" against his agency in an attempt to prevent it from giving approval to clinical trials which the National Cancer Institute is planning to carry out on the controversial anticancer drug laetrile.

Dr Robert Young, a clinical oncologist with the FDA, echoing concerns which have already been expressed and debated within the NCI, claims that such trials would be in violation of accepted ethical codes since no firm scientific evidence yet exists that the drug has any beneficial effects.

Both the FDA and the US medical establishment maintain stiff opposition to the use of the drug, which is derived from apricot kernels and is currently taken by thousands of cancer patients in the nation. The agency has issued notices warning that laetrile is "worthless" and that its cyanide content in particular is potentially dangerous.

However despite this uncompromising stand, FDA commissioner Dr Donald Kennedy, previously professor of human biology at Stanford University, has come under pressure from those who argue that although previous studies do not provide any conclusive evidence that laetrile has a beneficial effect, neither is there sufficient evidence to rule out such an effect.

The clinical trials have been proposed by the NCI following a retrospective study of laetrile patients which the institute, having frequently rejected the idea in the past, agreed to carry out last year.

Over 90 cases claiming evidence of a beneficial effect of laetrile treatment were submitted in response to a nationwide appeal, and a detailed study of 67

of these by a panel of oncologists revealed six patients who had received laetrile as their primary treatment, and were judged to have had a clinical response.

Announcing the results of the study last autumn, the NCI said that these would not normally be sufficient to suggest that a drug merit clinical tests over other candidate drugs. However it added that "because of widespread public use and interest in laetrile, the NCI will proceed with plans to evaluate the drug".

Subsequently the institute filed an "investigational new drug application" (IND) with the FDA in December. And although the agency has taken considerably longer than normal to approve the application—there have been detailed discussions with the NCI both about the protocol for the experiment, as well as on technical details about the quality, stability and purity of the substance to be tested—this is expected to be granted within a few weeks.

Dr Young's objection to granting approval is that "there is not adequate scientific data that would justify the

test of the drug in human beings." In an interview with *Nature* last week he cited in particular the lack of evidence, accepted by the NCI, of the drug's efficacy in animal tests; and even the retrospective tests, he says, provides "no scientific data from which you can responsibly conclude that it was the taking of the drug that mitigated the disease".

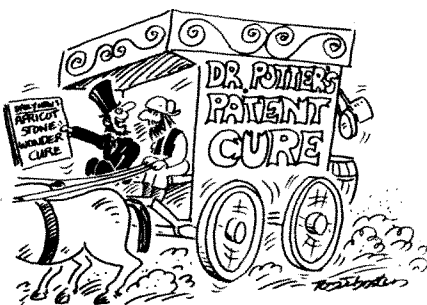
In his petition, which Dr Young says reflects the feelings of a number of laboratory staff in the agency, he requests that laetrile should not be exempted from the provision of the Food and Drug Act, which require reports "adequate to justify the proposed clinical testing". And he criticises the agency for using a "sociopolitical argument", rather than a solid scientific case, to justify the trials.

If the FDA approves the cancer institute's IND, a six-month study will be carried out involving between 150 and 300 patients with different types of cancer; participation will be restricted to patients in whom all known therapies have been attempted.

Meanwhile the Supreme Court has agreed to rule on whether the FDA has the right to bar the use of laetrile as an anti-cancer drug, following the decision of a lower court that the need to show a drug is both "safe" and "effective" before being approved for sale or distribution does not apply to drugs used by terminally ill cancer patients.

In appealing the decision to the Supreme Court, the FDA has claimed that this ruling seriously limits its power to protect the public from unsafe and ineffective drugs, adding that it would be "virtually impossible to restrict the use of laetrile to the terminally ill".

David Dickson



"To the nearest apricot farm!"

Space shuttle: costs and technical delays raise political doubts

AFTER almost 10 years of preparation, technical delays and uncertainties are turning the final development stages of the National Aeronautics and Space Administration's Space Shuttle programme into something of a political cliffhanger.

A succession of last minute technical problems, although not unusual in a project as complex as developing a re-usable launch vehicle able to place and subsequently retrieve objects from an earth orbit, are receiving wide press attention and close public scrutiny. And Congress has already told NASA that, in a year of tight budgets, the agency cannot be guaranteed of receiving the extra \$185 million it has requested for covering the extra work involved. Current estimates of the cost are 8-9% higher than originally estimated in 1971. If granted, the additional money would take the total science budget for the financial year 1979 above a previously agreed ceiling.

If NASA does get the extra money, it is confident that Space Shuttle can be in orbit by the end of the year. Even though it publicly admits that the chances of meeting the planned 9 November launch date are "relatively low", it has criticised the National Academy of Sciences which suggested a launch date of April 1980, as being "unduly pessimistic".

But if NASA does not get the money—and the request is being closely scrutinised by the Senate budget committee, in particular since money for space research comes under the heading of discretionary funding—then the agency could be in deep trouble.

Under such circumstances, according to associate administrator John F. Yardley, the first manned orbital flight would be delayed an additional six to nine months. The delivery of production orbiters would be delayed up to one year, and this could seriously disrupt planned launch schedules.

Among the projects affected could be the European Space Agency's Spacelab, whose first launch is currently scheduled for August 1981. Initially planned for launching at the end of this year, cost increases for Spacelab—some made necessary by changes in the design of the Space Shuttle—have already exceeded the limit above which participating nations are entitled, if they wish, to withdraw.

Since being given the go-ahead by President Nixon in 1972, the Space Shuttle programme has had to run a continuous gauntlet of congressional criticism. Among past critics is Vice-



President Walter Mondale, today silent on the issue, but in 1972 claiming that the Shuttle was many times worse than supersonic transport "in the magnitude of its cost, in the folly of its concept, and thus in the damage to the country."

Such opposition has kept the research and development programme to a skeletal minimum, a far cry from the lavish spending on the Apollo moon programmes. Mr Yardley complains that requirements carried out in 1974, 1975 and 1976 have taken their toll, and that "further reductions in programme content are not considered possible".

Funding pressure has forced hard managerial decisions on NASA, and some of these were commented on by an *ad hoc* review group set up last year at the request of a senate committee by the National Academy of Sciences under the chairmanship of Dr Eugene E. Covert, professor of aeronautics and astronautics at Massachusetts Institute of Technology.

In an initial report last March, the committee pointed out that unlike previous programmes, the development strategy for the Shuttle programme as devised by NASA was "success oriented", and based on the assumption that each piece of development hardware and its subsequent verification test would succeed at the first attempt.

"It may be that this strategy has already saved time and money However, if or when malfunctions occur during the testing of the operational engine, new hardware may need to be designed, constructed and retrofitted, causing delays in the programme", the report said.

Such indeed has turned out to be the case. There have been problems, for example, both with the turbine blades and with the heat exchanger in the Shuttle engine—being developed by Rocketdyne—the latter being a component which the review group singled out for concern. And the temporary tiles which broke loose during a flight last week and damaged some of the fitting had been made necessary by un-

expected delays in fitting the permanent tiles—of which there are 5,000, each requiring 650 man-hours of work.

There have also been problems with weight. Design changes in the launcher vehicle have caused problems for, for example, the Galileo orbiter probe to Jupiter, planned for launch in 1982, but on present specifications now requiring 109% of the engine's theoretical thrust.

The big question now is how smoothly the rest of the pre-launch development programme goes. NASA admits that its present schedule is a "success oriented schedule with a minimum of contingency time", but adds that "it has been our experience in the past that such success schedules result in minimum cost and earliest actual dates",—in other words that even if unrealistic, planning for sooner rather than later helps to keep the pressure up.

The Covert committee is less optimistic. "We think it highly likely that there will be more problems before the first orbital flight", one member of the committee said last week. This sanguine perspective led the committee last month to recommend next spring as a more realistic date. NASA says that this is based merely on "intuitive feeling".

Meanwhile NASA officials are busy trying to persuade Congress to give them the extra money needed to let the shuttle programme proceed to its conclusion as planned. The supplemental request is being examined for Senator Muskie by the General Accounting Office of Congress. The 1980 request for NASA contains no new space science starts largely because of the pressure on Shuttle funding.

There is already talk of NASA possibly needing a supplemental to its 1980 budget, even though it may mean the agency coming back to Congress cap in hand. Ten years ago, the money would have been provided eagerly—today no-one is taking bets on the outcome.

David Dickson

West Germany: eyeball to eyeball over nuclear fuel reprocessing

NEXT week, critics and supporters of nuclear power will be meeting in Hanover, West Germany to analyse proposals to build a nuclear fuel reprocessing and waste disposal facility at a site 5 km from the East German border near the village of Gorleben in Lower Saxony. The forum for the analysis is to be a week of public hearings organised by the state government of Lower Saxony.

Twenty-five expert nuclear critics, 20 of them from outside West Germany, will face 25 nuclear supporters in a 'round table' discussion: an audience of 200 will listen in. The ostensible aim of the hearings is to inform the public and to help Ernst Albrecht, premier of the Lower Saxony government, decide whether or not to grant a licence for construction of the facility.

Already there are fears that the hearings will get off to a bad start. Some of the critics are suspicious of the state government's motivation for holding them: they think that pressure from the federal government means that construction of the plant is inevitable. They are also critical of the delay in deciding on a format for the hearings and of the lack of public information on them. Three weeks before them, the names of the 25 pro-nuclear experts had not been released, although the anti-nuclear names had been known for a long time.

Criticism has also been levelled at the way in which the 200 places in the audience have been allocated. Apparently about 100 places are to be taken by state and government authorities, 20 by representatives of the nuclear industry, 30 by the press, only three by members of local environmentalist groups and one by a representative of the district authority. The remainder are for members of parliament of Lower Saxony. With no spare places for interested members of the public and only 30 press places, it is argued, the hearings are not truly public.

The proposal under discussion is for the construction of a plant capable of reprocessing all of West Germany's spent nuclear fuel. It would include spent fuel storage ponds and plants for fabricating mixed oxide fuel, uranium finishing, vitrifying high-level radioactive waste as well as reprocessing 1,400 tonnes of spent fuel per year. Final disposal of all radioactive waste would also be on the site in salt domes a few hundred metres below the surface. The total cost is estimated at DM12,000 million.

The nuclear industry, amongst others, expects the decision reached by the Lower Saxony government over the licensing of the plant to be crucial to the future of nuclear power in West Germany. In the summer of 1977, the federal government announced that no new reactors could be licensed until the problem of disposing of spent nuclear fuel—*Entsorgung*—had been solved. Since then, reprocessing has generally been assumed as the only way of satisfying that condition. Unless an alternative can be found (and the nuclear critics hope to show next week that there is one) a veto of the reprocessing plant is expected to mean an end to the expansion of nuclear power in West Germany.

Plans for the proposed facility, or *Entsorgungszentrum*, were first submitted to the Lower Saxony government in March 1977, by DWK, a company set up by the West German utilities specifically to build it. At the same time, plans for the underground waste storage facility were submitted by the federal Physical-Technical Institute. A 3,000-page safety report, which accompanied the two applications, was examined by the Commission for Reactor Safety and the Commission for Radiological Protection, two advisory bodies to the federal Ministry for the Interior. In October 1977, both bodies published their comments on the safety report saying that they found it reliable and acceptable. The federal government, believing that the disposal of spent nuclear fuel was under control, began to talk about expanding the nuclear programme.

Meanwhile, local people in Gorleben started to protest about the proposal. They felt that the two commissions had been biased in their assessments of the safety report. In addition, DWK had started to try to purchase land from local landowners by offering them a very high price if they sold by a certain date. If they missed the dealing, DWK threatened to start expropriations procedures to procure the land for a much lower price.

To date only 40% of the land has been sold. Of the remaining 60%, the vast majority is owned by one man—Count Bernstorff, a remnant of the German aristocracy and prominent figure in the local landowner association (which has been very active in organising the protest).

At the beginning of 1978, the landowners association began to put pressure on the Lower Saxony govern-



ment to set up a committee of independent nuclear experts to review the proposals. After approaching one or two foreign experts to gauge support for the idea, the association drafted a list of experts it wanted to sit on a review committee. After elections in June of last year, when local environmentalist groups took 18% of the votes in Gorleben and 3.9% in the surrounding area, the state government agreed to invite the experts to spend 30 days producing their own report on the proposals and safety report.

In September 1978, 20 expert nuclear critics from France, Norway, Sweden, the UK and the US met in Hanover for the first time. By the beginning of March they had prepared a 2,200-page report and had submitted it to the Lower Saxony Ministry of Social Affairs. The report consists of eight chapters each prepared by different experts. The first chapter is a general introduction, the second discusses routine emission of radiation and radiological protection, the third accidents, the fourth safeguarding of fissile materials, the fifth the technical problems of reprocessing, the sixth waste management, the seventh final disposal and the eighth decommissioning. Among those taking part in the review were Walt Patterson and Amory Lovins of Friends of the Earth, Frank Barnaby of the Stockholm International Peace Research Institute, Paul Sieghart of the International Commission of Jurists and Professor O. Lindstrom of the Royal Institute of Technology, Stockholm.

Although the members of the review panel started work last September, they did not receive their contracts until the end of December. Several of them claim that during discussion of the terms of the contract they had requested that the report be published before the public hearings. However, the contracts said that the report had to be withheld from publication until 10 July.

A significant achievement of this action has been to increase dissatisfaction with the way the review and hearings are being run. The state government is committed to take some decision on the future of the reprocessing plant in May or June, before

the July publication date. Some feel this defeats the object of the review as a public information exercise.

More generally, there are feelings that the government would not seriously contemplate calling a halt to the plant whatever the outcome of the hearings. The formal licensing procedure was begun in 1977, and although it has now almost ground to a halt,

it is thought that the Lower Saxony government would be unlikely to stop it abruptly. Even if licensing were now to proceed as fast as possible, it would be 1981 before it were completed. What seems most likely, therefore, is a 'vague' decision.

Whilst the critics and nuclear industry are discussing the Gorleben proposals next week, the people of Gorle-

ben, who do not have an invitation to the hearings, will be taking part in a demonstration march from Gorleben to Hanover. The Lower Saxony government is expecting the affair to be violent for it is reported that 100 prisoners have been transferred from local gaols to make way for the recalcitrant villagers.

Judy Redfearn

US panel approves research on *in vitro* fertilisation

THE US is likely to end a three-year moratorium on research on the *in vitro* fertilisation of human ova following the conclusions of a federal advisory board that there are no ethical objections to such research being carried out on federal funds.

The ethics advisory board was established last year by Mr Joseph A. Califano Jr, Secretary of Health Education and Welfare, to advise him on whether work along the lines of the British research workers Mr Patrick Steptoe and Dr Robert Edwards should be permitted to proceed in the US. On reaching its conclusion, however, the board declined to say whether it felt that such research should in fact receive federal support.

Having heard evidence from scientists, religious leaders, anti-abortion groups, infertile couples and others, the board recommended that the work should be permitted provided that a number of requirements were met. These include:

- that research be aimed primarily at establishing the safety and efficacy of procedures for *in vitro* fertilisation;

- that no embryo be sustained in laboratory conditions for more than 14 days;

- that both the participants in the research—which would include clinical trials—and the public be informed if there is any evidence that such research is having ill-effects.

Although these provisions, and the decision to recommend that the moratorium be lifted, were agreed unanimously by the 13-member board of lawyers, scientists and others, there was less agreement on the priority which *in vitro* fertilisation should be given as a research topic.

Several members suggested that it should come low on the national priority list, and one suggested that the government should not fund any such research. Others disagreed, claiming that the research might soon become a "high priority" if there is wide demand from doctors to adopt the new techniques.

The board therefore agreed not to recommend whether HEW should fund such research, accepting that in addition to ethical concerns, this also

raised scientific, economic and political considerations.

Funds for 'test-tube baby' research have been held up for more than three years in anticipation of the advisory board's report, although the members of the board were only announced last summer. Congress placed a total ban on all research involving fetuses in 1974, and although this ban was lifted one year later, the position with respect to *in vitro* fertilisation has remained uncertain.

The only application at present lodged with HEW for carrying out such research is from Dr Pierre Soupart of Vanderbilt University, who has been waiting since 1975. The ethics board suggests that Dr Soupart's application now be forwarded to the National Institutes of Health for scientific review.

The board also recommended that the secretary of HEW should encourage, and even possibly provide financial support for, the development of a model law outlining the legal status of children born as a result of *in vitro* fertilisation. □

Spanish universities unhappy with new autonomy law

SPAIN'S universities have long sought autonomy from government control—and they should achieve it as one of the first actions of Spain's new government. But it may not prove to be the prize once dreamed of.

The first duty of the new government will be to debate the laws outlined in Spain's new constitution; and one of these is a law granting university autonomy: but was the law well-framed?

Government officials, political parties, and university professors are unanimous in their belief that the universities need rapid attention: they suffer from overcrowding, graduate unemployment, poor teaching, and lack of research. Furthermore the universities do not have the power to distribute their own funds, to select their staff or students, or to organise their own teaching. Every decision has to pass through the central Ministry of Education.

Even before the Constitution was written and approved, the Ministry of Education and Science was preparing a

draft of a law for autonomy, and—despite strong criticism—it was forced through the Council of Ministers and entered parliament the day before its dissolution.

The law as it has been published is a long and in certain aspects detailed text. It deals at length with the structure of university management (Senate, Rector, etc.) and with the teaching staff; but it deals only briefly with the administrative staff and research.

Two changes introduced by the new law are financial autonomy, and the separation of academic and economic authorities. The law also allows the existence of private universities, which would interest certain religious societies.

But many points in the law have received criticism from the universities—such as the system of selection of teaching staff, which remains almost untouched. For example, an aspirant to full professorship (Catedrático) must spend three years as associate professor (Adjunto), a regulation which bars many non-academic professionals

whose expertise might benefit a university.

The law also describes—in detail—the composition of the various university institutions, making it difficult to apply to any new university, while some fundamental problems—such as the role and funding of research, the selection of students, and increases in funding or staff, are left untouched.

The parliament formed after the elections held on 1 March is almost identical to the previous one, and the centrist party will most likely be charged with the task of forming the new government. It will probably back the existing law as created by the Ministry.

In the universities it is hoped that the law may produce some positive changes, but it is regretted that a general debate and a deep rethinking of the function of the universities in Spain has not taken place. If the law is approved as it is, it may be felt by the teaching community as a lost opportunity.

Pedro Puigdoménech

Bulgarian industry tackles environment

BULGARIAN industry is involved in a crash programme of anti-pollution measures, according to Mr Vordan Tsvetanov, Deputy Minister of Machine Building, who visited Britain recently.

The visitors, who included the Minister of Machine-Building, Mr Toncho Chakarov, came to discuss increased trade and cooperation in internal combustion engines, hydraulics and electro-technical equipment. Although pollution problems did not enter specifically into these discussions, Mr Tsvetanov told *Nature* that Bulgaria was making "wide use" of the anti-pollution experience of other countries and was naturally very interested in possible cooperation "especially as far as production facilities are concerned".

According to Mr Tsvetanov, new industrial complexes in Bulgaria pose little threat to the environment. A special government body "has a decisive say" in all matters touching on potential hazards "as early as the planning stage", deciding on the siting of the plant and the anti-pollution measures and equipment to be used. The main problem, he said, is that of existing plants which do not meet the more stringent modern requirements; Bulgarian industry is developing very rapidly and, accordingly, "everything has to be brought up to standard as soon as possible".

His own ministry, said Mr Tsvetanov, was particularly concerned with the purification of gaseous and liquid effluent. There is a "very substantial investment" in anti-pollution equipment, he added, "approximately 5%" of all capital invested in industry. He stressed, moreover, that his ministry's work is only part of an over-all plan for environmental protection.

Asked whether Bulgaria had made any major innovations in conservation and anti-pollution measures, Mr Tsvetanov said that it was "very difficult to say" if Bulgaria had achieved any major breakthrough.

This was a surprisingly modest remark in view of the numerous successes announced in the Bulgarian media. During the past month, Bulgarian radio has described new closed-cycle effluent-free production processes for copper, zinc, lead and molybdenum developed by the Bulgarian Academy of Sciences, a "superior" new coagulant for use in water purification, likewise from the academy, and an "efficient" method of recovering mercury from effluent, developed by the Sofia Institute of Chemical Engineering.

Vera Rich



Tamarugo, a high-protein leguminous plant which survives Chile's Atacama desert. Research on species such as this could bring great benefits.

"A goldmine" for scientists in third world

"THE vast majority of agricultural research has been on north temperate species in north temperate research stations. This research has been done by the countries that need it least." Yet, 85% of all plant species grow in the tropics. This is "a goldmine of new material waiting to be worked", declared Arthur Bell, Botany Professor at King's College, London.

He was speaking in London Saturday at a symposium on "What kind of science for African Development?". The meeting is intended to be the first of a series run by the UK African Studies Association.

Bell also argued that research has concentrated on cereals, because they are more important in north temperate zones, whereas legumes are more important for developing countries, which are usually tropical. Legumes fix their own nitrogen, reducing the demand for chemical fertilisers, they are rich in protein, and they grow in the most inhospitable places.

Of the 17,000 species of legumes, most grow in the tropics. But few kinds of legumes are eaten, because most are toxic to man. Some have been bred over the centuries: lima or butter beans now contain less than 3% of the cyanide of their natural Javan ancestor.

He suggested that research should concentrate on already existing minor crop plants to make them more acceptable. "One can improve a plant in its own habitat with many fewer gene changes than by bringing a plant from far away in Rothamsted", UK experimental station.

One example, based on his own research, is the *Lathyrus* of which most species are toxic. (The common sweet pea is a *Lathyrus*). In India, various *Lathyrus* species are commonly used

for food, but if eaten in large amounts cause a paralysis of the legs known as Lathyrism. Researchers found that the toxin was oxalyl-L-lysine. Bell showed that it was easy to use high voltage electrophoresis to test for this acid, and that not all *Lathyrus* species contained it. In a paper in *Nature* more than 15 years ago, he suggested that India substitute non-toxic *Lathyrus* species.

He now admits that this was a naïve suggestion. The three commonly eaten toxic species grow well and were rich in protein. But a variation of his suggestion was adopted. Indian agricultural scientists used high voltage electrophoresis to find that there was great variation in the toxin within the species, and are now substituting less toxic varieties. Similar work has been done in Australia to make *Leucaena* less toxic to animals.

Bell's concern about the research bias toward the developed countries was picked up by other speakers. Indeed, the conference convener Paul Richards noted that although Bell's work was of direct use of developing countries, it was done in London rather than a developing country.

Barbara Harriss of the University of East Anglia, in a paper on the Sahel submitted to the symposium, pointed out that research funded by Western agencies tended to support particular policy aims. For example, the World Bank was looking for technological packages that the first world could export to the third. And aid donors tended to support government marketing and agricultural structures. Yet as a rule neither of these help the poorest peasants. Indeed, research has ignored what the peasants actually eat—their nutrition and preferences and what factors affect their food consumption.

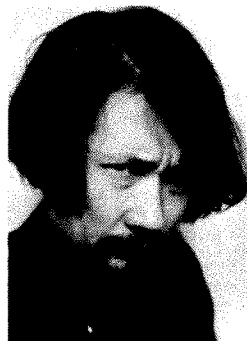
Joseph Hanlon

Joseph Hanlon is a development journalist specialising in third world science.

news in brief

Japanese physicist in UK fights deportation:

Dr Tetz Yoshimura is neither a political refugee nor a victim of economic or racial pressures—simply a mathematical physicist who wishes to pursue his researches unhampered by the materialistic rat-race of modern society. For this reason he left his native Japan in 1963, and, after holding a number of academic appointments in various countries came to the UK in 1971. Britain, however, is now proving a reluctant host, and Dr Yoshimura is fighting a deportation order.



After two years as a research fellow at the University of Essex and Kings College, London, Dr Yoshimura adopted a freelance life, concentrating on his research and supporting himself by freelance translation and the like. By drastically paring down expenditure on creature comforts, he claims that he can maintain himself on £12 per week, and that his average income is usually comfortably double this sum.

The Home Office, however, demand proof of a regular £100 per month before they will give him the papers necessary for permanent residence. Furthermore, since his original entry permit described him as a "student", his original application for permanent residence was rejected, apparently as a matter of routine.

Dr Yoshimura is an active researcher in axiomatic quantum field theory with papers published in 1978-79 in the *Physical Review*, *Journal of Mathematical Physics* and *Physics Letters*. This line of work is hardly one to engage the interest of the average civil servant. Nor, indeed, is his spartan lifestyle. Lord Avebury, the Liberal spokesman on immigrant problems, who is greatly interested in Dr Yoshimura's case told *Nature*: "The Home Office want everyone to fit into their procrustean rules. If you have rigid rules, there will always be cases where for reasons of humanity and common-sense, exceptions ought to be made. Dr Yoshimura's case is an example of this!"

US asked to reduce occupational radiation levels on basis of study of British dock-yard workers: A Washington lobby group has asked President Carter to implement a ten-fold reduction in the permissible exposure to radiation of US workers on the basis of a British study of nuclear dock workers which has demonstrated that low level radiation can result in a significant increase in chromosomal damage (*Nature*, 15 February, p. 531).

In the letter, Dr Sidney Wolfe and Alexander Harris of the Health Research Group claim that almost five thousand workers in US atomic power plants were exposed to the levels of radiation that, in the British study, induced a four-fold increase in the incidence of damaged chromosomes over a ten-year period.

According to the group, the total radiation exposure in US atomic power plants increased 23% between 1976 and 1977, while the number of operating plants increased by only 8%, from 39% to 42%. Furthermore the number of workers exposed to more than 0.5 rem increased by 25% over this period to a total of 16,712. This is the level to which permitted exposure levels have been suggested should be reduced by Dr Edward Radford, chairman of the National Academy of Sciences committee on the Biological Effects of Ionising Radiation, whose own report is to be published within the next few weeks.

● The Nuclear Regulatory Commission last week ordered the temporary shutdown of five East Coast nuclear power plants following evidence that a "simple arithmetical error" in a computer formula may have resulted in cooling system pipes being made six times too weak to withstand the effects of an earthquake.

GMAG notice on self-cloning will exempt certain experiments:

Following a meeting on March 16, GMAG are expected to release a statement within the next two weeks on their requirement for notification of so-called 'self-cloning' experiments. Unsurprisingly, they have apparently decided to 'exempt' experiments in which genes from *Escherichia coli* are cloned in *E. coli*, genes from *Bacillus subtilis* in *B. subtilis*, and yeast genes in yeast. Those wishing to carry out such experiments will still be required to inform GMAG of their intention and will be expected to carry them out under conditions of 'good microbiological practice'. Also under consideration are proposals to handle in a similar way work with certain rigorously characterised recombinant DNA clones that have been agreed to carry no possible hazard.

MRC taken to High Court: A further action has been started by solicitors for Dr Amiram Ur (21/28 December 1978) against the Medical Research Council. In addition to proceedings before the industrial tribunal for unfair dismissal a High Court writ served last week claims damages for breach of contract and wrongful dismissal, damages for losses due to negligence, breach of duty by the MRC in its conduct of Ur's application for tenured employment, and damages for "unlawful interference with the plaintiff's economic interests." The writ also names Professor I. M. Glynn of the Physiological Laboratory of Cambridge as co-defendant. Glynn was chairman of Ur's tenure committee.

Dr Amiram Ur's solicitors will now serve a statement of claim setting out in detail the allegations and the defendants will have to serve a defence. If the Court finds against the MRC damages could range from a payment of costs with a symbolic half penny award to the full amount claimed. Both the MRC and Professor Glynn had "no comment" to make on the action. Meanwhile the industrial tribunal reconvenes in London this week for another two weeks of hearings.

Geophysicists asked to write to Congress representatives to protect budget increases:

Members of the US geophysics community have been asked to spend six minutes working on letters to Congress representatives—urging them to support funds for basic science—for every hour spent preparing grant proposals, contracts and project justifications.

This suggestion of a commitment to "tithing" has been made by Professor Allan Cox, professor of geophysics at Stanford University and president of the American Geophysical Union. Writing in the union's newsletter, Professor Cox says that after several lean years of funding, the increase in funds for basic science promised by President Carter in his budget proposals for 1980 are necessary just to stay even.

"Moreover I would argue that to stay even we need to establish better communications with Congress", he says, pointing out that historically the basic research community—especially the academic part of it—has been "notoriously ineffective" in supporting its spokespersons in Washington. Such support is needed to protect the promised increases during the Congressional review process, he says.

According to Professor Cox, scientists are the only ones who can provide support for the administration's claim that their research is a good long-term national investment. "The pace of the budget process is such that a short letter now will be more useful than a long letter later."

Many members of the geophysics community were taken by surprise last year when Senator William Proxmire cut almost all funds for research into lunar samples brought back by the Apollo moon missions from the budget of the National Aeronautic and Space Administration.

US environmentalists provide some "good news" about energy: Adequate investment in energy saving techniques could allow the US economy to reduce its energy demand by 30-40%, and require only a 10-15% increase in energy usage over today's levels to maintain a healthy, expanding economy in the year 2000, according to a report published last week by the Council on Environmental Quality.

Techniques for increasing energy productivity include substituting heat pumps for electric resistance heating in homes, using greater insulation and more efficient appliances and machines in home and industry, and developing the use of total energy systems to generate heat and electricity simultaneously in commercial buildings, the report says.

"With a determined national effort to conserve energy, we can do well, indeed prosper, on much less energy than most people imagine," the chairman of CEQ, Mr Charles Warren, said on releasing the report, titled *"The Good News About Energy"*.

AAAS begins action against human rights violations in Argentina: The American Association for the Advancement of Science has decided to circulate widely its report and resolution on human rights violations in Argentina. The author of the report, Bruce Alan Kiernan, AAAS Human Rights Coordinator, observes that "Argentina continues systematically, in my view, to violate basic international standards of human rights: the right to security of person, to freedom from torture, to the right of trial and minimum standards of legal defense and appeal and humane standards of punishment". Of particular concern is the matter of disappeared persons, estimated by Kiernan to be between 20,000 and 30,000 people including many scientists—most of whom are believed dead. "40% of Argentine physicists have been kidnapped and some disciplines such as psychiatry no longer exist," says Kiernan. Senior advisors to President Videla admitted to Kiernan that many people in military custody were thrown out of helicopters, a tactic learned from American practice in Viet Nam. "I heard testimony of individual experiences in jails and detention centres and these accounts included descriptions of torture techniques that can only be described as horrendous." The AAAS resolution calls for scientific organisations to initiate on-site investigations in Argentina "on an urgent basis" and to release their results to their governments. The resolution also calls for the release and resettlement of falsely imprisoned Argentine scientists. The AAAS has asked the Human Rights Committee of the Organisation of American States to make a separate report on the condition of scientists. The AAAS Committee on Scientific Freedom and Responsibility regards the situation as "extremely bad" and has been meeting for the past week to consider among other things a call to scientists and scientific organisations to cut off all scientific exchanges to Argentina until the government restores human rights.

Friends of the Earth steps up campaign against Torness: The Torness Point nuclear reactor site in Scotland, the target for militant anti-nuclear demonstrations last year, is under new criticism from Friends of the Earth. On Wednesday, in what is described as "an eleventh hour effort to save us from yet another nuclear blunder" FOE

delivered a ten foot diameter millstone, the largest in Britain, to Bruce Millan, Secretary of State for Energy along with a copy of its report. FOE calls the government's outlay of £742m for Torness "an economic disaster twice the magnitude of Concorde". The report details numerous deficiencies in the government's proposal including: inadequate costing (a further £200-300m will be needed for the distribution system); inadequate need projections (the Scottish Electricity Board had a 65% surplus above maximum demand last winter); inadequate design on the two previous advanced gas cooled reactors; and loss of jobs (in the local community). *Torness: Keep it Green*. By Michael Flood. Friends of the Earth, 9 Poland Street, London W1V 3DG. 85p.



Shirley Williams proposes short term contracts for lecturers: In a speech on 5 March at The Times Higher Education Supplement's Conference on Trends in Education into the 1990s, Shirley Williams, Secretary of State for Education and Science suggested that short term contracts for university and polytechnic lecturers may become a necessity. Basing her argument on demographic considerations,

Williams predicted that a current "hump" in student enrolment would peak in the universities in the mid-1980s. Half the number of lecturers required to teach this excess above "background" could then be expected to be employed on short term appointments. In addition, Williams feels that short term contracts could provide room for young lecturers and for dismissal of older lecturers who had not fulfilled their "early promise". Laurie Sapper, General Secretary of the Association of University Teachers said that Williams' proposal was "just a disguised way of saying that the universities should employ cheap labour".

US report says no solution yet to nuclear waste disposal: An interagency working group has told President Carter that there is still insufficient evidence to assess the feasibility of safely disposing of nuclear waste in underground repositories, and that such evidence could only be acquired by investigating the suitability of particular sites.

The group, which was set up by President Carter last year, said that current knowledge was adequate only to identify possible storage sites for further investigations. It also said that conservative engineering judgment, as well as providing multiple barriers between radioactive material and the environment, could reduce some of the risks involved and compensate for some of the uncertainty.

West German fast breeder report criticised: Dr Ulrich Steger, chief spokesman of the SPD working group for research and technology in the *Bundestag*, has fiercely criticised a recent report on the future of the fast breeder in Germany. The report, by Dr August Eitz of the Schnell-Brüter-Kernkraftwerksgesellschaft (SBK), deplores the delay in licensing the prototype SNR-300 fast breeder at Kalkar on the Dutch-German border, minimises the risk of accidents, and argues that nuclear proliferation is unavoidable except by international surveillance. The use of nuclear energy leads logically to fast breeders to conserve uranium stocks, Eitz says. But according to Steger, Eitz—in order to put the breeder in a favourable light—has offered "only stale growth-fetishism". According to Steger anyone looking for the reasons why nuclear energy is in a difficult situation would find some of them in Eitz's speech.

correspondence

INFCE looks for technical and institutional solutions

SIR,—I would like to make some corrections to the report entitled "INFCE halfway through" (8 March, page 112). Although the idea of INFCE was first suggested at the London Summit, it was set up in Washington in October 1977 and involves not only the Western industrialised nations but the Soviet Union, several of the East European States and quite a number of developing countries. It is incidentally organised into eight working groups, not twelve.

At the time INFCE started, the proliferation risks of reprocessing were indeed judged differently by on the one hand the United States and Canada and on the other all the major nuclear power users (not simply the UK as your article suggests). These countries all felt that the case for reprocessing must take into account environmental and economic factors; and that as spent fuel contained plutonium, spent fuel storage also carried proliferation risks. However, as a result of studies in INFCE it is now apparent that some intermediate fuel storage is necessary because it is most unlikely that world reprocessing capacity will be sufficient to deal with all spent fuel arising for perhaps another 20 years, and some spent fuel will therefore have to be stored for a considerable time.

Your article is correct in implying that there is no simple technical fix to the proliferation risks arising from the growing spread of civil nuclear power. But we are also engaged in an examination of institutional measures to counter the dangers of proliferation. The International Atomic Energy Agency has already shown itself to be an eminently valuable institution, especially its safeguards system, and it may well be that internationally controlled plutonium storage and internationally controlled spent fuel storage can be important further additions to world confidence.

Yours faithfully,

SIR HERMANN BONDI

Chief Scientist,
Department of Energy, London.

Defence R&D and SRC budgets are not comparable

SIR,—I was interested to see your editorial (1 March, page 1). Most members of the scientific community in the UK will agree with you that the "difficult question"—the minimum rate at which expenditure by the research councils should grow so as to sustain the health of research in the country—should be addressed with urgency. It is good to see a lead from the ABRC, for it is not only sustenance that is at stake; there is a vital need to repair the attritions of the past decade or more.

I regret, however, that you should have confused this important problem with a less than adequate examination of the relativities of government R&D resource allocation. The defence R&D budget and SRC expenditure set out to do quite different things: SRC remains, I believe, dedicated to the promotion of research of 'timeliness and promise', and the research councils accept responsibility—as they must—for the nurturing of the research function in universities, polytechnics and their own laboratories. Less than 1% of defence R&D expenditure can be identified with the support of innovative research: a matter of considerable concern to myself and my colleagues here. As the 1979 Defence White Paper—or the more recent Review of Cmnd 5046—indicates, more than 70% is invested in British industry (including, for instance, the important area of electronics) and is dedicated to the short term task of developing new equipment essential to the Armed Forces and national defence. Thus about 25% of the R&D budget finds its way to the defence establishments, and this too is overwhelmingly dedicated to short and medium term development work.

Moreover, I fear that your misunderstanding of the equivalence (or lack of it) between defence R&D and the SRC budgets reads over to your remarks on the competence of personnel in our R&D establishments. It will be clear from the above that the criteria for judging the ability and performance of scientists and engineers in defence laboratories must, in general, be quite different from those applied to my colleagues in the universities. This is not to say, of course, that MOD scientists inhabit a world isolated from the wider scientific community. We are always looking for ways of promoting contact; but communication is already frequent and fruitful, much of it through the Defence Scientific Advisory Council, which monitors very carefully the programmes of all R&D laboratories and which submits independent annual reports to the Secretary of State. There exist other forms of "peer review", internal and external, both for programmes and for individual scientists and engineers.

There may, in fact, be less complacency in defence about the need to repair the innovative function than there is elsewhere. The R&D establishments have suffered considerable erosion for a number of years, during which their work has been directed away from the long term innovative task. It would be unfair to attack them now on the basis of a spurious comparison of their contribution to microbiology or solid state physics, or whatever, with those emerging from research sponsored by the research councils. The latter are conducted for different purposes, and are different in kind.

Yours faithfully,

RONALD MASON

Chief Scientific Adviser,
Ministry of Defence, London.

Pitldown stains

SIR,—In your correspondence columns (22 February, page 596) Dr L. B. Halstead recalls that he refuted my suggestion (*The Times*, 7 November 1978) that Sollas may have used potassium bichromate to harden fossils to which he applied his serial sectioning technique. I wrote a letter in reply, but it was not published owing to the temporary cessation of *The Times*. I accept the validity of the evidence which Halstead found in the records of the University Museum, Oxford, showing that all the fossils to which Sollas applied serial sectioning were hard by nature, and therefore required no artificial hardening. The truth of the matter is that after the passage of some 70 years there is nothing to be gained by speculating on the purpose for which Sollas ordered the packet of bichromate crystals. As I pointed out in my letter to *The Times* of 7 November, Charles Dawson himself stated that he soaked some of the Pitldown bones in a solution of potassium bichromate, allegedly for the purpose of hardening them. Only by special pleading could anyone argue that this chemical was ordered by Sollas for delivery to him in Oxford, after which (one would have to suppose) it was forwarded to Dawson in Lewes with instructions as to how it might be used. These are the thoughts of a Jabberwocky! Dawson would have had no difficulty in obtaining potassium bichromate from a pharmacy in Sussex.

Researches by Dr (now Sir Frank) Claringbull and Dr M. H. Hey at the British Museum (Natural History) (Further Contributions to the Solution of the Pitldown Problem, *Bulletin of the British Museum (Nat. Hist.)*, Geology series, vol 2, no 6 (1955)) showed that the Pitldown cranial bones had been artificially stained by treatment with a solution of an iron sulphate. The additional use of a bichromate solution can now be understood because it would serve to aid the oxidation of a ferrous iron salt and thus the production of rusty staining. So the bones, which were originally of pale biscuit colour, in the end matched the dark reddish-brown Pitldown gravel in which they were supposed to have been found.

Dawson is known to have carried out experiments on the staining of bones and flints. He may well have required advice from somebody with greater knowledge of chemistry than he himself possessed, but it is unlikely that he would have sought help from the Oxford Professor of Geology. He was in a position to obtain chemical advice locally: in his first paper on Pitldown, read to the Geological Society in December 1912, Dawson thanks Mr S. A. Woodhead, Public Analyst of East Sussex, for chemically testing a piece of the Pitldown cranium and reporting that no organic matter could be detected in it (recent analyses of the cranial bones showed protein residues represented by 0.2–1.9% nitrogen).

Yours faithfully,

KENNETH P. OAKLEY

Oxford, UK,

news and views

Aetiology and genetic control of natural scrapie

from Richard H. Kimberlin

SCRAPIE disease, a neurological disease found naturally in sheep, is generally considered to be caused by a transmissible agent. Recently, however, Parry¹, on the basis of studies of the incidence of natural scrapie in commercial flocks has reiterated his long-held belief^{2,3} that scrapie is a disease solely of genetic origin, controlled by an autosomal recessive gene. Although Parry does not dispute the existence of a transmissible scrapie agent he believes it to be "formed *de novo* in each affected animal by the metabolic activity of the natural recessive gene", and considers it irrelevant to the disease, or at least not involved in its spread.

In view of his comments it may be useful to re-examine the role of the transmissible agent in scrapie. Many scientists in the field consider that scrapie is caused by a transmissible agent, with host genetic factors exerting some kind of control on the development of clinical disease. In my opinion this interpretation is not only supported strongly by available data but it does not conflict in any way with Parry's interesting results.

Evidence for a transmissible agent

There is no doubt that the injection of brain homogenates from affected sheep will transmit the disease to other sheep after long incubation periods⁴. The transmissible agent can be filtered^{5,6} and experimentally passaged in sheep to extremely high dilutions of original inoculum⁷, thus showing the presence of a replicating, virus-like agent. Experimental forms of scrapie have been produced in many species⁸, mice in particular, and it is notable that mouse-passaged agent can also be injected into sheep to produce scrapie^{9,10}. This evidence alone provides a substantial reason for concluding that the transmissible agent causes the natural disease.

Many different strains of scrapie agent have been isolated in mice from natural sources⁴ and it is now known that some of these strains undergo mutation on serial passage¹¹. Strain

variation and mutation are the expected attributes of a pathogenic microbial agent. There is a serious problem with Parry's suggestion "that the scrapie TSEPA [transmissible spongiform encephalopathic agent] is formed *de novo* in each affected animal by the metabolic activity of the natural recessive gene" because one has to explain how a single host gene can produce biologically different strains of agent, sometimes in the same animal⁴.

However, the main issue raised by Parry is not the existence of the scrapie agent but whether or not it is naturally infectious. I would like to summarise three major studies that strongly indicate natural spread of agent from one animal to another.

In one experiment¹², 11 Blackface sheep were close-penned from birth until 3 to 4 years of age with a succession of naturally affected sheep. Four died of intercurrent disease before they reached an age at which scrapie first occurs, but three of the remaining seven developed scrapie. In the same study¹², 17 goats were exposed under similar conditions and 10 of these developed scrapie. As considerable care was taken to ensure that the exposed animals were obtained from scrapie-free stock the high proportion of scrapie cases in Blackface

sheep (43%) and in goats (59%) strongly suggests contagious spread.

In a second experiment¹³, the exposed Scottish Blackface sheep came from a well documented flock in which no cases of scrapie had been seen in over 18,000 sheep during 13 years. However, when some of these Blackface sheep were kept in life long contact with a Suffolk flock with a high incidence of natural scrapie, and managed under normal commercial conditions, 21 (28%) of the exposed sheep developed scrapie. It is extremely difficult to explain such a high level of scrapie in the Blackface sheep except by contact spread of agent.

Even more impressive data have been obtained from the USA with Angora, Nubian and Toggenburg goats and Rambouillet, Targhee, Hampshire and Suffolk sheep, all from scrapie-free flocks. It should be noted that no field cases of scrapie have been found in Angora goats or in Rambouillet or Targhee sheep in the USA. Yet when these and the other animals (total 140) were put in contact with scrapie-affected flocks, five developed the disease¹⁴. These 140 animals were bred within their own groups in the scrapie environment and produced 358 progeny that survived for at least two years. Of these, 9% (27%) developed scrapie. The figures for individual

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2. Parry, H. B. *Nature* **185**, 441 (1960).
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17. Dickinson, A. G., Young, G. B. & Renwick, C. C. *ibid.*
18. Dickinson, A. G., Stamp, J. T., Renwick, C. C. & Rennie, J. C. J. *Comp. Path.* **78**, 313 (1968).
19. Nussbaum, R. E., Henderson, W. M., Pattison, I. H., Elceck, N. V. & Davies, D. C. *Res. vet. Sci.* **18**, 49 (1975).
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breeds ranged from 14 to 39% for sheep and from 26 to 61% in goats¹⁴. Again, the conclusion that contact spread of scrapie occurred seems inescapable.

Genetic control

The evidence that scrapie is an infectious disease is clearly incompatible with a disease of genetic origin. However there is no reason why genetic factors in the host should not control the process of infection or the development of disease once infection has taken place and this was the conclusion drawn from other studies of genetic factors in natural scrapie^{4,13}.

There are three major studies showing that the probability of a lamb's developing scrapie depends more on the eventual scrapie status of the ewe than of the ram¹³⁻¹⁵. These observations of maternal bias indicate transmission of infectious agent from ewe to lamb and there is evidence from other sources that agent is probably passed to the embryo^{4,16,17}. However the extent of this maternal bias will depend on many factors, not least of which is the level of contagious spread of agent, which if relatively high may obscure maternal transmission. Hence the fact that Parry finds no evidence for maternal transmission of agent may simply mean that in some situations it is not of paramount importance.

The only other area where there are conflicting data concerns the nature of the genetic control exerted by the host on the development of disease. Parry's results¹ indicate that the natural disease in his flocks is controlled by an autosomal recessive gene whereas another study¹³ showed a more complex and unresolved genetic control of natural scrapie. Two further investigations^{18,19} demonstrated that the development of clinical scrapie in Cheviot and Herdwick sheep following injection with the SSBP/1 source of scrapie agent was mainly controlled by a single gene with the fully dominant allele conferring susceptibility. A broadly similar genetic control of the natural disease in these two flocks is indicated by the subsequent appearance of natural scrapie which is largely confined to the susceptible lines^{4,20}. This diversity of findings cannot easily be explained at the moment but the following observations may be relevant.

First, given that scrapie is caused by an infectious agent, then variables such as the strain of agent, the relative importance of maternal and contagious spread of infection and the age of the animal when exposed, could conceivably involve different genetic components.

Second, the genetic studies of experimental scrapie in Cheviot¹⁸ and Herdwick¹⁹ sheep used the same source

of scrapie agent and gave similar results. However it has now been shown that the response of genetically selected lines of Cheviot sheep to an agent designated CH1641 is quite different from that found with SSBP/1 (refs 4,10). This means that the genetic control of experimental sheep scrapie varies with the strain of agent.

The third point follows from this and concerns the interaction seen between different strains of mouse-passaged scrapie agent and the murine gene *sinc* which controls incubation period^{21,22}. The gene has two alleles, *s7* and *p7*. Many agent strains (for example) ME7, 22C, 139A) have a shorter incubation time in mice homozygous for *sinc*^{s7} than in *sinc*^{p7} mice, but agents such as 22A have the reverse properties. When comparisons are made of incubation periods in *s7p7* mice, the interactions between agents and the alleles of the *sinc* gene range from no dominance or partial dominance of one or other allele, to full dominance and even positive overdominance in which the incubation period is longer in the heterozygote than in either of the homozygotes^{21,22}.

If this level of complexity applies to the natural disease then it would be quite wrong to expect the genetic control of scrapie to be the same in all situations and the fact that different studies have given different results may be a reflection of this. □

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Conversion of mouse fibroblasts into adipocytes

from Franklin H. Portugal

AN important new avenue of research on lipid metabolism and obesity has been opened up through the conversion of mouse fibroblasts into adipocytes under defined culture conditions. In 1974, Green and Kehinde (*Cell* **1**, 113; 1974) observed a few cells with lipid droplets in resting cultures of 3T3 mouse fibroblasts. They suggested that these observations indicated the establishment of clonal lines of fibroblasts that have a high frequency of conversion into adipocytes when their growth is arrested. Because low concentrations of bromodeoxyuridine block this conversion without affecting other specialised cell functions such as collagen synthesis, the conversion process

involves differentiation of the fibroblast.

In contrast to the fibroblast, the adipocyte acquires the signet ring appearance of adipose cells, accumulates triglycerides, and loses the ability to revert to the growing state. Insulin, L-adrenaline, N⁶, O^{2'}-dibutyryl cyclic AMP and isoproterenol alter the incorporation of precursors into triglycerides in the adipocytes. During the conversion from fibroblast to adipocyte, there is a 40-50-fold increase in the incorporation of ¹⁴C-acetate into triglycerides. This increase is paralleled by an increase in lipogenic enzyme activities including acetyl-CoA carboxylase, ATP-citrate lyase, and fatty acid synthetase (Mackall *et al. J. biol. Chem.* **251**, 6462; 1976). For one of these enzymes, acetyl-CoA carboxylase, the increase in activity is due to an increase in the cell enzyme level as determined by measuring the change in immunotitratable enzyme. Lipoprotein lipase, a lipolytic enzyme, also increases rapidly during the post-confluent period (Eckel *et al. Biochem. biophys. Res. Commun.* **79**, 720; 1977). The conversion of 3T3 fibroblasts to adipocytes is also sensitive to agents that affect cyclic nucleotide levels, such as prostaglandin F₂ and 1-methyl-3-isobutyl xanthine (Russell & Ho *Proc. natn. Acad. Sci. U.S.A.* **73**, 4516; 1976). Adenylate cyclase of adipocytes compared with the enzyme in preadipocytes is markedly stimulated by isoproterenol and shows a response to adrenocorticotrophic hormone (Rubin *et al. J. biol. Chem.* **252**, 3554; 1977). Near-physiological levels of insulin stimulate the conversion of glucose into carbon dioxide and lipid only in the adipocyte.

Serum factors also seem to play a part in the conversion of fibroblasts. Recently, Kuri-Harcuch *et al. (Cell* **14**, 53; 1978) reported that in serum-supplemented medium, 3T3 fibroblasts can still apparently differentiate into adipocytes even in the absence of biotin which is essential for fatty acid synthesis. They became spherical, the activity of NAD-linked glycerolphosphate dehydrogenase increased 300-fold and the activity of lipoprotein lipase increased 50-fold. But without biotin the cells were unable to increase the rate of acetate incorporation into triglycerides.

Subsequently Kuri-Harcuch and Green (*Proc. natn. Acad. Sci. U.S.A.* **75**, 6107; 1978) have identified a non-lipid adipogenic factor in serum. In the presence of this factor, cells grown to confluence become spherical, increase their malic enzyme and glycerolphosphate acyltransferase activities, and accumulate triglycerides, whereas in its absence none of this occurs. Fetal calf serum is much richer in this factor than calf serum or the other

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mammalian sera that were tested. This serum factor differs from biotin in that it regulates cell shape and differentiation of cell enzymes.

Négrei *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75**, 6054; 1978) now report that they have established a clonal line of adipose cells from epididymal fat pad cells of genetically obese C57BL/6J *ob/ob* mice. At the resting stage, these converted fibroblasts accumulate triglycerides. Differentiation of these cells is accompanied by increased lipoprotein lipase, acid:CoA ligase, and acyl-CoA:diglyceride acyltransferase activity. They respond to hormones including adrenaline, corticotropin, and insulin. Bromodeoxyuridine almost totally prevents the presumed induction of the triglyceride-pathway enzymes. Conversion of *ob* 17 cells to adipocytes apparently differs in several respects from the conversion of 3T3 cells obtained from the Swiss mouse embryo. These differences may include sensitivity to insulin and biotin as well as lower values for fatty acid biosynthesis.

The fact that these adipocyte cells in culture show many of the same characteristics as adipocytes *in vivo* suggests that they can be useful models

for understanding the factors that control obesity. Comparisons of adipocytes from genetically and non-genetically obese mice may help to clarify the genetic factors that regulate obesity. Furthermore, studies of lipid metabolism that are difficult with intact animals are now simplified. For example, the use of adipocytes in culture suggests that the appearance of lipoprotein lipase is not substrate-induced by the presence of serum triglycerides or lipoproteins. These cells are also important for distinguishing true lipogenic enzymes such as pyruvate carboxylase from lipogenic-type enzymes such as aldolase. An understanding of how fibroblasts are converted into adipocytes should also increase our understanding of the mechanisms that control cell differentiation. In addition, these cells can increase their number of insulin receptors whereas most systems show either no change in response to increased insulin levels or decrease their number of insulin receptors. Thus, the conversion of fibroblasts into adipocytes will also be useful for determining the factors controlling receptor development. □

mean atomic weight is effectively constant to within 2%, and Shankland (*Geophys. Surv.* **3**, 69; 1977) has suggested on the basis of a study of chemical and crystallographic properties of supposed mantle materials that "the mantle appears to be chemically homogeneous". Above 700 km the mean atomic weight is apparently more variable because of the occurrence of phase transitions at several depths; but Schubert and Turcotte (*J. geophys. Res.* **76**, 1424; 1971) have already shown that convection is not thereby prohibited. In summary, then, there appears to be no significant reason why convection should not occur anywhere in the mantle.

The case for whole-mantle convection as such was recently boosted by the detailed studies of Davies (*Geophys. J.* **49**, 459; 1977) who used the arguments quoted above, among others. And it has now been given further support by Elsasser *et al.* (*J. geophys. Res.* **84**, 147; 1979) who have set the scene for the decision that must ultimately be made between, on the one hand, convection down to 700 km only, and, on the other, convection down to the core-mantle interface. As Elsasser and his colleagues point out, the "entire question of mantle convection has lately entered a new stage" in view of the conclusion by Rochester *et al.* (*Geophys. J.* **43**, 561; 1975) that precession provides too little power to drive the core dynamo, which must therefore be driven either by radioactivity-induced thermal convection or by the progressive falling out of Fe and Ni at the boundary of the inner core.

It follows from this (cutting out many stages of the argument) that, if radioactive heat transfer in the mantle is small (as is widely supposed), there must be a hot thermal boundary layer at the base of the mantle, for the quantity of heat leaving the core will be greater than the mantle can dissipate by conduction alone. The build-up of heat that this implies will then result in the detaching and upward motion of parts of the boundary layer material, thereby setting up a system of motion in the mantle. From this starting point, and the deduction that the temperature at the core-mantle interface is $4,000 \pm 500$ K, Elsasser *et al.* go on to develop a model of whole-mantle convection that works in the sense that, for all its assumptions, it is consistent with global heat flow, plate sizes, plate speeds, mantle viscosity and certain seismic data.

The picture thus conjured up is one of extreme simplicity—whole-mantle convection moves continents and sea floors and leads to surface volcanism whilst whole-(outer)core convection generates the lower boundary condi-

Mantle convection: shallow or deep?

from Peter J. Smith

A QUESTION that has exercised the minds of geophysicists on and off for more than 40 years now, is that of the vertical scale of convection in the mantle, assuming that convection takes place there at all. Thoughts on this subject may be divided conveniently into three periods. During phase 1 the pioneers (Vening, Meinesz, Holmes, Runcorn for example) evidently took it for granted that convection would be mantle deep, a view that prevailed generally (at least among the supporters of continental drift) up to the early 1960s. But the rapidly growing appreciation of the special properties of the asthenosphere—in particular its relatively low viscosity—gradually convinced many, but not all, that convection limited to the upper mantle was a viable alternative. Not that convection was regarded as necessarily being limited to the asthenosphere itself—that is, to a maximum depth of about 250 km. Some took the view that 700 km or so should be regarded as a lower limit because earthquakes occur to that depth, largely in subduction zones where lithosphere is descending. Either way, however, it

did not seem unreasonable to suppose that convection stopped well short of the core-mantle boundary at 2,900 km. That was phase 2.

Phase 3 began sometime during the past two or three years, marking a period of maturity during which both shallow and deep convection hypotheses must battle it out on more equal terms.

For example, by the late 1960s a number of geophysicists had concluded that below the asthenosphere the viscosity increases rapidly with depth in the mantle, thereby, it was suggested, making convection in the deeper mantle impossible. But ideas on that have now changed, largely as the result of the work of Cathles (*The Viscosity of the Earth's Mantle*, Princeton University Press, 1975) and Peltier (*Geophys. J.* **46**, 669; 1976) who have made convincing cases (by different routes) for a mantle viscosity more or less constant at about 10^{22} poises below the asthenosphere.

Another condition that would preclude whole-mantle convection would be a variation of gross chemical composition with depth. But Watt *et al.* (*Geology* **3**, 91; 1975) have concluded from free oscillation data that between 700 km and 2,900 km in the mantle the

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tions for mantle convection as well as the geomagnetic field. Moreover, as volcanism has evidently been occurring and the geomagnetic field has evidently existed for at least 3×10^9 years and probably getting on for 4×10^9 years, the convective systems would appear to be almost as old as the Earth. As Elsasser and his coworkers concede, "this is in line with Tozer's often expressed view that since planets are convectively unstable and since the radioactive heat of their interiors can only be removed by convection (for example, Tozer *Phys. Earth Planet. Int.* 6, 182: 1972), most planets can be expected to convect most of the time".

Obviously an excellent case can be made for whole-mantle convection. Whether it actually occurs or not is more difficult to prove. □

Reproduction in flowering plants

from A. J. Richards

INTRODUCING the International Symposium on the Reproduction of Flowering Plants, held recently in New Zealand*, E. J. Godley (DSIR, Christchurch) discussed the special characteristics of the New Zealand flora. One question posed was whether the proportion of unisexual flowers found in New Zealand is as uncommon as has been previously supposed. Twelve per cent of the 1,800 species of indigenous flowering plants are dioecious (with stamens and pistils on different plants) compared with 2% of indigenous UK species. However, high frequencies of unisexual plants are common in tropical forests (as shown in Costa Rica recently by K. S. Bawa (University of Massachusetts)) and are found in all temperate countries south of the Equator. Dioecy is generally regarded as being a secondary phenomenon, hermaphrodite flowers being the primitive type in the flowering plants, so it seems unlikely that dioecy is a hang-over from the ancient southern continent of Gondwanaland. Perhaps the high level of variability and vigour engendered in outbreeders was favoured during the establishment phases of the southern land-masses, as they became increasingly isolated from the mainstream of flowering plant evolution to the north.

Godley also discussed the predominance of white and yellow flowers in

New Zealand, so well-known in the hebes, olearias and celmisias cultivated in the UK. He quoted Cockayne, stating that of attractive flowers, over 60% are predominantly white flowered in New Zealand (the comparable figure for the UK is 25%). Certainly, the experience of a casual visitor from Europe, faced with white gentians, white buttercups, white willowherbs and white forget-me-nots bears this out. Godley thinks that white flowers, mostly of an unspecialised nature, might also be representative of an ancient, isolated southern continent, and many anthecologists would agree that this simple syndrome ties in well with the paucity of pollinators that a land-mass, isolated so early, might be expected to demonstrate. Specialisation of flowers will only succeed if a rich flower-visiting fauna is present.

Until recently, gynodioecy, in which pistillate and hermaphrodite plants are mixed, was thought to be uncommon. A number of workers showed that it is much more frequent than previously realised. Theoretically, it is difficult to model a balanced gynodioecy; either the disadvantage to pistillate flowers of requiring cross-pollination for seed to set, and having fewer potential pollen sources; or the advantage of not bearing the reproductive cost of stamens, is likely to upset the balance. This is compounded by the tendency for male sterile genes to be carried cytoplasmically, and thus to be inherited maternally. It was suggested that in stable gynodioecious conditions, nuclear-controlled male fertility restorers help natural selection in the maintenance of a balance between male fertile and male sterile plants. Marianne Philipp (University of Copenhagen) provided some interesting data to show how this balance might operate selectively.

X. Delanny (University of Louvain) echoed the work of other speakers in showing just how cryptic gynodioecy can be. Most European species of thistle are gynodioecious, but the over-familiar creeping thistle, *Cirsium arvense*, is usually fully dioecious although morphologically the flowers appear hermaphrodite. Females show early abortion of pollen, despite normal looking anthers, and males have perfectly functional pistils, apart from the pollen-receptive papillae on the stigma, which fail to develop.

As in so many other sciences, reproductive genetics seems to be emerging with difficulty from a model-building phase, and there are still more ideas than facts. However, this is much less true with respect to recent work on the physiology of sexual fusion in flowering plants. Using technically outstanding light and electron micrographs

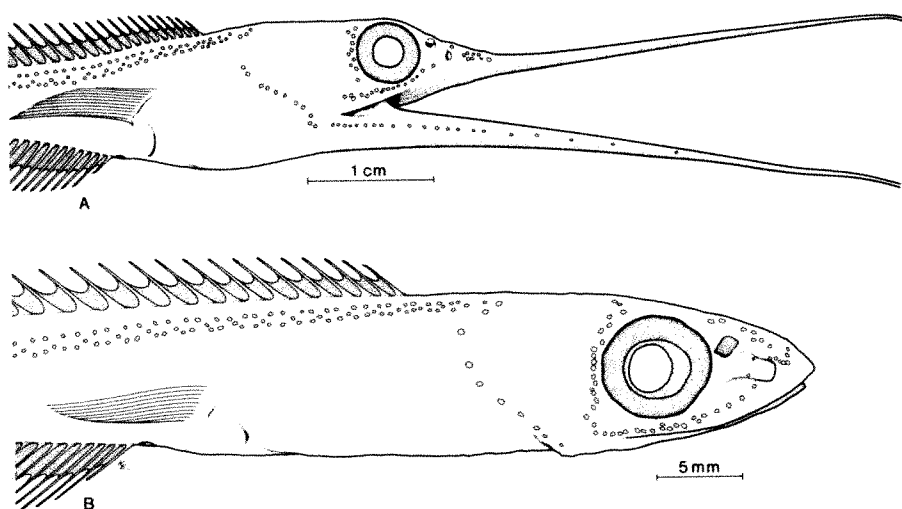
of cotton ovules W. A. Jensen (University of California, Berkeley) has followed the fate of the male gamete immediately before fertilisation in fascinating detail. The generative nucleus of the pollen tube is a true cell, surrounded by a plasma membrane. On entering the embryo-sac, the pollen tube forms an association with one of the synergid nuclei. This synergid grows and migrates to the egg nucleus, carrying the male cell which has been discharged by the tube into the synergid. Plasma membrane between the male cell and the egg are sequentially broken down, finally permitting controlled fusion. The remains of the synergid subsequently throws out the second male nucleus towards the fused polar nucleus (with which it will fuse to form the triploid endosperm). The polar dikaryon has already fused on the stimulus of pollination, and this fusion is probably triggered by secondarily produced gibberellic acids. In a similar way, the growth and change of appearance of the receptive synergid is apparently triggered hormonally, and can be induced by auxin placed on the stigma in the absence of pollen. There is now good evidence that the directional chemotaxis of the pollen tube occurs in response to a calcium gradient, according to evidence achieved with the use of ion-analysing microprobes.

Ultrastructural work has also thrown light on the control of apospory, a form of agamospermy (seeds without sex) in the New Zealand grass *Cor-dateria*. Melva Philipson (DSIR, Christchurch), showed that sexual meiosis is able to proceed towards the formation of megaspores and an embryo-sac, protected by a callose sheath. In aposporous conditions, the callose breaks down early, and the megaspore mother cell degenerates, allowing the formation of vegetatively budded, maternal embryo-sacs, which develop embryos autonomously, identical to the parent.

There was much more to report at this conference: a mass of exciting anthecological and breeding system work is emerging from the botanical wealth of Australia, which with over 20,000 species of flowering plants, and a host more waiting to be described, bids fair to replace California as the evolutionary field laboratory of the world. Exciting new techniques were also in evidence, especially in the fields of cell fusion studies, somatic gene transmission, embryology, using new clearing techniques to replace tedious sectioning, and the use of artificial dried 'seeds' cloned from tissue cultures. □

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*The International Symposium on Reproduction in Flowering Plants, organised by the Royal Society of New Zealand was held on 5-10 February, 1979 at the University of Canterbury.



Head of *Nemichthys scolopaceus*: A, ♀; B, ripe ♂.

Snipe-eels unravelled

from a Correspondent

THE snipe-eels are a family of eels found in mid-water in deep sea throughout all the temperate and tropical oceans. They derive their common name from the extraordinary elongation of the jaws which resemble a snipe's bill, except that the tips curve outwards in the vertical plane. In common with almost all groups of eels there has been much confusion in the nomenclature and systematic arrangement of the group although for several reasons the snipe-eels have been exceptionally complicated. A recent study by J. G. Nielsen and D. G. Smith on the eel family Nemichthyidae (*Dana-Report* No. 88, 1; 1978) will, however, go a long way to clearing up the confusion in these fishes.

One of the problems with these eels is that they live in mid-water and until the development of effective mid-water nets in the middle of the present century, most of the specimens seen by naturalists were accidental captures made while hauling bottom trawls. Indeed, it is not infrequent for deep-water fishermen to find snipe-eels entangled by their slender 'beaks' in the wings of a bottom trawl. Most of the specimens described by early ichthyologists were thus isolated captures and often not in good condition (*Nemichthys scolopaceus* grows up to 1 m in length, is about 2 cm deep at its deepest part, and less than 1 cm wide—so general fragility might be expected). Another potent source of confusion arose from this fragility, such as when one species was described as new to science on the grounds that there was a ridge of skin on the dorsal surface of the snout—a feature which Nielsen and Smith show to be due to shrinkage of the skin. These and other factors all lead to a thorough con-

fusion within the family, and it seems from the historical account of Nielsen and Smith that the group was fated to attract workers who failed to appreciate critical differences and even similarities. Since 1848, when the first genus *Nemichthys* was described, no fewer than eleven other genera have been proposed in the family, and the plurality of species has been even greater. In the current revision this has been reduced to three genera; only nine species are recognised.

A major factor in the artificial boost in the number of species was that mature male snipe-eels look totally dissimilar to females and immature fish. This sexual dimorphism was demonstrated by Smith and Nielsen (*Steenstrupia* 4 (1), 1; 1976) in a preliminary note, but the phenomenon is discussed in greater detail in their current revision. At sexual maturity male snipe-eels lose the characteristic snipe-bill, lose their teeth in the process, develop tubular anterior nostrils, change colour, and their pectoral fins move posteriorwards. Sexually mature females may also change colour and partially lose their teeth. So great are the differences between males and females that for over 50 years they have been placed in separate genera, and by some workers even in separate families and suborders!

It seems probable that the males at least spawn once and die soon after. The scarcity of short-nosed snipe-eels in collections suggests that they are rare in nature. However, as Nielsen and Smith point out, fishing gear may select in favour of the females, which having teeth and long snouts are more likely to become tangled in netting than are the toothless, short-snouted males. No doubt their study will stimulate further investigation of the ratio of male to female snipe-eels.

The fascinating shape of the jaws is briefly discussed but no new light is

shed on the means by which these fish capture their food, which is mainly crustacean. An earlier suggestion by G. W. Mead and S. A. Earle (*Proc. Calif. Acad. Sci.* 38 (5), 99; 1970) was that the long, tooth-studded non-occlusible jaws might be used to entangle the antennae of the sergested and hoplophorid shrimps which they eat. The backward-pointing teeth would ensure that any trapped animal could only move towards the gullet, and yet the awkward divergence of the upper and lower jaws hardly seems to be an efficient method of capturing food.

Nielsen and Smith's study is a major advance in clarifying the confusion in taxonomy of the snipe-eels, but it is unfortunate that it appeared at almost the same time as the publication of a study by E. S. Karmovskaya (*Trudy Inst. Okeanol.* 109, 186-210; 1977) on the taxonomy and distribution of the genus *Borodinula*, with the description of a new species. Nielsen and Smith refer to this paper in a postscript added in proof, pointing out that Karmovskaya's new species is a species they consider was described as long ago as 1916, while *Borodinula* is a synonym of *Avocetina*. Evidently the era of confusion in snipe-eels is not yet over.

Equatorial upwelling rates

from M. Tomczak Jr.

ESTIMATES of vertical velocities in the ocean have long been a matter of interest in oceanic science since the supply of nutrients for the surface layer where primary production through photosynthesis can take place, almost exclusively depends on renewal from below. Apart from the important coastal upwelling regions along the coasts of California, Peru, North-west and South-west Africa, there is clear evidence for strong upwelling caused by a divergence in the surface layer flow along the Equator. To estimate the productivity of the region, however, is difficult because upwelling rates, that is, the amount of water which upwells into the surface layer per unit time interval cannot at present be measured directly. Average vertical velocities are believed to be of the order of a few centimetres per day or less.

Theoretical estimates of upwelling rates are usually based on the calculation of 'Ekman layer suction', the amount of water absorbed into the surface layer from below in order to compensate for local losses due to divergences in the surface currents. Since surface currents are wind-driven it is possible to calculate this effect

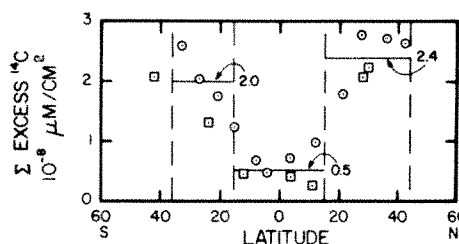


Fig. 1 Radiocarbon content of the Atlantic Ocean, integrated over the water column. The dashed lines define the equatorial and temperate zones in the study of Broecker, Peng and Stuiver (*op. cit.*) from which the figure is taken.

from data on the wind stress distribution over the ocean and some effort has been devoted to a more detailed knowledge of this important parameter (Leetma & Bunker *J. mar. Res.* **36**, 311; 1978). Application of these considerations to the equatorial zone leads to upwelling rates of $15\text{--}20 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ for the Atlantic Ocean which means that the upward flux of water into the surface layer, distributed over the entire Equatorial Atlantic Ocean, amounts to about one quarter of the transport of the Gulf Stream along the American coast. In a recent paper by Broecker, Peng and Stuiver (*J. geophys. Res.* **83**, 6179; 1978) the same order of magnitude is derived from the distribution of radiocarbon which entered the ocean after the bomb tests between 1955 and

1965. Their result is of particular interest because it represents a method of assessing upwelling rates which is completely independent of any meteorological parameter and, if it can be accepted as reliable, could provide a valuable reference value for other calculations.

Broecker, Peng and Stuiver start from the fact that the radiocarbon content of the Equatorial Atlantic Ocean is low by a factor of about 4–5 compared with the temperate zones and proceed to consider a number of models on the assumption that the water of the surface layer in the equatorial zone is removed sideways into the temperate zones and replaced by upwelling. In the simplest of their models the equatorial zone is treated as a well-mixed homogeneous layer of 150 m depth between 15°N and 15°S . The choice of the lateral boundaries seems natural from the observed bomb radiocarbon distribution (see Fig. 1). In the vertical, other models use a succession of layers with a 100 m top layer and three 50 m layers below. The resulting upwelling rates vary between 15.6×10^6 and $20.2 \times 10^6 \text{ m}^3 \text{ s}^{-1}$.

Encouraging as these results might seem, the problem with them is that the models could well be called an oversimplification of the actual situation. It is known and has been reported in *Nature* by Düing *et al.* (257, 280; 1975) in a review of the GATE Equatorial Oceanographic Experiment that

the equatorial current system between 15°N and 15°S does not display a simple homogeneous structure (Fig. 2). Broecker, Peng and Stuiver acknowledge the fact and state that their results should be taken as averages over the entire area. It might well be questioned whether an average over two westerly currents and three easterly ones has any significance. More important is the neglect of all zonal transport in the model. The authors calculate, on the basis of their upwelling rate, a vertical velocity of about 30 m yr^{-1} and a residence time of 4.7 yr for the water in the surface layer. Now, even a modest flow of 25 cm s^{-1} crosses the Equatorial Atlantic from east to west (or, in the Undercurrent and in the Countercurrents, from west to east) within less than 300 d. This shows that there is an appreciable inflow of water into the area by advection and that most of the water which enters the surface layer from below at a rate of 30 m yr^{-1} leaves the area at the eastern or western ends rather than through the poleward boundaries. The effect can of course be accounted for by assuming a higher upwelling rate. But this would almost certainly result in a discrepancy between the results of the two independent methods.

It seems, then, that we are still some way from reliable estimates of upwelling rates. The proposal of Broecker, Peng and Stuiver to use radiocarbon observations for an independent calculation is a very valuable one. Bomb radiocarbon concentrations will remain above natural background levels for another decade at least. It is certainly worth collecting more radiocarbon information to provide a good data base for an improved model of equatorial upwelling rates. □

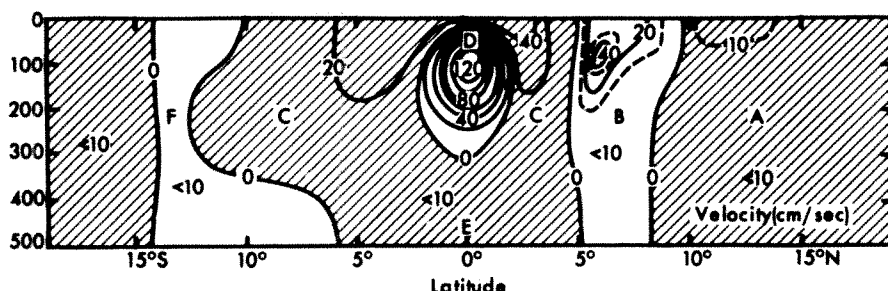


Fig. 2 A cross-section of the equatorial current system, showing the zonal component of velocity for the central Pacific Ocean. Westward flow is shaded, letters refer to individual components of the current system: A=North Equatorial Current, B=North Equatorial Countercurrent, C=South Equatorial Current, D=Equatorial Undercurrent, E=Intermediate Equatorial Current, F=South Equatorial Countercurrent. From Philander (*Rev. Geophys. Space Phys.* **11**, 513; 1973).

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A hundred years ago

Tides in the Bay of Fundy

HAVING resided for some years in the neighbourhood of this bay, I am able to give a little information respecting its tides. The bay splits into two at its inner end. One of these branches leads through a narrow channel into the broad basin

of Minas. The other, called Chegnecto Bay, is not interrupted by any such contraction, and is therefore more favourable for the formation of very high tides. This bay itself divides at its upper end into two, and one of these, called Chepody Bay, contracts very gradually for some thirty miles inland, forming the estuary of the Petitcodiac River. This is the place where the highest tides occur, and as far as I have been able to learn, their maximum height is 70 feet. A powerful "bore" is formed by the incoming waters. The captain of the steamer *Emperor*, which plied between St. John, N.B., and Windsor, N.S., informed me that the highest tide in any part of Minas Basin was about 55 feet.

A PETROLEUM spring, one boring of which has yielded 2,000 kilos in twenty-four hours, has been discovered at Pohar, in Austrian Poland.

ACCORDING to the report of H.M.'s Consul for Hiogo and Osaka, the Japanese claim that petroleum has been known in Japan for over 1,200 years, and it would certainly be curious if the numerous springs which exist in certain localities should have escaped notice in their immediate neighbourhood. It is doubtful, however, whether it was ever utilised, and certainly no attempt was made to refine it before the arrival of foreigners. From *Nature* **19**, 20 March, 458, 473; 1879.

articles

Neogene relative motion between the Pacific plate, the mantle, and the Earth's spin axis

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The northward rates of motion for the central equatorial Pacific during the past 24 Myr vary between ~ 20 – 30 mm yr^{-1} based on the migration of sedimentary facies, $\sim 30 \text{ mm yr}^{-1}$ based on the age and orientation of the Hawaiian ridge, and ~ 50 – 60 mm yr^{-1} based on palaeomagnetically determined sediment and basalt palaeolatitudes. To resolve the differences between hot spot and palaeomagnetic results we may need to invoke a long-term drift in the orientation of the geomagnetic field, or a northerly component of motion of the Hawaiian hot spot relative to the Earth's spin axis.

SIGNIFICANT differences between Neogene rates of motion of the Pacific plate relative to: (1) the Hawaiian hot spot, (2) the Earth's spin axis determined from palaeomagnetic data, and (3) the Earth's spin axis deduced from the distribution of equatorial sediment facies, suggest that one or more of the assumptions inherent in each method may be invalid.

Calculation of palaeolatitudes from palaeomagnetic data is usually based on the assumption that, when averaged for several thousand years, the Earth's magnetic field approximates that of a geocentric dipole coincident with the Earth's spin axis. This assumption is supported by palaeomagnetic results such as those of Opdyke and Henry¹, which are based on a worldwide distribution of sediment cores. Other studies conclude, however, that the time-averaged geomagnetic field is better represented by a dipole field slightly displaced into the Northern Hemisphere², or by a pair of unequal axial dipoles³. If either of the latter two results is valid, then palaeolatitudes determined assuming geocentricity will be systematically in error. At low latitudes, however, there is little difference between the geocentric and nongeocentric models in terms of the rate at which field inclination varies with latitude. Because most of the palaeomagnetic data used in this study are from latitudes of $< 10^\circ \text{ N, S}$, the choice of models has no significant effect on palaeomagnetically determined rates of plate motion, and, therefore, cannot be the source of discrepancy between palaeomagnetic results and either hot spot or facies migration results. It is not known whether the average pole positions of the geomagnetic field have remained coincident with the geographic poles over periods of time exceeding several tens of thousands of years. At present, however, there is no evidence of long-term net motion of the north geomagnetic pole towards the Pacific, which is the direction of movement that would be required to reconcile the present palaeomagnetic results with the less rapid northward rates of Pacific plate motion relative to the Hawaiian hot spot.

Other factors that may alter or obscure initial palaeomagnetic

remanence and thereby produce spurious palaeolatitudes, are magnetic overprinting, caused by chemical alteration in either rocks or sediments, and in non-indurated sediments, acquisition of systematic inclination error, caused by post-depositional grain rotation and compaction. Chemical alteration is usually recognised as systematic differences between the direction or magnitude of normal and reversed intervals within a magnetostratigraphic section, or by the behaviour of the remanent magnetism during alternating-field demagnetisation analysis. No such evidence of chemical alteration is observed in the present palaeomagnetic data, and Hammond *et al.*⁴ have shown that there is no discernible compaction-related rotation of palaeomagnetic vectors in equatorial Pacific pelagic radiolarian-rich clays as old as 30 Myr. Their results showed consistent inclination trends within individual chronologically overlapping cores recovered north and south of the Equator.

The present distribution of relatively thick equatorial sedimentary facies, originally deposited in equatorial regions of upwelling and high biological productivity, has been used^{5–8} as another means to determine the Cainozoic motion of the Pacific plate relative to the Earth's spin axis. Isopachs delineating progressively older sediments are systematically offset to the north. Poles and rates of rotation are determined by backtracking the locus of maximum sediment thickness, for a discrete time interval, to its original equatorial deposition site. It is difficult to quantitatively assess the reliability of plate motions based on the migration of equatorial sedimentary facies other than by their consistency with other independently derived results. The accumulation of sediment on a moving plate is governed by the interaction of dynamic factors including upwelling-induced surface productivity, the width of the area of upwelling, erosion, and CaCO_3 solution. Relatively brief hiatuses within a sedimentary section, due to nondeposition, dissolution, or erosion, for example, may be cumulatively significant as well as difficult to detect. Van Andel and Whaley⁹ have pointed out that maxima and minima in rates of sedimentation can vary in response to factors unrelated to the latitude of the deposition site. There is little doubt, however, that the distribution of equatorial sedimentary facies is strongly influenced by the motion of the Pacific plate.

According to the hot spot hypothesis of Wilson^{10–12} and Morgan¹³, linear volcanic chains are formed as the sea floor moves over magma sources below the lithosphere. Morgan¹³ originally defined 20 hot spots and postulated that they were surface manifestations of convection plumes that originate near the core-mantle boundary. He also suggested that hot spots might move relative to each other at maximum rates of about 5 mm yr^{-1} . Recent reconstructions (refs 14–17 and Morgan, personal communication) confirm that little or no relative motion is required between hot spots, suggesting that hot spots can be used as a global coordinate system to which plate motion

can be referred. Volcanic chains that were formed at hot spots can be used to determine the motion of plates relative to this coordinate system. Because the Hawaiian ridge–Emperor seamount chain is the most prominent and best studied hot spot trace on the Pacific plate, it provides the best estimate of the plate's motion with respect to a hot spot coordinate system. Epp's¹⁸ analysis used only this volcanic chain, and, therefore, made no *a priori* assumptions about the relative age of other volcanic chains on the Pacific plate. The poles Epp¹⁸ found, however, seem to be representative of other hot spot traces on the plate, suggesting that there has been little relative motion among Pacific hot spots for at least the past 25 Myr. In other studies, Clague and Jarrard⁷ used the Hawaiian, Cook–Austral, and Guadalupe chains to determine a pole position; their analysis was amended by Dalrymple *et al.*¹⁹ to incorporate a revised radiometric age for Midway. Minster *et al.*¹⁴ determined a pole position and rate of rotation from a systematic inversion of all available relative motion data and azimuths of hot spot traces. The poles and rates of rotation thus determined can be used to determine the northward motion of the Pacific plate relative to the hot spot coordinate system.

Palaeomagnetism of Pacific deep-sea sediments

The northward motion of the Pacific plate during the late Mesozoic and the Cenozoic seems to be responsible for the discrepancies, found for older sediments and volcanic rocks, between measured palaeomagnetic inclinations and those characteristic of the present latitude of the sampling site. Latitudinal migration of the Pacific plate has been inferred from results of paleomagnetic surveys of Pacific seamounts²⁰, palaeolatitudes from drilled basalts²¹, and palaeolatitudes from both drilled and cored sediments^{4,22}.

In the present study 27 chronologically overlapping piston cores, which collectively span the past 25 Myr, have been used to determine the northward motion of the central equatorial Pacific. The cores were recovered from the Central Pacific Basin (average depth 5.0–5.5 km), where extensive erosional surfaces make possible the recovery of relatively old sediments by piston coring²³. All of the cores contain homogeneous brown,

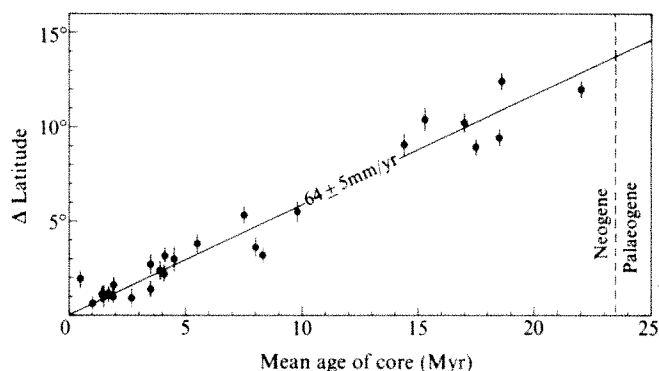


Fig. 1 Progressive increase, with increasing age, between the observed mean palaeolatitude and the coring site latitude (Δ latitude) for chronologically overlapping cores from the Central Pacific Basin. Vertical bars represent the standard error for the mean palaeolatitude of each core. Age span and Δ latitude value for the cores are shown in Table 1.

radiolarian-rich clays (Table 1). Average core length is about 13 m and average sediment accumulation rate is about 3 mm per 10^3 yr. This relatively low accumulation rate means that each sample from the cores contains, on the average, a depositional record spanning about 6,000 yr. Magnetic secular variation is therefore largely averaged out within single samples.²⁴ The cores were dated by correlating their radiolarian and magnetostratigraphy to the time scale of Thayer and Hammond^{25,26}. The K76 core series (Table 1; Fig. 1) is fully orientated, which allows an unambiguous determination of both polarity and the hemisphere in which the cores were deposited²⁷.

The stability of depositional magnetic remanence was established by standard alternating-field partial demagnetisation analysis of at least six samples from each core^{4,28}. After the stable remanence was resolved, individual inclination values in each core were converted to palaeolatitudes by use of the geocentric dipole formula. A mean palaeolatitude was calculated for each core by first determining the mean palaeolatitude for each polarity interval, and then dividing the sum of the absolute values of the polarity interval means by the total number of polarity intervals.

The mean palaeolatitude for each core was normalised for its core site latitude⁴ and then plotted against the mean age of the core (Fig. 1). Each core was sampled at 10-cm intervals, so the average northward rate indicated in Fig. 1 is based on nearly 3,500 discrete palaeolatitude determinations. A linear regression of the differences between mean palaeolatitude and coring site latitude (Δ latitude) versus mean age, assuming mean core age to be the independent variable, indicates an average rate of palaeolatitude change for the central Pacific of about 64 mm yr⁻¹ throughout the Neogene. This rate agrees with that determined in a similar manner by Hammond *et al.*⁴ (using seven cores) for the interval 0 to –13 Myr, but is considerably slower than their previously determined rate of about 110 mm yr⁻¹ for –13 to –21 Myr. The distribution and resolution of the present data preclude a reliable determination of variations in the rate of northward motion during the Neogene, although such variations may have occurred as a result of reorganisation of plate boundaries and change in the rate and/or direction of seafloor spreading. At present, palaeomagnetic data are insufficient for reliable estimation of an average northward rate of plate motion during the Palaeogene, but the Neogene rate shown in Fig. 1 is compatible with approximately 30° northward displacement of the Pacific plate since the Cretaceous^{5,20}.

Comparison of results

Since northward rates of motion for different locations on a plate vary with distance and azimuth to the pole of rotation, DSDP Site 166 (3.76° N, 174.08° W), located in the Central Pacific

Table 1 Cores taken from the Central Pacific Basin

Core no.	Coring site	Water depth (m)	Recovery (m)	Age span of core (Myr)*	Δ latitude (deg)
S68-24	2.0° N, 178.8° W	5291	21.5	16.7–27.0	12.0
M70-10	1.6° N, 166.3° W	5200	9.3	16.4–17.5	10.2
M70-14	0.7° N, 162.5° W	5200	11.3	0.1–0.8	1.9
M70-16	3.2° S, 160.3° W	5505	11.2	0.3–3.6	1.6
M70-17	7.5° S, 161.5° W	4730	11.5	3.7–12.4	3.6
M70-38	2.3° S, 171.0° W	5360	15.8	16.4–21	12.4
M70-39	2.5° S, 173.3° W	5412	21.8	2.3–4.8	2.7
M70-76	3.0° N, 174.9° E	5015	12.9	12.5–16.4	9.1
K72-36	2.4° S, 177.8° W	5545	9.8	16–19	8.9
K72-37	2.1° S, 178.9° W	5310	12.2	3.9–11.0	5.3
K72-38	5.4° N, 164.4° W	4658	12.1	0.0–2.9	1.0
K72-46	2.3° N, 164.2° W	5235	12.3	0.6–4.7	0.9
K72-48	4.2° N, 165.8° W	5477	12.0	1.6–5.3	1.4
K76-03	7.6° S, 169.7° E	4745	8.1	0.0–2.7	1.1
K76-04	7.1° S, 171.0° E	5000	15.8	0.4–3.4	1.1
K76-06	4.7° S, 173.4° E	4735	11.0	14–16	10.4
K76-07	4.4° S, 174.1° E	5170	11.7	3.6–4.6	2.2
K76-08	2.8° S, 177.9° E	5290	11.7	3.0–4.7	2.3
K76-09	2.8° S, 177.9° E	5295	10.6	3.0–6.0	3.0
K76-10	1.1° S, 179.5° E	5355	11.8	1.3–4.8	3.2
K76-12	0.9° N, 178.6° W	5195	11.7	3.0–11.0	3.8
K76-14	2.7° N, 178.7° W	5390	11.1	0.2–2.1	0.6
K76-15	2.9° N, 178.5° W	5260	11.3	5.3–11.2	3.2
K76-16	2.9° N, 178.5° W	5380	17.9	0.4–3.3	1.0
K76-17	4.6° N, 176.5° W	5170	17.0	13–24	9.4
K76-18	4.6° N, 178.6° W	5240	16.9	8.0–11.5	5.5

*Ages greater than 5 Myr are based on correlations of each core's radiolarian- and magnetostratigraphy to the Neogene sediment-based reversal time scale of Thayer and Hammond^{25,26}. For this interval, a conservative estimate of the accuracy of a given age is about ± 0.5 Myr (ref. 33).

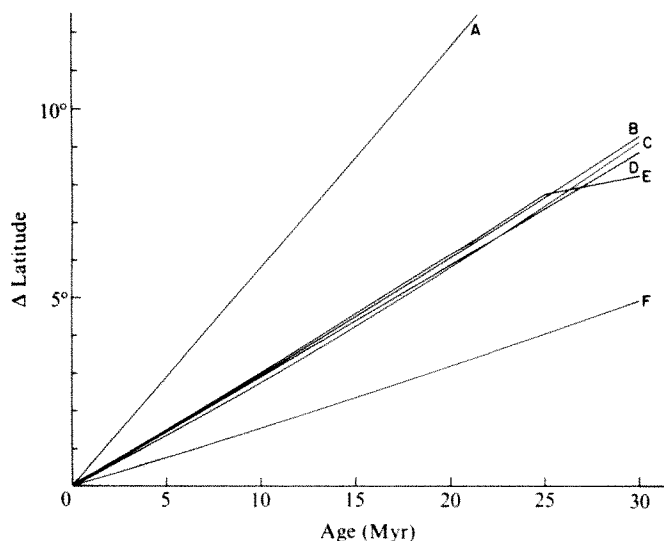
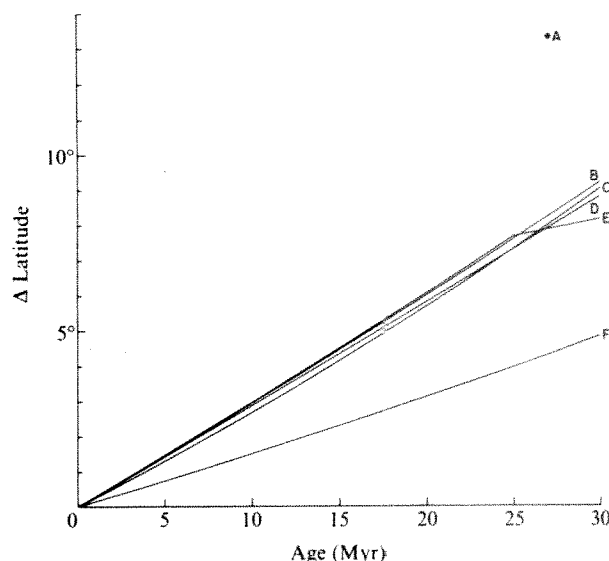
Table 2 Pole positions and rate of rotation for various age ranges determined by different authors

Author	Pole		Age range (Myr)	Rate of rotation (deg Myr ⁻¹)
Epp	65° N	40° W	0-23	0.86
	59° N	54° W	23-42	0.67
Minster <i>et al.</i>	67.3° N	59.4° W	0-10	0.83
Clague and Jarrard and Dalrymple <i>et al.</i>	69° N	68° W	0-42	0.83
van Andel	67° N	59° W	0-25	0.83
	67° N	59° W	25-50	0.25
Lancelot and Larson	67° N	45° W	0-40	0.5

Basin (and central to the location of the palaeomagnetically studied sediment cores), was chosen as a convenient location where palaeomagnetically-derived northward rates of plate motion could be compared with the northward rates of motion determined from migration of sediment facies and from hot spot traces. Using the pole positions and rates of rotation determined by the investigators listed in Table 2, Site 166 was rotated from its present location to its position at -24 Myr. In Fig. 2 the change in latitude (Δ latitude) of Site 166 determined by these rotations is compared with the palaeomagnetically determined change in latitude. The palaeomagnetic data were fit by a straight line which indicates a northward motion of 64 mm yr⁻¹. The other rotations show a slight increase in the northward rate of motion with age which results from the change in position of DSDP Site 166 relative to the poles of rotation. The northward rates determined from the hot spot analyses of Epp¹⁸, Clague and Jarrard⁷ together with Dalrymple *et al.*¹⁹, and the plate inversion analysis of Minster *et al.*¹⁴ are similar and about 30 mm yr⁻¹. The consistency of these analyses supports the result.

The sediment facies analysis by van Andel⁶ requires a similar northward motion, 33 mm yr⁻¹, at Site 166. The more recent equatorial facies analysis of Lancelot and Larson⁸, on the other hand, indicates a considerably slower northward rate, about 20 mm yr⁻¹, for the same time interval and location. Winterer's⁵ sediment facies analysis (not shown) yields a northward velocity of about 43 mm yr⁻¹.

There are relatively few other Neogene palaeomagnetic results from the Pacific plate which can be compared with the foregoing results. Grommé and Vine²⁹ determined an average palaeolatitude from samples of 13 basalt flows recovered in a

**Fig. 2** Change in latitude versus age for DSDP Site 166 from: A, sediment palaeomagnetism; B, Minster *et al.*¹⁴; C, Epp¹⁸; D, Clague and Jarrard⁷ with a rate of rotation determined by Dalrymple *et al.*¹⁹; E, van Andel⁶; F, Lancelot and Larson⁸.**Fig. 3** Change in latitude versus age for Midway. Midway data from Grommé and Vine²⁹ and Dalrymple *et al.*¹⁹. Other lines from the same sources as in Fig. 2.

deep drill core from Midway Island (28.2° N, 177.6° W). Thus Midway is another location where the Neogene northward motion of the Pacific plate determined by the above analyses can be compared (Fig. 3). The northward velocities at this location differ only slightly from the Site 166 results, and the discrepancies between the various analyses are just as pronounced. The palaeomagnetic data represented by point A in Fig. 3 indicate that Midway Island has been displaced northward by ~13° during the past 27 Myr. Using the average rate of northward displacement of Midway during this interval of time (52 mm yr⁻¹) to constrain the Hawaiian ridge model of Epp¹⁸, an average northward velocity, for the Central Pacific Basin, of 55 mm yr⁻¹ is obtained. Thus the Midway palaeomagnetic rate is consistent with the Central Pacific Basin palaeomagnetic results in that it also is significantly more rapid than the other rates shown in Fig. 2.

Results of a recent study by Marshall³⁰, of mid-Cretaceous to mid-Miocene basalts recovered at several north Pacific DSDP sites, are consistent with the Midway and the Central Pacific Basin palaeomagnetic results inasmuch as they, too, indicate a higher rate of northward plate motion than do hot spot and sediment facies studies.

Discussion

The results in Figs 2 and 3 show significant and consistent discrepancies among independently derived Neogene rates of northward motion of the Pacific plate. Palaeomagnetically derived rates are roughly twice as fast as those determined by backtracking equatorial sedimentary facies to their original deposition sites.

The sediment facies analyses describe the motion of the plate relative to the spin axis, and therefore should be consistent with the palaeomagnetic analyses. As shown in Figs 2 and 3, however, the predicted rates of northward motion based on sediment migration are considerably slower than those based on palaeomagnetism of both sediments and basalt. The inherent difficulties in sediment facies analyses discussed above may be responsible for the differences among the van Andel⁶, Lancelot and Larson⁸ and Winterer's⁵ results as well as the difference between the sediment facies results and the palaeomagnetic results. If palaeomagnetically determined rates do provide an accurate indication of plate motion relative to the spin axis, the agreement between facies results and those based on hot spots may be fortuitous.

The palaeomagnetically derived rate of about 64 mm yr⁻¹ appears reliable from the standpoint of the consistency and the

large amount of data from which it was determined. Likewise, the agreement between the results of Epp¹⁸, Minster *et al.*¹⁴, and the combined work of Clague and Jarrard⁷ and Dalrymple *et al.*¹⁹ strongly indicates that 30 mm yr⁻¹ is a reliable rate for the northward motion of the Pacific plate relative to the hot spot coordinate system. If the Earth's magnetic field is fixed with respect to the spin axis and the palaeomagnetic results indicate the true velocity of the Pacific plate with respect to the spin axis, then, to resolve the difference between the palaeomagnetic and hot spot rates, the Hawaiian hot spot must have moved northward relative to the spin axis at an average rate of about 30 mm yr⁻¹. The implications of this motion for the motion of the mantle with respect to the spin axis depends on the origin of hot spots. If hot spots are fixed with respect to the asthenosphere, then any hot spot versus spin axis motion requires only that the asthenosphere move relative to the spin axis. If, on the other hand, hot spots are the surface manifestations of plumes that ascend from the core-mantle boundary³² then the entire mantle must be moving relative to the spin axis. As the total vector of motion of the mantle is unknown, the absolute mantle velocity cannot be determined; however, if this motion is the result of some mantle-wide convection system, there should be southerly motion at some other location. Burke *et al.*¹⁷ found that hot spots in the North Atlantic have moved northward relative to the spin axis, and hot spots in the South Atlantic have moved southward. To conserve matter, the mantle under Asia must be moving southward relative to the spin axis; however, the southward motion of South Atlantic hot spots suggests a more

complicated convection pattern. An intermediate possibility is that different plumes come from different depths in the mantle. This conceivably could allow a complex mantle convection system while retaining the fixed nature of hot spots.

An alternative explanation for the differences between plate motion rates, especially in view of the close agreement between van Andel's⁶ results and the hot spot results, is that the palaeomagnetic results are systematically biased with increasing age. As compaction-related inclination error and tectonic tilting of the Central Pacific Basin can be ruled out, the possibility that the north geomagnetic pole was moving towards the Pacific at a rate of approximately 30 mm yr⁻¹ during the Neogene must be considered. Andrews³² analysis of polar wander during the Mesozoic and Cainozoic indicates that the field was moving relative to the Pacific plate during the Neogene, but the apparent motion was in essentially the opposite direction needed to reconcile the palaeomagnetic, sedimentary facies, and hot spot results. If Andrews³² conclusion is correct, the palaeomagnetic rates, relative to the Hawaiian hot spot, are even more rapid than indicated in Figs 2 and 3.

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Tidally generated internal wave packets in Massachusetts Bay

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Observations in Massachusetts Bay of high-frequency internal wave packets indicate that they are caused by lee waves generated outside a submarine bank at the Bay's seaward margin during ebb tide. The lee waves propagate into the Bay as the tide turns to flood, steepen nonlinearly, and develop into the packet. A 200-kHz acoustic back-scattering system detected the evolution of the packets. Large overturning events were observed acoustically and in density profiles. Plankton distributions undergo strong vertical displacements and mixing associated with the wave packet passage.

HIGH-FREQUENCY internal wave packets in Massachusetts Bay^{1,2} (Fig. 1) have been studied to understand their generation and propagation, to describe the biological variability in the plankton caused by their passage, and to further evaluate the use of high-frequency acoustic backscattering systems as remote sensors of fluid processes in the ocean. Previous work on the physical properties of internal wave packets generated by tidal flows over topography¹⁻⁶, by atmospheric forcing of internal surges^{7,8}, or from unknown causes⁹⁻¹¹, has concentrated on packet propagation. Fewer generalisations seem possible about packet generation because it is more dependent on the particular forcing field and the local topography. D. Farmer and J. Smith (personal communication) and also T. Maxworthy¹², have developed conceptual models for the topographical generation

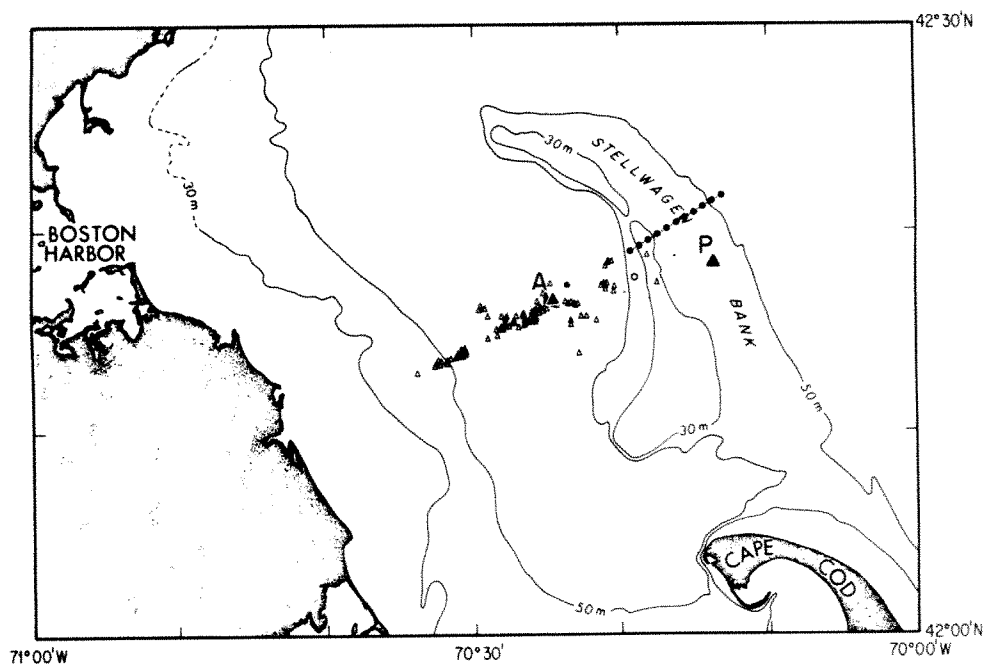


Fig. 1 Experimental area in Massachusetts Bay. A and P mark mooring sites. ●, Locations of Sippican T-11 expendable bathythermograph profiles. △, Positions at which internal wave packets were acoustically observed. *, Position at which acoustic record of Fig. 4a was obtained. Site P was a subsurface mooring (float at 22 m; water depth nominally 35 m) with a General Oceanics 2011 film-recording current meter at 25 m depth. Site A was a subsurface mooring (float at 22 m; water depth nominally 80 m) with an Aanderaa RCM4 current meter at 25 m depth, and a surface mooring carrying an Aanderaa TR1 thermistor chain with sensors at 3-m intervals between 5 and 32 m. ○, The deployment location of VCM2 (Fig. 3); VCM3 (Fig. 3) was very near site A.

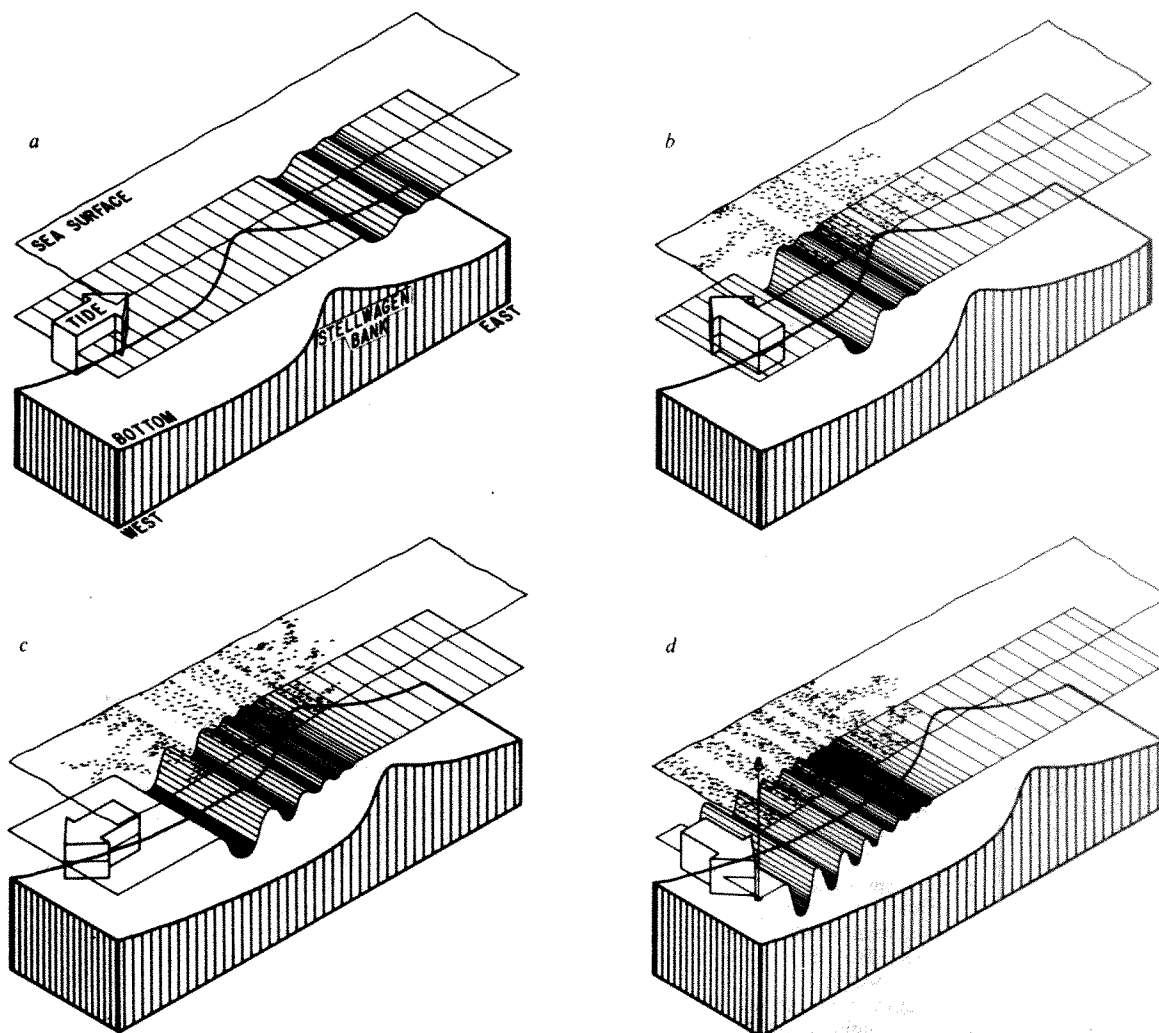


Fig. 2 Schematic diagram of internal wave packet generation as a lee wave during ebb tide over Stellwagen Bank (a), followed by nonlinear steepening after the tide turns (b). The turn of the tide allows the packet to propagate over the bank towards shore. Undulations then develop behind the steep front (c and d). See Fig. 4a for 200-kHz acoustic record of a developed packet.

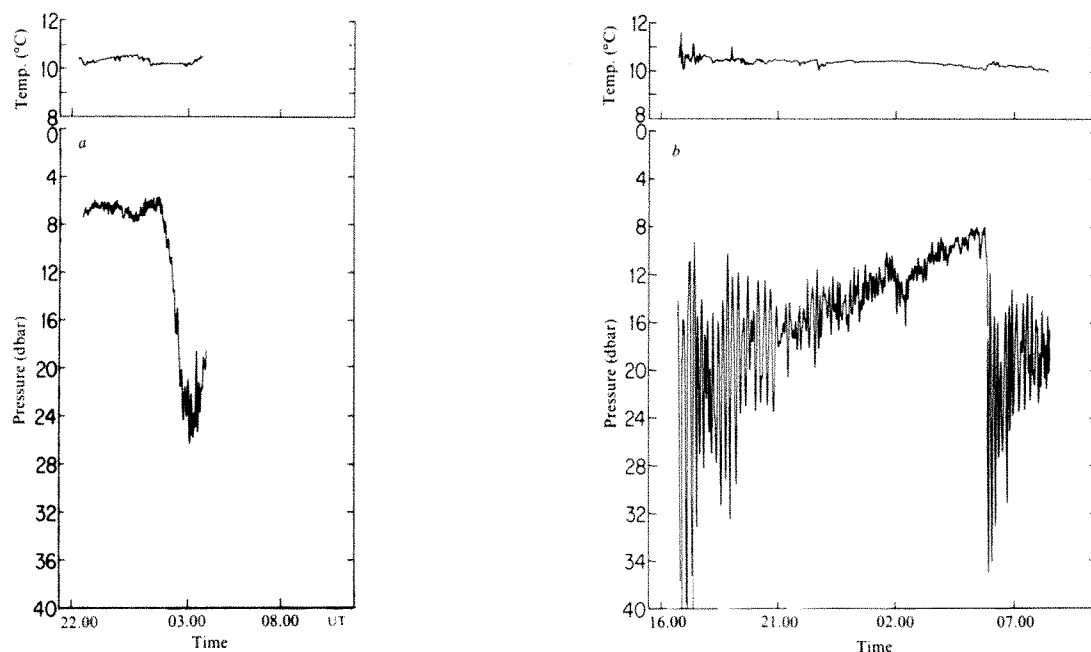


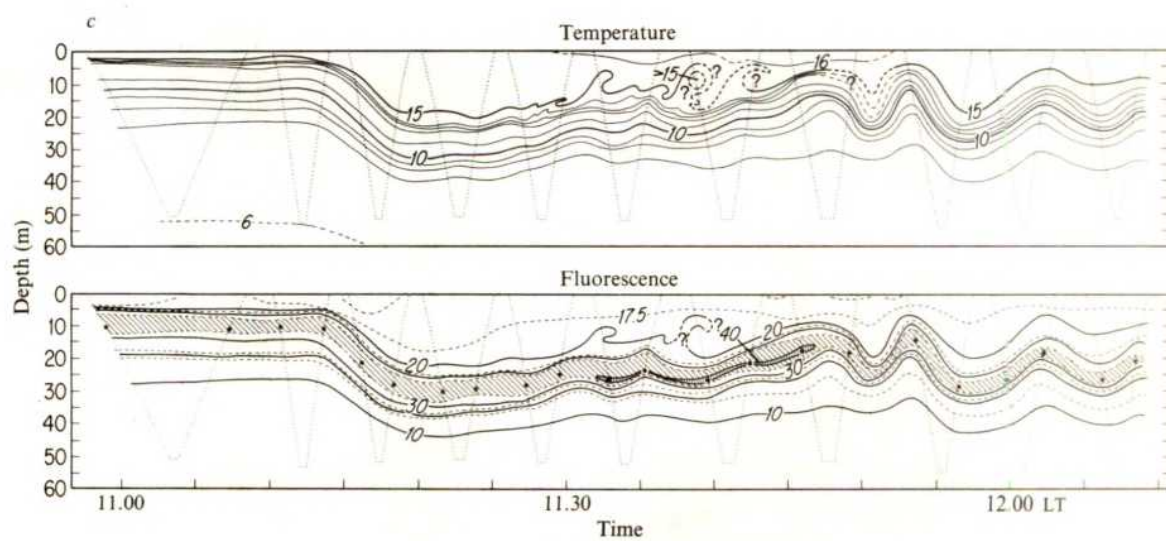
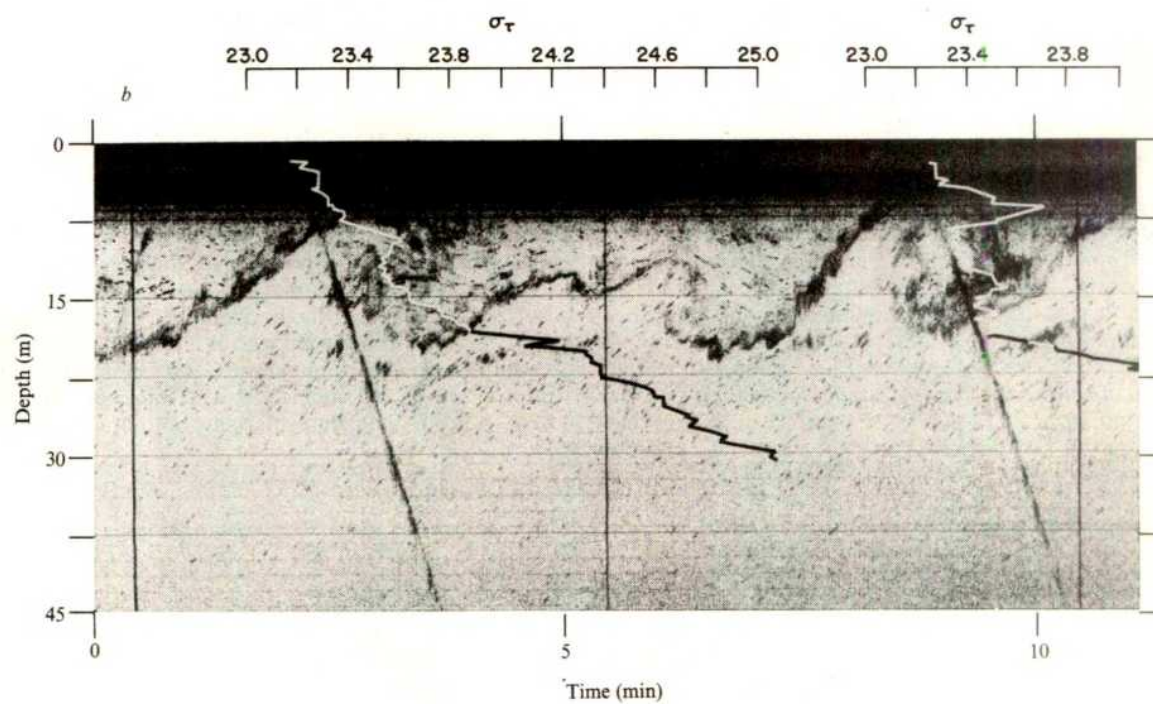
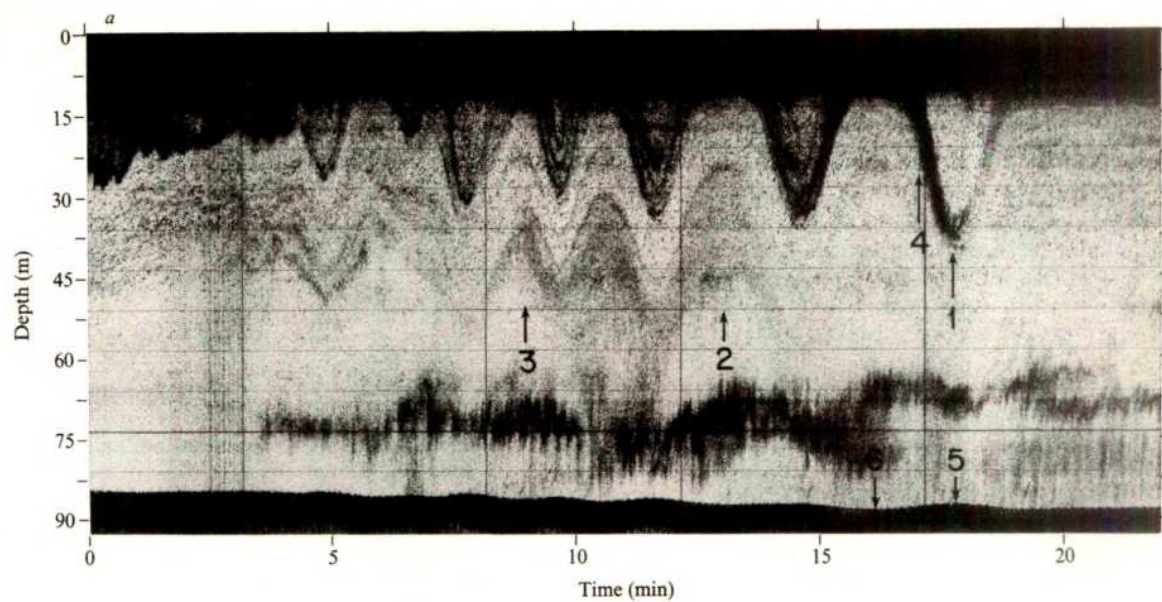
Fig. 3 Temperature and depth records from a modern version of the Swallow float vertical current meter (VCM)^{17,18} at positions shown in Fig. 1 *a*, VCM2 on 31 August; *b*, VCM3 1 September. VCM2 drifted about 0.6 km WSW and VCM3 about 2.3 km SSW during their respective deployments. The VCM3 trace shows the two internal wave packets that followed the single packet of the VCM2 trace. The VCM internally records temperature, pressure and rotation (vanes on the instrument rotate it about a vertical axis when there is vertical flow relative to the instrument). An acoustic signal from the VCM allows shipboard tracking while the instrument is deployed. Because the temperature is nearly constant at 10.2 °C (sensor time response <1 min), and because the rotation showed insignificant vertical currents past the VCM (less than 20 cm vertical water displacement), the VCM depth accurately portrays the fluctuating depth of the 10.2 °C isotherm. The record of isotherm depths from site A-moored thermistor chain during the VCM3 packet and results of CTD vertical profiling both substantiate this interpretation.

of internal wave packets by a time-varying tidal flow which are good descriptions of our observations in Massachusetts Bay. Specifically, the ebb tide flow over Stellwagen Bank (Fig. 2) generates a lee wave on the eastern side. When the tide turns to flood, the lee wave propagates into Massachusetts Bay (heading about 240 °T), steepens due to nonlinear effects¹³, and develops undulations due to an imbalance of dispersion and nonlinear effects¹⁴. In analogy to other locations^{3,4}, we would expect to observe wave packets outside Stellwagen Bank due to lee waves inside during flood tide. As most of our observations were inside the Bank, we did not see outgoing waves. They may not be present because of the asymmetry of the Bank and tidal flow.

Lee wave propagation

The occurrence of lee waves on the eastern side of Stellwagen Bank depends on an ebb tide flow with a subcritical internal Froude number¹⁵. Our measurements, together with those of ref. 16, showed the flow to be subcritical using either a two-layer fluid or a continuous-gradient model. The presence of the lee waves was substantiated by a transect of 11 bathythermographs across the Bank during ebb tide (Fig. 1). The 10 °C isotherm (in the thermocline) descended from 8 m over the Bank to 20 m at about 7 km to the ENE with oscillations that may be the expected lee waves.

Fig. 4 *a*, 200-kHz acoustic record of an internal wave packet observed at the position marked * in Fig. 1. The period of the waves are Doppler shifted by the ship's speed of about 2.5 knots (128 cm s^{-1}) as it travelled from the rear to the front of the packet parallel to the packet's propagation direction (240°; left to right in figure). The seasonal thermocline is displaced by 30 m (arrow 1) and the stratified point scatterers (zooplankton?) at 30 to 40 m are displaced more than 20 m (arrows 2 and 3). The heavy acoustic backscattering in the vicinity of arrow 4 and extending in an oscillatory pattern throughout the figure is possibly caused by turbulent mixing in the thermocline. The asymmetry of the wave field, with narrow troughs and broad crests, is determined by the depth of the mixed layer²⁰⁻²¹. Similar asymmetrical waves have been observed acoustically in the lower atmosphere²². The heavy scattering near 75 m is probably caused by euphausiid and mysid shrimp. The oscillations in bottom depth (arrows 5 and 6) are due to variations in the travel time of the sound pulses resulting from the thermal structure of the waves; that is, the water column above arrow 6 is mostly cold (slower speed of sound). *b*, The 200-kHz acoustic record of overturning instabilities associated with the internal wave packet (propagating from right to left) at site A (Fig. 1) between 11.30 and 11.41 LT, 30 August 1977. This portion shows two overturning events; the direction of overturning (left to right) is opposite that of packet propagation. Superimposed are the corroborating density profiles obtained by repeated vertical casts of the CTD. The instrument's path is seen as the oblique traces in the acoustic record. The ship was drifting during the observations. The winds during these observations (and for most of the experiment) were $<2 \text{ m s}^{-1}$. *c*, Profiles of temperature and chlorophyll fluorescence for the same wave packet as in Fig. 4*b*, but between 10.58 and 12.10. The vertical exaggeration is about twice that of the acoustic record; isotherms and 'isofluors' are drawn between profile data points using the acoustic imagery as a guide. The wide hatching depicts fluorescence values greater than 37.5 arbitrary units, the narrow hatching greater than 40.0 units. ●, Within the hatched areas are at the depth of the chlorophyll maximum. The light level at the chlorophyll maximum layer before the packet arrival was 18% of ambient (range 14–22%) [Secchi Disk depth: 11 m, extinction coefficient $k = 0.17$]. Immediately after arrival, the mean level was 3% (range 1–13%). Four hours later, the mean light level was still only 3.5%. During the time shown the ship was drifting with the local currents such that the observations (11.15 to 11.30 LT) are along the first trough of the wave packet, then slowly scanning back (after 11.30) through the packet as the current turned from west to SSW.



Although we believe that only lee waves were formed over Stellwagen Bank during our observations, certain conditions (sufficiently high Froude number) could generate a turbulent internal hydraulic jump. This has been observed in fjords with blocking sills³. The potential thus exists at Stellwagen Bank for strong biological effects due to the mixing. In its absence, however, the propagation of the lee wave into Massachusetts Bay seems to be a wave phenomenon with no significant net transport of water or plankton by the waves alone.

Two sets of data (Fig. 3) support the picture of wave propagation shown in Fig. 2b and c. The 10.2°C isotherm at the front of a newly developed wave packet (VCM2) initially deepens at about 18 cm s⁻¹, with few oscillations occurring during the next hour. At a later stage of wave development (VCM3), the isotherm deepens at 38 cm s⁻¹ with strong following oscillations. Numerous 200-kHz acoustic records¹⁹ obtained between Stellwagen Bank and site A also support the model. Neither of the VCM floats was transported by the wave packets; this supports the conclusions of the preceding paragraph.

The propagation of internal wave packets has been described by a form of the Korteweg-de Vries equation^{2-4,7}. Possible solutions include a modulated, undulatory wave packet in which the modulation has the form of solitary waves. The record of isotherm depth from VCM3 (Fig. 3) fits this solution; the 8–10-min period oscillations have a modulation envelope of about 90 min period; about three envelope oscillations are distinguishable. Long period modulations are also present in the thermistor chain record from site A.

Wave packets were repeatedly observed with the acoustic system (see Fig. 4a) as they propagated through Massachusetts Bay. Some of these acoustic records show large shear instabilities (Fig. 4b). These are similar to turbulent shear flow in the atmosphere²³ and laboratory²⁴⁻²⁶. Densities calculated from vertical profiling conductivity-temperature-depth (CTD)²⁷ casts through two events (Fig. 4b) show strong inversions. This supports the interpretation of these structures as overturning instabilities. The direction of overturn, being opposite to the wave propagation direction (Fig. 4b), argues for a shear opposed to the wave propagation²⁶, that is, the surface velocities are

against the waves, the deep velocities are with them. We have no direct measurements of shear, but note that 2×10^{-2} s⁻¹ levels have been observed at the site¹⁶.

Redistribution of chlorophyll and zooplankton

Figure 4c shows the overturning events reflected as inversions in the vertical profiles of temperature and chlorophyll fluorescence. These events redistribute the chlorophyll and zooplankton. As no nutrient data were collected, we do not know if the surface layer is enriched with nutrients mixed from below. Because the geographical regions within which internal waves break are restricted both spatially and temporally, the vertical effects of mixing would be reflected in heterogeneous horizontal distributions of chlorophyll, zooplankton, and, if nutrients are affected, primary production.

Phytoplankton production may be affected by the interaction of the diel light cycle with the chlorophyll depth variations caused by internal wave packets acting over the semi-diurnal tidal cycle of 12.4 h. A mathematical model of internal wave effects on a chlorophyll maximum layer at the depth of the 10° isotherm showed the maximum daily peak illumination level (12 h day length) experienced during a 2-week tidal cycle varies by a factor of 2.3, whereas the minimum total daily integrated illumination is 67% of the maximum. In these conditions, production is affected both by simple light limitation over the 2-week tidal cycle²⁸ and by the more complex physiological effects of rapid light variation during any one day²⁹.

Zooplankton distributions were sampled at site A using a Longhurst-Hardy plankton recorder (LHPR)³⁰. These data and the simultaneous acoustic records showed no evidence for any behavioural response rapid enough to suggest that the plankters are other than passive markers of the wave field. Plankton distributions taken by the horizontal LHPR tows, however, do vary markedly with the passage of internal waves. In Fig. 5, the abrupt change in the temperature and salinity signatures of the internal waves at sample 50 are mirrored by chlorophyll fluorescence and appendicularians, but not by amphipods and

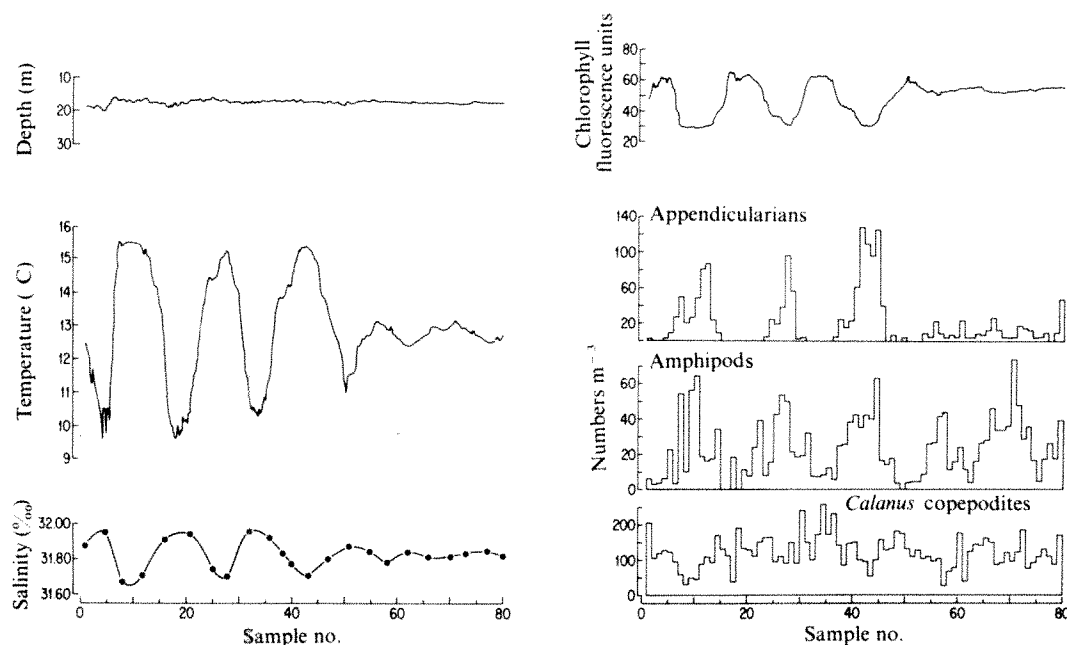


Fig. 5 Data from the Longhurst-Hardy plankton recorder tow taken at constant depth (17.5 m) through the first part of the internal wave packet at site A between 09.31 and 09.52 LT 28 August 1977. The record begins just after the leading edge (first trough) of the packet passed the ship. The direction of the tow was into the wave packet propagation; the tow speed was about 75 cm s⁻¹; tow length 980 m; sample length 12.2 m; mean volume filtered per sample 0.25 m³ (no net was used preceding the recorder box). The depth of the tow, temperature, salinity and chlorophyll fluorescence records are from the CTD and *in situ* fluorometer mounted on the plankton recorder vehicle. Other tows (oblique), taken to determine vertical distributions, showed the appendicularians and amphipods to be restricted to the warmer surface waters, whereas the *Calanus* copepodites occurred in greatest abundance in deeper water of temperature less than about 12–14 °C.

Calanus copepodites. Another tow taken at 1.5 m through the first three crests following the leading edge of an internal wave packet showed abundant *Calanus* copepodites at the surface in the third crest; water of almost the same temperature, but with few copepodites present, occurred at the surface in the preceding two crests. The 200-kHz acoustic records accompanying all the tows provided a valuable picture of the vertical distribution throughout the water column of scattering from both organisms (that is, point scattering) and from possible temperature and velocity fine structure.

Isotherm following has been suggested as a way of removing the confounding effects of internal waves on studies of plankton pattern³¹. The rapid rate of isotherm depth variation (see Fig. 3) makes such a task very difficult. Our observations show that variability of periods similar to those of the internal waves is effectively removed, but there is increased short period variability due to unavoidable excursions above or below the target isotherm depth and the impossibility of following isotherms in regions of overturning. Long period (greater than the internal wave period) variations in horizontal abundance, however, are clearly evident; these would not be easily detected by towing at constant depth.

Conclusions

The generation of the wave packets can be explained by the models of Maxworthy and Farmer (Farmer's internal hydraulic jump is more violent than our observations) and the steepening and propagation are consistent with earlier studies. We see suggestions of envelope behaviour in the modulation of the wave packets, and our acoustic observations show large overturning instabilities substantiated by density inversions in the CTD profiles. Strong inhomogeneities in the vertical and horizontal plankton distributions are caused by the passage of the internal waves; the waves also have potential effects on primary production in the Bay. Acoustic records (200 kHz) are particularly valuable in providing a real-time kinematic view of

the scattering field due to the combination of plankton and internal waves.

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Pliocene footprints in the Laetoli Beds at Laetoli, northern Tanzania

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Recent excavation of the tuffs of the Laetoli Beds in Tanzania has revealed the presence of a large variety of footprints from the Pliocene. Many of these prints can be correlated with fossilised remains of Pliocene animals found in the same area.

It was stated previously¹ that the name Laetoli would be used in preference to either Garusi or Vogel River for the area where the Laetoli Beds are exposed. Laetoli, as stated then, is an anglicisation of the Masai word Laetoli and was first used by Kent². The Tanzanian authorities have now asked that the term Laetoli should be dropped in favour of Laetoli. The name Laetoli will be used for the area, but the Pliocene deposits will continue to be known as the Laetoli Beds, as established in 1976¹.

The Laetoli Beds (Figs 1, 2) are dominantly tuffs which have a maximum known thickness of 130 m and are divisible into upper

and lower units. Nearly all the fossils have come from the upper unit which is 45-60 m thick.

In 1975 three marker tuffs had been identified in the upper unit of the Laetoli Beds¹. Since then, more than a dozen widespread air-fall tuffs (Fig. 3) have been recognised, permitting detailed correlations.

Description of the footprints tuff

The footprints tuff is divisible into two units of differing lithology and structure. The lower unit, 7-8.6 cm thick, is relatively uniform in thickness and is characterised by widespread ash layers of even thickness, commonly with rainprinted surfaces. The upper unit, generally 5-7 cm thick, thins over the higher areas of the lower unit and thickens in depressions to eliminate the undulations preserved by the mantle bedding of the lower unit.

Several unusual features of these tuffs can be explained by composite ash falls of natrocarbonatite ash and melilitite lava globules. The ash must have been cemented rapidly to have

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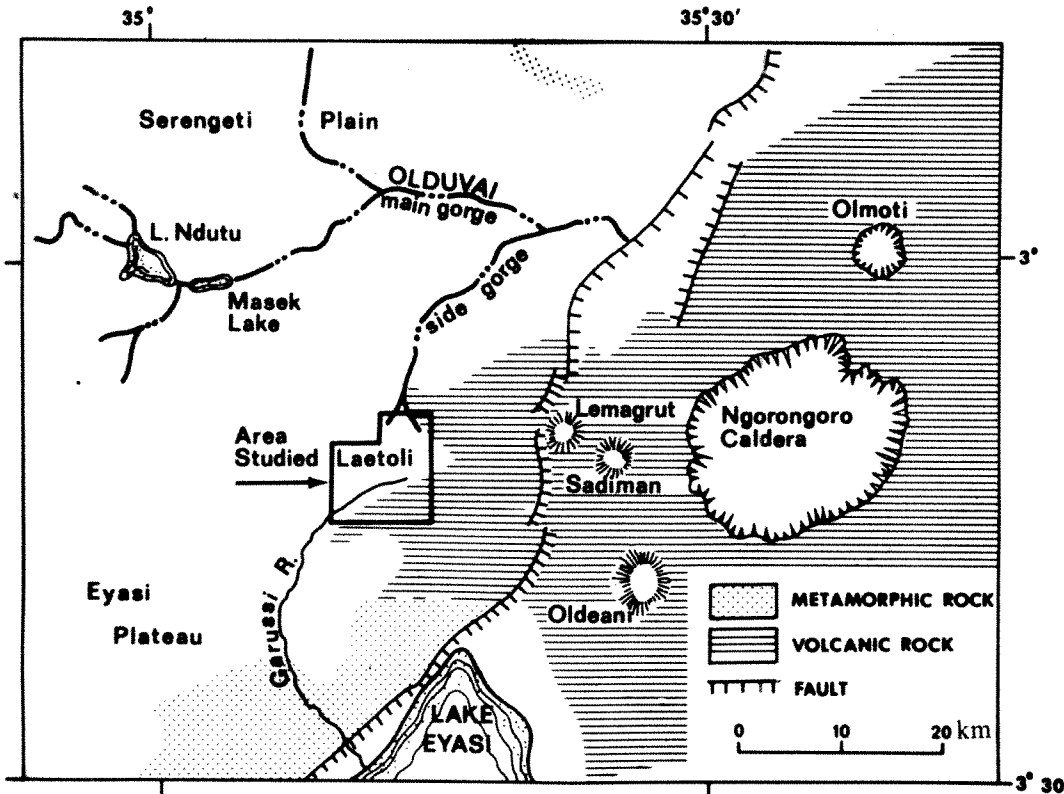


Fig. 1 Map of the southern Serengeti and volcanic highlands showing the position of the Laetoli area.

prevented erosion of the sand-sized lava globules by wind in this semi-arid climate in which 80–85% of the sediment was wind-worked. Natrocarbonatite ash would have dissolved incongruently in rainfall to yield soluble carbonates, which would have crystallised under the heat of the Sun to cement the ash layer in a few hours.

Footprints were made on at least six different surfaces but are by far the most common at two levels (Fig. 4). Prints of birds and

hares are common to all levels, but prints more than about 10 cm in diameter have been found only in the upper unit. Particularly striking is the number of elephant prints in the higher levels compared to their apparent absence below.

On the basis of the available evidence a tentative history of the footprint tuff begins near the end of the dry season and continues into the rainy season. The first showers of ash fell on a relatively bare, nearly flat landscape with scattered *Acacia* trees. The ash layers which constitute the lower unit were cemented by intermittent showers near the beginning of the rainy season. A few times between showers extruded eolian sediments, together with some of the air-fall ash, were redeposited by wind. The sharp contact at the base of the upper unit may mark the onset of the rainy season. Stratification in the upper unit is compatible with sheetwash produced by heavy showers, but is unlike that of the lower unit in which individual ash layers vary little in thickness over a distance of 5 km. The smaller amount of calcite in the upper unit may have resulted from either heavy rains or a smaller original content of carbonatite ash, or both. Thus, the abrupt appearance of footprints of elephants and other large animals in the upper unit may represent, at least in part, their migration which accompanies the rainy season.

The 1975 and 1976 field seasons at Laetoli were devoted to study of the geology of the area and the collection and excavation of fossil vertebrates and molluscs from the Laetolil Beds and later deposits. The age of the upper, fossiliferous part of the Laetolil Beds was established at 3.6–3.75 Myr (ref. 1).

While visiting the Laetoli camp during 1976 Dr Andrew Hill observed a number of depressions in the surface of a fine-grained tuff exposed in a river bed. These proved to be footprints of birds and mammals ranging from elephant and rhinoceros to carnivores and hares, which had been exposed by natural erosion and weathering.

The first site where footprints were observed (site A in Fig. 2) lies just south of the Garusi River in fossil Locality 6. An area of ~490 m² has been exposed by natural erosion and by excavation. To the south-west, at a second site (C) there are ~156 m² of the footprint-bearing tuff exposed. Both these localities were studied in detail in 1977. Five further areas where the footprint-bearing tuff is well exposed are also known but have not yet been completely studied.

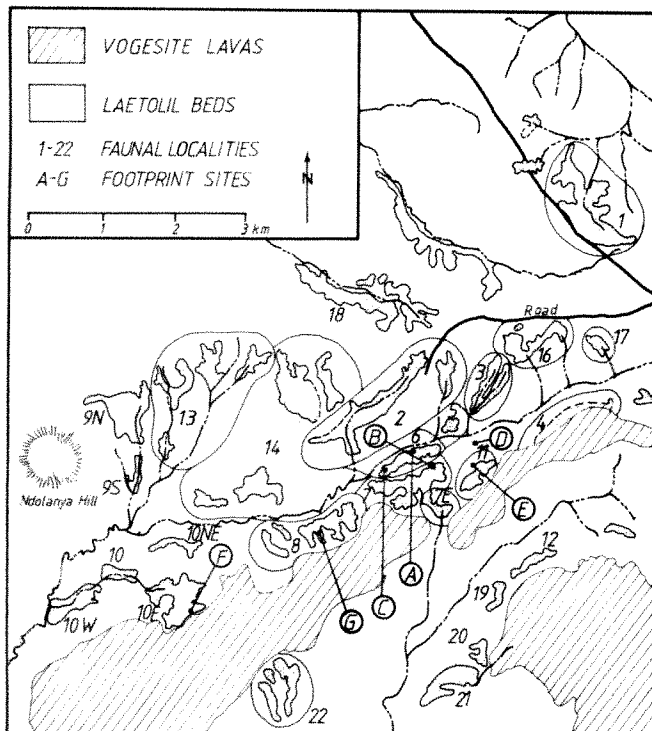


Fig. 2 Map of the fossil localities and footprint sites at Laetoli.

Table 1 Mean percentages of mammalian specimens from various sites

Bovidae		Equidae	4.4%
(of which 15.1% are		Suidae	3.6%
<i>Madoqua</i> , dik-dik)	43%	Proboscidea	3.4%
Lagomorpha	14.4%	Rodentia	3.3%
Giraffidae		Carnivora	3.1%
(including both a large			
and small species, as			
well as <i>Sivatherium</i>)	11.2%		
Rhinocerotidae	9.7%		

Avifauna, Cercopithecidae, Hominidae and Pedetinae were omitted from this list.

Deeply worn game tracks or pathways can be seen crossing the footprint areas at two sites. These were clearly used repeatedly by animals to reach some objective and on modern behavioural patterns it is likely that they were made by the game going to and from water holes.

Only a proportion of the animals represented in the fossil prints have been identified so far. Investigation of present-day game tracks in National Parks is underway to provide comparative information. In general, however, the fossil record agrees well with the footprints. A Musukuma tracker was employed to assist in identifications. He assisted considerably in identifying to family and generic levels, particularly in the case of Bovidae, which are commonly represented in both the footprint and fossil records.

A partial breakdown of the mammalian specimens recovered from the Laetoli Beds in 1975 was published¹ and may usefully be given here. Additional material was recovered in 1976 and 1977, but the overall proportions of various groups remain close to the first figures (Table 1).

The footprints that have been recorded are briefly described below, with notes on the fossil material where it seems to be related.

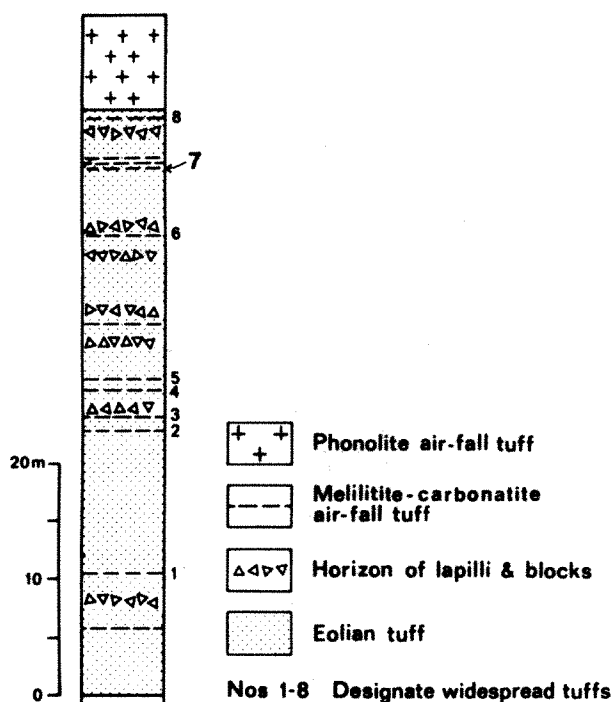


Fig. 3 Columnar section of the upper unit of the Laetoli Beds at Locality 1 showing air-fall tuffs and horizons of lapilli and blocks. The present report is based on studies of the lower part of Tuff 7, termed the Footprint Tuff. Tuffs 6, 7 and 8 were designated Tuffs a, b and c by Leakey *et al.*¹. The pholite tuff was designated Tuff d.

Diplopoda

A single track approximately 20 cm long is known at site (A).

Fossil record: a small fragment of fossilised centipede was found at Locality 4.

Avifauna

(1) *Struthio* sp. Two isolated prints at site (A).

(2) *Phasianidae*, cf. Guinea fowl. Numerous tracks occur at all sites, generally in trails of four or more. They compare closely with tracks of the living helmeted Guinea fowl, common in the Laetoli area today. Average length of eight fossil prints 62 mm, of nine modern prints 60 mm.

(3) Similar but smaller tracks, averaging 45 mm in length, possibly of francolin.

Fossil record: numerous fragments of ostrich eggshell are known but no skeletal remains. Clutches of eggs comparable in size to those of modern Guinea fowl have been found at Locality 10.

Primates

Cercopithecidae: Tracks are known at three localities.

At site (C) there is a single trail comprising six hind foot prints with a digit protruding to one side. Each of these prints is accompanied by a roughly circular impression, always to the left (Fig. 5). When first discovered these prints were interpreted as knuckle impressions, but more thorough cleaning has revealed traces of the palms of the hands and they are undoubtedly prints of the forefeet. In the hind feet the longest digit is central and the prints range in length from 20.1 to 14.7 cm with an average of 17 cm. The width varies from 10.9 to 8.1 cm with an average of 9.9 cm (excluding the great toe). Stride length varies from 34 to 46 cm with an average of 41 cm. (Stride is here interpreted as the distance between the posterior margin of successive heel prints of the same foot.)

The second trail is at site (D). It was made by a single animal and is 4 m long. There are prints of both hind and forefeet. All are lightly imprinted on a surface which was clearly wet and slippery when the animal walked over it. The average length of the hind prints is 14.5 cm and of the forefeet prints 11 cm. Stride length averages 27.7 cm. These prints are not only smaller than those at site (C) but are relatively broader, with very narrow heel impressions.

At site (F), in fossil Locality 10, there are at least four sets of tracks going in slightly different directions, as do the tracks of present-day baboons when they move in a troop. The average measurements of the hind feet in each of the four trails range from 15.2 to 10 cm and of the forefeet prints from 7.6 to 4 cm (excluding the great toe).

Fossil record: a number of cercopithecoid mandibles and teeth and a few postcranial fragments have been provisionally attributed to *Papio* sp. and a colobine. Most are as small or even smaller in size than those of a living female baboon, but two mandibular fragments, a calcaneum and the distal ends of a humerus and femur are considerably larger than in any living baboon and compare in size with *Theropithecus oswaldi*. The difference in size between the prints described above is compatible with that of the known fossils, although age and sex differences are factors to be considered.

Hominidae: Three trails believed to be hominid are known at sites (A) (Fig. 6) and (G) (Figs 7, 8), in fossil Localities 6 and 8. (1) At site (A) there are five prints in a trail 1.5 m long (Fig. 6). Natural erosion has almost entirely exposed two of the prints, but the remaining three are still filled with matrix of the overlying deposits. The exposed prints are short and broad, 15.5 cm long and 10.5 cm wide. The stride is also short with an average length of 31 cm. The gait was somewhat shambling, with one foot crossing in front of the other. Unlike the cercopithecoid prints, the longest digit is the great toe, situated as in the human foot.

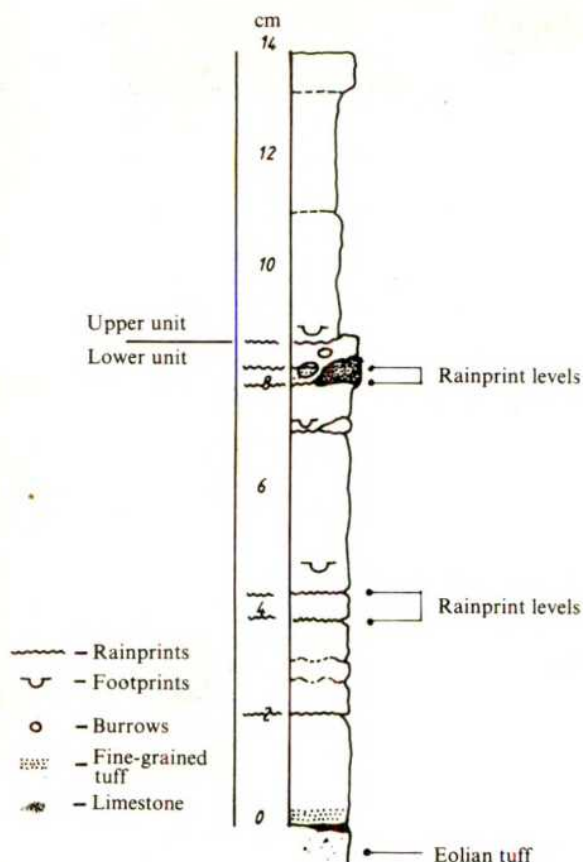


Fig. 4 Generalised columnar section for footprint tuff at site A.

It has been suggested that these prints might have resulted from the superposition of a hind on forefoot impression, or vice versa, either of a quadrupedal animal or a knuckle-walking primate. Careful examination of the original prints *in situ* reveals no indication of superposition, while the last interpretation invokes the hypothetical existence of an animal which is not present in over 5,000 fossil specimens.

(2) At site (G) there are trails left by two individuals travelling north (Figs 7–9). The trails are parallel and ~25 cm apart, too close for the hominids to have walked abreast. They followed the same line or pathway but it is possible that they did not pass by at the same time as there is a noticeable difference in the conditions of the two sets of prints. Those of the smaller individual are sharp and well defined, indicating a firm, compact surface, whilst those of the larger individual, with one notable exception, are blurred at the edges and enlarged, as would be the case if the surface had been dry and dusty. At one point the smaller individual appears to have paused and made a half-turn to the left before continuing in a northerly direction.

In a number of prints the original surface in which they were made has been eroded, leaving only indentations in the underlying deposits. Thus, the measurements of these prints do not accurately reflect the dimensions of the original impressions and are not included.

The site is now being excavated and the trails have so far been found to extend for a distance of 23.54 m. Trail 1 contains 22 prints and Trail 2 contains 12 prints. The average length/breadth of the prints in Trail 1 is 18.5 × 8.8 cm and in Trail 2 is 21.5 × 10 cm. Average stride length in Trail 1 is 38.7 cm and in Trail 2 is 47.2 cm. Note that the longitudinal arch of the foot is well developed and resembles that of modern man, and the great toe is parallel to the other toes (Figs 7, 8 and 9).

Fossil record: two mandibles, parts of 2 maxillae, partial deciduous and permanent dentitions as well as a partial infant

skeleton and a number of isolated teeth have been recovered from the Laetolil Beds. They bear considerable resemblances to the material collected from the Afar in Ethiopia, although the Ethiopian material is substantially later.

Leporidae

Innumerable tracks of hares occur at all sites.

Fossil record: mandibles and other remains attributed to *Serengetilagus* sp. are abundant.

Carnivora

(1) Viverridae, indet. Series of small carnivore prints with non-retractile claws occur at all sites. They are generally lightly imprinted and rather faint. Average length/breadth 34 × 27 mm. Other small, rounded prints without claw marks, suggest genets. Average length/breadth 28 × 27 mm.

Fossil record: a variety of Viverridae are known among the fossils, some were described by Dietrich³, those from the recent collections are being studied by Mme G. Petter.

(2) Hyaenidae. Hyenas have left a series of well-defined and relatively long trails containing large numbers of prints. Eight trails are known and two gaits are represented. Subject to research on living hyaenas, these appear to be a walk and a slow canter or lope. There is uniformity in size, depth and stride within each of the four trails measured, although it is evident that the animals varied in individual size. Average length/breadth/depth measurements:

Trail C.22 (19 prints)	122 × 94 × 12 mm
Trail C.27 (30 prints)	125 × 102 × 22 mm
Trail C.31 (11 prints)	102 × 87 × 13 mm
Trail C.39 (18 prints)	111 × 100 × 18 mm

Fossil record: in a preliminary report, M. G. Leakey noted the existence of three species of hyena from the Laetolil Beds: (1) *Hyaena bellax* (Ewer 1954); (2) *Hyaenictis* cf. *preforlex* (Hendey 1974); and (3) *Lycyaena* sp.

(3) Felidae. One trail of 12 prints at site (A), and several isolated prints appear to correspond in size to those of a serval



Fig. 5 Print of cercopithecoid fore and hind foot at site C.

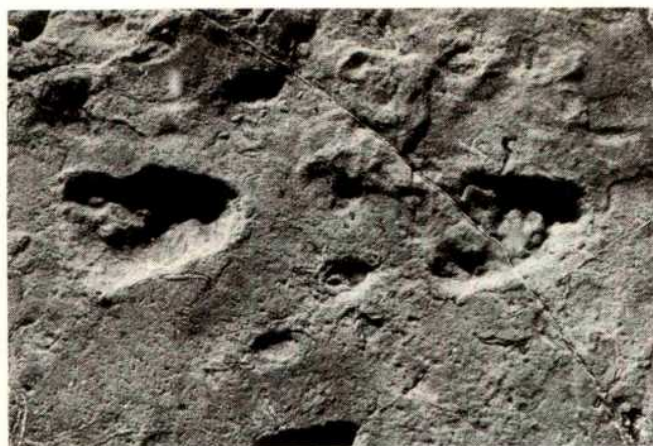


Fig. 6 Hominid footprints at site A.

cat. Average length/breadth/depth $65 \times 52 \times 13$ mm. Average length of stride 29 mm.

Fossil record: remains comparable to *F. serval* and *F. caracal* have been noted in the 1974–76 collections.

(4) *Machairodontinae*. Two prints at site (A), measuring 134×115 mm and 110×115 mm probably belong to a large sabre-tooth cat, as no other felid of comparable size occurs in the fossil material.

Fossil record: The presence of a large sabre-tooth cat, cf. *Homotherium* is indicated by two teeth.

Proboscidea

The proboscidean prints appear to belong to *Loxodonta exoptata* with the exception of one particularly well-preserved print in a trail of four at site (C) in which the phalanges and metapodials were more nearly vertical than in other known proboscidean prints and may be of a *Deinotherium*. The *Loxodonta* prints include a number made by juvenile animals as well as some that are usually large by present-day standards. The average for 15 measured prints, mostly adult, is $420 \times 346 \times 34$ mm.

Fossil record: both *Loxodonta exoptata* and *Deinotherium cf. bozasi* occur in the Laetolil Beds, but the latter is relatively scarce. A high proportion of the *Loxodonta* teeth are from juvenile animals.

Equidae

Only two equid trails are known. They have only recently been discovered and have not yet been fully studied. Both are at site (G), one on either side of the hominid trails but travelling in an opposite direction, to the south. The best preserved trail is 4.97 m long and contains 15 prints, nine of which can be measured. They range in length from 9.9 to 7.8 cm with an average of 8.6 cm and in width from 10.6 to 8.3 cm with an average of 8.8 cm. The animal appears to have changed gait during this trail.

Fossil record: numerous teeth, postcranial material and some incomplete mandibles have been found. All can be attributed to *Hipparion* sp.

Rhinocerotidae

Both *Ceratotherium* and *Diceros* must be represented among the many prints of rhinoceros, but no distinguishing features have been observed to date except on the basis of size. A trail at site (A) is one of the longest known, measuring 22 m in length. It contains 31 prints, 23 of which are double, with the hindfoot superimposed on the front. At the western end of the trail, where the animal changed gait, the prints become single and are irregularly spaced. Comparison with modern prints of *Diceros bicornis* at Olduvai show very close similarity. Average length/breadth/depth of double prints in trail: $416 \times 273 \times 31$ mm. Single prints, forefoot $246 \times 248 \times 25$ mm, hindfoot

$256 \times 228 \times 37$ mm. Isolated single fore and hind prints in the adjacent game trail are unusually large and may be of *Ceratotherium*. They measure $285 \times 310 \times 42$ mm and $400 \times 270 \times 33$ mm respectively.

Fossil record: on the basis of the early collections only *Ceratotherium* was believed to be present in the Laetoli fauna. But a skull of *Diceros* has now been found, as well as numerous teeth and some postcranial material.

Chalicotheriidae

Two Chalicotheres prints were found at site (C). They are deeply indented and measure $250 \times 155 \times 63$ mm and $243 \times 110 \times 46$ mm. The prints comprise impressions of three digits and of the palm. The digits have left symmetrical rounded grooves and the emplacement for a claw can be seen on one. The prints are 1.30 m apart.

Fossil record: a few specimens which can be referred to *Ancylotherium cf. hennigi* have been recovered. They include a calcaneum, astragalus and some phalanges.

Suidae

Thirteen suid prints at site (A) have been measured. They are comparable in size to prints of the living warthog. Average length/breadth/depth measurements are $55 \times 47 \times 11$ mm.

Fossil record: only two Suidae are known from the Laetolil Beds, *Potamochoerus* sp. and *Notochoerus euilus*. *Hylochoerus* and *Phacochoerus* were previously believed to be present in the early fauna, but during 1974–77 have only been found in the more recent deposits. On size, the prints are probably of *Potamochoerus*, as *Notochoerus* was a larger animal than the living warthog.

Giraffidae

(1) Three trails made by single animals are known. They consist of 6, 7 and 11 prints respectively. There are also 19 other prints attributed to giraffe. In one trail, the animal has dragged its feet after each step and left scuffed grooves up to 96 cm long. Average length/breadth/depth measurement for the prints in the trails are $190 \times 151 \times 29$ mm, $202 \times 144 \times 10$ mm, $211 \times 150 \times 17$ mm and $205 \times 150 \times 28$ mm. Averages for the isolated prints are $208 \times 155 \times 19$ mm.

Fossil record: the prints are of similar size to those of the modern giraffe and can be attributed to *G. jumae* which occurs as a fossil.

(2) Small giraffe cf. *G. stillei*. There are no prints which can positively be allocated to this animal, although its fossil remains are by far the most common giraffid. However, there are many prints, including three trails, which were identified as 'eland' by the tracker. No eland is known in the fossil fauna, nor is there any other bovid in the collection of a size suitable to have made these prints. For the present, it seems justifiable to assign these prints to the small giraffe. They are abundant at site (A), where there are three trails consisting of 21, 14 and 5 prints, as well as 72 isolated prints. The average length/breadth/depth measurements for the prints in the three trails are $140 \times 107 \times 14$ mm, $139 \times 104 \times 13$ mm and $128 \times 94 \times 16$ mm.

Fossil record: if the prints are correctly identified, they would belong to the animal originally named *Okapia stillei*, now known as *Giraffa stillei*.

(3) *Sivatherium*. No prints could be identified. The fossils consist mostly of isolated teeth and foot bones.

Bovidae

(1) Bovini cf. *Simatherium kohllarseni*. These prints consist of large, rounded and generally deeply indented tracks resembling those of the living African buffalo. They are clearly made by Bovini and can be attributed to *S. kohllarseni*, as this is the only bovine in the fossil record. The prints are represented by four trails containing 16, 12, 10 and 5 tracks, as well as 40 additional

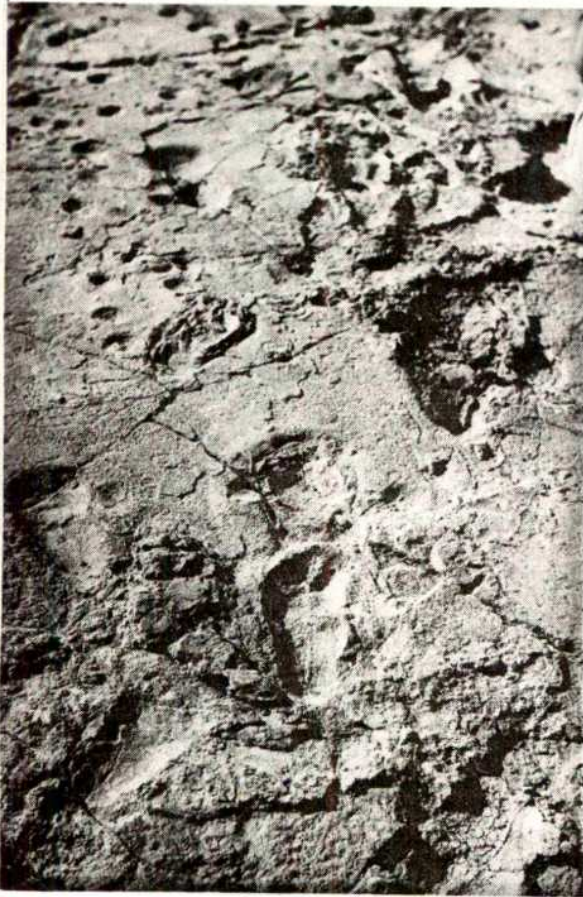


Fig. 7 Dual trail of hominid footprints at site G.

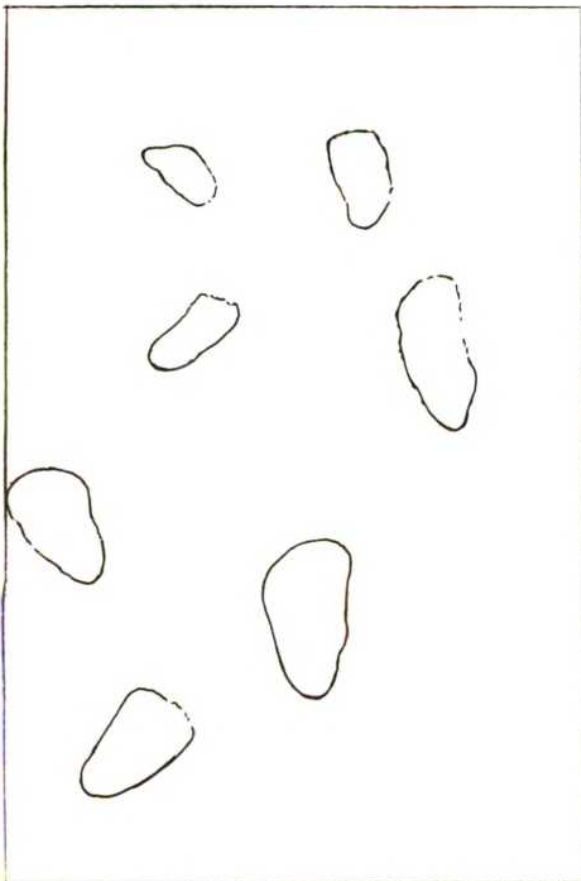


Fig. 8 Outline of footprints shown in Fig. 7.

prints, either single or in pairs. The average length/breadth/depth measurements for the 43 prints in the trails are $185 \times 149 \times 25$ mm.

Fossil record: a number of horn cores and teeth have been found, including a cranium associated with both horn cores. (2) *Hippotragus* sp. Seven single prints from sites (A) and (C) were identified by the tracker as 'roan antelope'. They are characterised by widely splayed, elongate hoof-marks. The average length/breadth/depth measurements are $109 \times 95 \times 16$ mm.

Fossil record: three horn cores and some teeth, collected during 1975 are believed to be a hippotragine and have

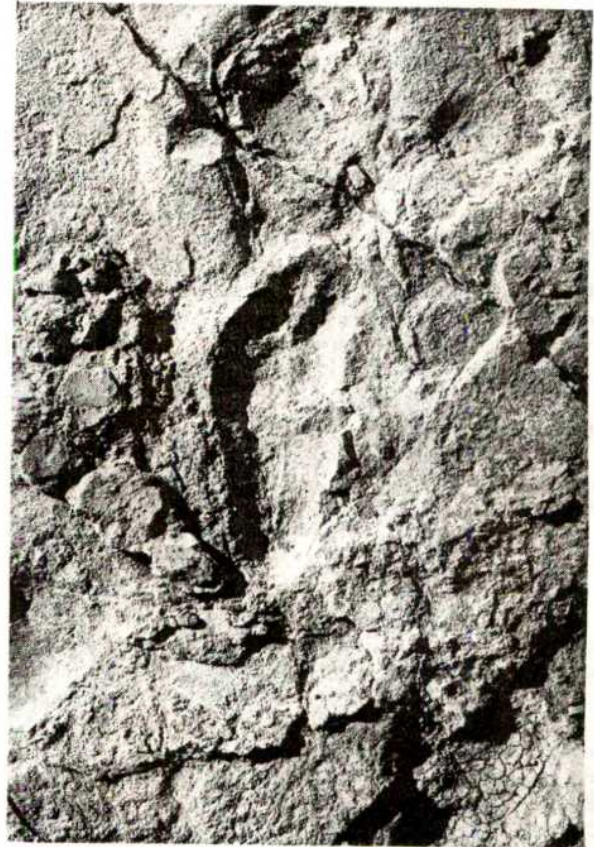


Fig. 9 Left footprint of larger individual in dual trail at site G.

provisionally been attributed to *Praedamalis deturi*. A horn core and teeth in the earlier collections may also belong to this species which is smaller than the living roan antelope.

(3) Alcelaphini. Eighteen prints at site (A) and two at site (C) were identified by the tracker as hartebeest. The prints at site (A) are both smaller and shallower than those at site (C). Average length/breadth/depth for the former are $80 \times 60 \times 14$ mm and for the latter $103 \times 80 \times 23$ mm.

Fossil record: *Parmularius* sp. has been identified on a frontlet with horn cores, teeth and other fragmentary horn cores. There is also a cranium collected in 1959 which has been attributed to a larger species of alcelaphine. A third species may also be represented.

(4) Small antelopes and gazelles. Prints of dik-dik (*Madoqua*) are rare although dik-dik are the most abundant single species of fossil Bovidae. This anomaly may be explained by the fact that dik-dik are one of the Bovidae who do not require to drink

water, subsisting on moisture from vegetation, while tracks of other Bovidae were probably made going to or from water holes.

Fossil record: *Neotragini*? and *Raphicerus* sp. (Steenbuck) are provisionally identified. *Madoqua* is very abundant. *Antilopini*: the gazelle appears to be *G. janenschii*.

Conclusions

The greater part of the fossil fauna from the Laetoli Beds is recorded in the fossil tracks. In all, over 20 taxa are represented. The preservation of the footprints can be attributed to an unusual and possibly unique combination of climatic, volcanic and mineralogic conditions. The available evidence indicates that the episode took place during a brief period, probably during the onset of a single rainy season which happened to coincide with the eruption of light ash showers from the nearby volcano Sadiman.

The locomotor pattern displayed by the trails of hominid footprints is still under examination but it is immediately evident that the Pliocene hominids at Laetoli had achieved a fully upright, bipedal and free-striding gait; a major event in the evolution of man which freed the hands for tool-making and eventually led to more sophisticated human activities. Moreover, evidence supplied by cranial parts of the somewhat later but related hominid fossils from the Afar in Ethiopia (dated between 2.6 and 3 Myr) indicates that bipedalism outstripped

enlargement of the brain. To have resolved this issue is an important step in the study of human evolution, as it has long been the subject of speculation and debate.

With the hands free and available for purposes not connected with locomotion it is perhaps surprising that no form of artefact has been found. But the concept of tool-making may well have been beyond the mental ability of these small-brained creatures. Any 'tools' or weapons used must have been solely of perishable materials as the Laetoli Beds are devoid not only of artefacts but of all stones other than volcanic ejecta.

Further exploration of sites and analysis of material will continue in 1979, but it is evident that Laetoli will give an unique perspective into hominid environment during Pliocene times.

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The chick ovomucoid gene contains at least six intervening sequences

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A 15-kilobase pair EcoRI chick DNA fragment, containing both the termination codon UGA and the 5'-portion of the structural ovomucoid gene, has been cloned in λ phage Charon 4A by in vitro packaging. Restriction mapping and electron microscopic analyses of this cloned DNA have revealed that the structural ovomucoid gene sequences are separated by at least six intervening sequences.

OVOMUCOID is a major egg-white protein, and its synthesis in the chick oviduct is regulated by the steroid hormones oestrogen and progesterone¹⁻³. Using a specific hybridisation probe comprised of a complementary DNA synthesised from purified ovomucoid messenger RNA, it has been demonstrated that the steroid-mediated induction of ovomucoid synthesis in the chick oviduct is preceded by the accumulation of its messenger RNA⁴. We have recently succeeded in the isolation and amplification of the structural ovomucoid gene by molecular cloning⁵. Using the cloned ovomucoid DNA as a specific hybridisation probe, we have shown that the kinetics of hormonal induction of ovomucoid mRNA accumulation in the chick oviduct is similar to that of ovalbumin mRNA⁶⁻⁹. To study the mechanisms by which steroid hormones regulate the expression of these oviduct genes, we attempted to define their sequence organisation within genomic chick DNA. We have recently reported the cloning of various *EcoRI* fragments of the natural ovalbumin

gene¹⁰⁻¹³. Detailed analysis of these cloned DNA fragments have shown that the structural ovalbumin gene is separated into at least eight portions by seven intervening sequences^{11,13,14}. The discovery of intervening sequences within eukaryotic genes has introduced a new level of complexity in the elucidation of the mechanism of gene expression. To substantiate the pattern for genetic organisation, we have attempted to clone the natural ovomucoid gene so that we might establish the sequence organisation of this gene.

The recombinant plasmid pOM100

Ovomucoid complementary DNA (cDNA_{om}) was previously synthesised from a partially purified ovomucoid mRNA preparation and cloned in the *PstI* site of pBR322 (ref. 5). Several recombinant clones have been demonstrated to contain inserted ovomucoid cDNA sequences by direct DNA sequencing. One of these recombinant plasmids, designated pOM100, contains about 650 base pairs of DNA, including the 3'-portion of the mRNA, and lacks about 125 base pairs at the 5'-terminus of the structural ovomucoid gene. A preliminary structure of the cloned ovomucoid cDNA (Fig. 1) has been established by restriction mapping⁵ and DNA sequencing (J.F.C., unpublished). It contains a *KpnI* site very close to the 5'-end of the inserted DNA. There is a unique *HincII* site and two cleavage sites each for *PstI* and *HaeIII* within the peptide coding portion of the gene. The ovomucoid structural gene also contains 138 base pairs of untranslated region at the 3'-terminus following the termination codon and there are unique *EcoRI* and *BamHI* sites

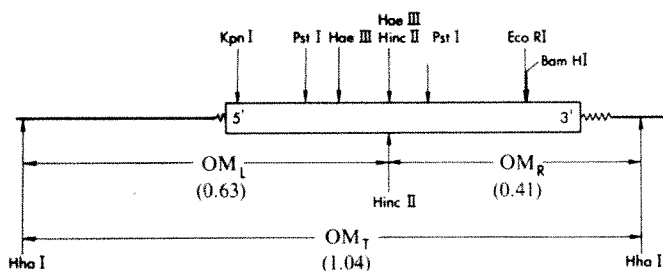


Fig. 1 Structure of pOM100 and the preparation of ovomucoid DNA fragments as specific hybridisation probes. □, Ovomuroid DNA insert; —, pBR322 DNA; wavy, dA-dT linkers. The various restriction endonuclease sites of the ovomucoid DNA insert are as indicated by the arrows. The numbers in parentheses indicate the size of the fragments in kilobases. 300 µg of pOM100 DNA was digested with *HhaI* (BRL) in standard conditions. The digestion products were separated by electrophoresis in a 1% preparative agarose gel. The 1.04-kilobase fragment (OM_T) was recovered from the gel by diffusion as described previously³⁶ and was further digested with *HincII* into two fragments of 0.63 (OM_L) and 0.41 (OM_R) kilobases, respectively. These fragments were again isolated by preparative gel electrophoresis. OM_T, OM_L and OM_R were labelled with [α -³²P]dCTP by nick translation to a specific radioactivity of about 2×10^8 c.p.m. per µg (ref. 15).

located in this region. The cloned ovomucoid DNA is not cleaved by the restriction enzymes *HhaI*, *HindIII*, *AvaI* or *TaqI*.

As the cloned ovomucoid DNA does not contain any *HhaI* cleavage site, a DNA fragment containing the entire inserted DNA fragment was prepared by *HhaI* digestion of pOM100 followed by preparative agarose gel electrophoresis (Fig. 1). This 1.04-kilobase DNA fragment (OM_T) was further digested with *HincII* into two DNA fragments containing the left (OM_L) and right (OM_R) halves of the cloned ovomucoid DNA. These DNA fragments were again separated from each other by preparative agarose gel electrophoresis and used as specific hybridisation probes after labelling by nick translation¹⁵.

Identification of chick DNA fragments containing the ovomucoid gene

Total chick DNA was digested with *EcoRI* and subjected to agarose gel electrophoresis. A large number of DNA fragments, heterogenous in size, can be visualised as a broad banding pattern in the gel after staining with ethidium bromide (Fig. 2A, lane a). The DNA fragments in the gel were transferred onto a nitrocellulose filter by the method of Southern¹⁶. The filter was treated according to the procedure of Denhardt¹⁷, and those DNA fragments on the filter containing structural ovomucoid gene sequences were identified by hybridisation with ³²P-OM_T followed by autoradiography¹⁸ (Fig. 2B, lane a). It is evident from this experiment that the structural ovomucoid gene sequences are contained within two *EcoRI* DNA fragments of 15 and 7 kilobases. The separation of the structural gene sequences into two *EcoRI* DNA fragments was not unexpected, as there is an *EcoRI* cleavage site within the structural gene. The order of these two DNA fragments was established by hybridising total *EcoRI*-digested DNA with OM_L and OM_R separately. The 15-kilobase DNA fragment hybridised with both probes, whereas the 7-kilobase DNA fragment hybridised with OM_R only (data not shown). Thus, the 15-kilobase DNA fragment contains the 5'-portion of the ovomucoid gene.

Cloning of the natural ovomucoid gene

The 15-kilobase DNA fragment was enriched from total *EcoRI*-digested chick DNA by preparative agarose gel electrophoresis. The gel was cut into 3-mm slices and DNA recovered from each gel slice was assayed for ovomucoid gene content by analytical gel electrophoresis followed by Southern hybridisation using

³²P-OM_T. The ethidium bromide staining pattern and autoradiogram of these individual DNA fractions are shown in lanes b-e of Fig. 2A and B, respectively. It is evident that the DNA fraction displayed in lane c is enriched for the 15-kilobase ovomucoid DNA fragment. This DNA fraction was thus used to clone the ovomucoid gene.

λ Phage Charon 4A contains three *EcoRI* cleavage sites and yields four DNA fragments on digestion with *EcoRI* (Fig. 2A, lane f). The two upper phage DNA arms were separated from the middle *EcoRI* DNA fragments by preparative agarose gel electrophoresis, and allowed to ligate in the presence of the DNA fraction enriched for ovomucoid DNA using T4 DNA ligase. The ligation mixture was used for cloning in a P3 facility by *in vitro* packaging according to the method of Sternberg *et al.*¹⁹ as modified by Faber, Kiefer and Blattner (personal communication). Briefly, λ lysogens were induced and cell extracts were made by alternate freezing and thawing and by sonication. The two protein extracts were mixed with purified λ A protein and incubated at 37 °C for 60 min and 5 µl of the ligation mixture in a final volume of 0.25 ml. The packaging mixture was plated in 12-µl aliquots in soft agar on 20 agar plates. Approximately 20,000 phage plaques were generated in this experiment. These phages were screened for the recombinant phage containing the ovomucoid gene by hybridisation with the OM_T probe using an amplification procedure as described previously¹⁰. Three phage plaques gave positive signals on the autoradiograms in this test. These phages were cultured individually and their DNA prepared. *EcoRI* digestion of DNA from any of the three phages produced two DNA fragments corresponding to the arms of Charon 4A and an additional 15-kilobase DNA fragment (Fig. 2A, lane g), which hybridised with the ovomucoid structural sequence OM_T on Southern analysis (Fig. 2B, lane g). This 15-kilobase DNA fragment of the natural ovomucoid gene was subsequently recloned in pBR322 in *Escherichia coli* χ 1776 and was designated pOM15.

Restriction mapping of the cloned 15-kilobase ovomucoid DNA

The 15-kilobase ovomucoid DNA (OM15) was excised from pBR322 DNA by *EcoRI* digestion of the recombinant plasmid pOM15 followed by preparative agarose gel electrophoresis. The excised ovomucoid DNA was further digested with a variety of other restriction endonucleases and those DNA fragments containing the left and right halves of the structural ovomucoid

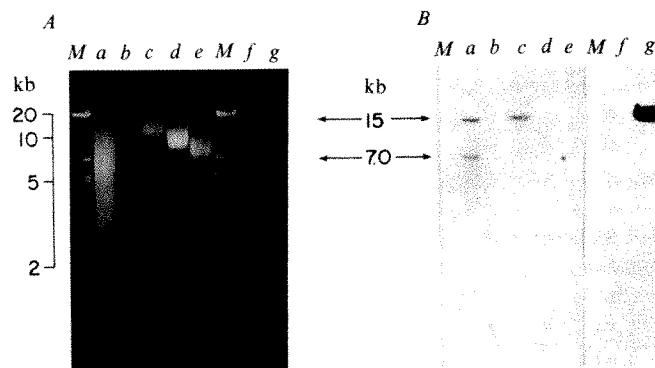


Fig. 2 Agarose gel electrophoresis of various DNA fractions and identification of ovomucoid DNA sequences by Southern¹⁶ hybridisation. A Shows the ethidium bromide stain of the gel and B the corresponding autoradiogram after the DNA was transferred from the gel to a nitrocellulose filter and hybridised to ³²P-OM_T. Lane M: 1 µg of *EcoRI*-digested wild-type λ phage DNA; lane a: 15 µg of *EcoRI*-digested total chick DNA; lanes b-e: 1.0 µg each of *EcoRI*-digested chick DNA extracted from various gel slices of a preparative gel; lane f: 0.5 µg of the *EcoRI*-digested Charon 4A λ DNA; lane g: *EcoRI*-digested DNA from one of the three recombinant phages that hybridised with ³²P-OM_T.

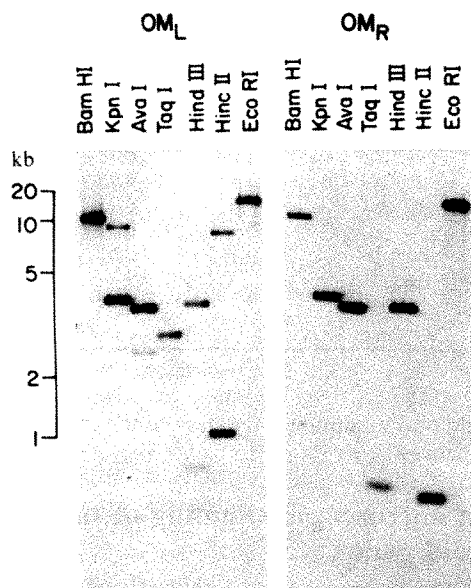


Fig. 3 Autoradiogram of 1% agarose gel containing OM15 DNA after digestion with various restriction endonucleases and Southern hybridisation. OM15 DNA was extracted from *EcoRI*-digested pOM15 by preparative agarose gel electrophoresis. 100 ng of purified OM15 DNA were digested by respective restriction endonucleases as indicated above each lane in the autoradiogram. Electrophoresis was carried out in 1% agarose gels and DNA was transferred to nitrocellulose. Hybridisation was carried out with ^{32}P -labelled OM_L (left panel) and ^{32}P -labelled OM_R (right panel), respectively.

gene sequences were identified by agarose gel electrophoresis followed by Southern hybridisation with the OM_L and OM_R probes, respectively. Some of the data are shown in Fig. 3 and summarised in Table 1. These data were used to construct a preliminary map of the natural ovomucoid gene as shown in Fig. 4. As expected, the OM15 DNA hybridised with both probes (Fig. 3). *HincII* digestion of OM15 DNA resulted in two DNA fragments of 8.0 and 1.1 kilobases that hybridised with OM_L and a unique 0.7-kilobase DNA fragment that hybridised with OM_R but not OM_L (Fig. 3). Thus, there is a *HincII* site (*HincII*_c) 0.7 kilobases to the left of the *EcoRI*_b site (Fig. 4). By virtue of the method used to prepare the two probes, the *HincII*_c site must be the unique *HincII* cleavage site present in the structural ovomucoid gene (Fig. 1). Limited sequence analysis has also established that the *EcoRI*_b site is the one present in the struc-

tural ovomucoid gene (data not shown). The unique *HincII* and *EcoRI* sites in the structural gene, however, are separated by only 0.2 kilobases of DNA. Thus, it seems that these two sites may be separated by an intervening sequence in genomic DNA. Furthermore, there should be a *HincII* site (*HincII*_a) 9.1 kilobases to the left of *HincII*_c. The *HincII*_b site, however, could be either 8.0 or 1.1 kilobases to the left of the *HincII*_c.

BamHI digested the OM15 DNA into a 10-kilobase DNA fragment hybridisable with both OM_L and OM_R (Fig. 3). In addition, there is a 0.3-kilobase fragment hybridisable only with OM_R, and resistant to *HincII* digestion (Table 1). Thus, there should be a *BamHI* site (*BamHI*_b) 0.3 kilobases to the left of *EcoRI*_b, and an additional *BamHI* site (*BamHI*_a) 10 kilobases to the left of *BamHI*_b (Fig. 4). The presence of these two *BamHI* sites predicts that a *HincII* digestion of the *BamHI* DNA fragments would result in a 8.0 and a 1.1 kilobase DNA fragment that would hybridise with OM_L only, and that the 0.7-kilobase *HincII* DNA fragment would be digested by *BamHI* into two fragments of 0.4 and 0.3 kilobases, respectively. These are exactly the results observed (Table 1). As there is no *BamHI* cleavage site between the *HincII* and *EcoRI* sites in the structural gene, these results confirm that there must be at least one intervening sequence separating these two restriction sites in the structural ovomucoid gene.

HindIII digested OM15 DNA into a 3.5-kilobase DNA fragment hybridisable with both OM_L and OM_R probes, as well as a 0.9-kilobase DNA that hybridised with OM_L only (Fig. 3). Thus, there should be a *HindIII* site (*HindIII*_b) 3.5 kilobases to the left of *EcoRI*_b, and an additional *HindIII* site (*HindIII*_a) 0.9 kilobases to the left of *HindIII*_b (Fig. 4). Indeed, the 3.5-kilobase DNA is cleaved by *HincII* into a 1.1 and a 0.7 kilobase fragment hybridisable with OM_L and OM_R, respectively, which are the same DNA fragments observed by digestion of OM15 DNA with *HincII* alone (Table 1). This experiment further suggests that the *HincII*_b site is 1.1 kilobases to the left of *HincII*_c and that there is no structural gene sequence between *HindIII*_b and *HincII*_b. In addition, there should be some structural gene sequence within the 0.9-kilobase *HindIII* DNA fragment (Fig. 4). The fact that the structural gene contains no *HindIII* cleavage site suggests that there may be more intervening DNA sequences within the natural ovomucoid gene.

KpnI digested the OM15 DNA into a 3.7-kilobase fragment hybridisable with both OM_L and OM_R and an additional 8.5-kilobase DNA fragment hybridisable only with OM_L (Fig. 3). Hence, there should be a *KpnI* site (*KpnI*_b) 3.7 kilobases to the left of *EcoRI*_b, and another *KpnI* site (*KpnI*_a) 8.5 kilobases to the

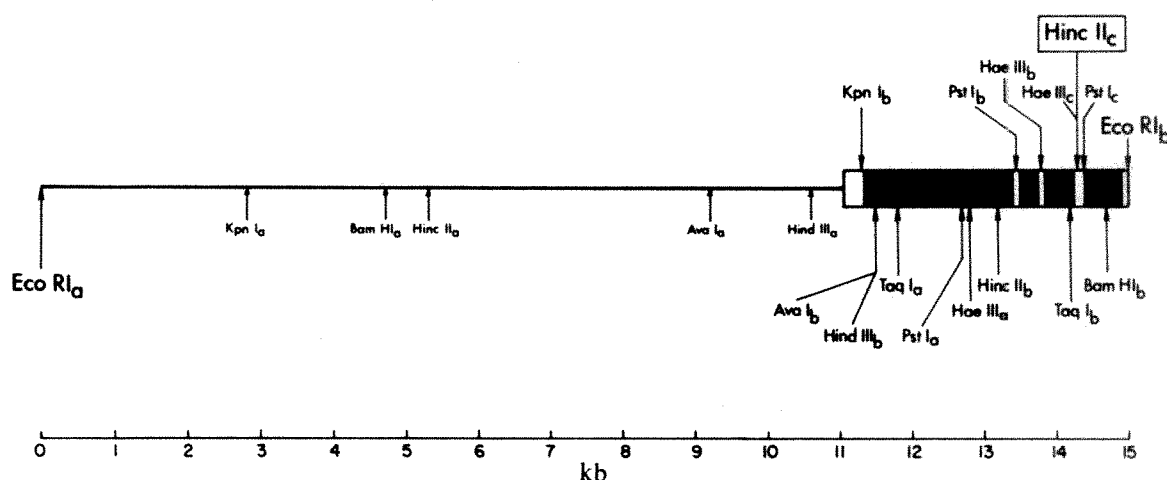


Fig. 4 Structural organisation of the natural ovomucoid gene (OM15). Ovomucoid structural sequences are represented by $\square$, intervening sequences by $\blacksquare$, and flanking DNA sequences by — . The arrows indicate various restriction endonuclease sites on the ovomucoid gene. Those above the schematic drawing indicate restriction sites present in the structural ovomucoid gene and those below are sites within the intervening or flanking DNA sequences. There are additional restriction sites present in the left-hand portions of OM15 DNA, but their positions have not been mapped and are therefore not shown.

Table 1 Sizes of ovomucoid structural sequence-containing fragments after restriction endonuclease digestion of OM15

	Size after digestion (kilobases)								Probe used
	<i>EcoRI</i>	<i>HindIII</i>	<i>BamHI</i>	<i>TaqI</i>	<i>AvaI</i>	<i>KpnI</i>	<i>HaeIII</i>	<i>PstI</i>	
- <i>HincII</i>	15.0	3.5	10.0	2.4	3.5	8.5	1.0	0.75	OM _L
		0.9			2.3	3.7	0.5	0.75	
+ <i>HincII</i>	8.0	1.1	8.0	1.1	2.3	6.0	0.6	0.7	OM _L
	1.1	0.9	1.1		1.1	1.1	0.5	0.3	
- <i>HincII</i>	15.0	3.5	10.0	0.75	3.5	3.7	0.7	0.65	OM _R
			0.3						
+ <i>HincII</i>	0.7	0.7	0.4	0.7	0.7	0.7	0.7	0.65	OM _R
			0.3						

left of *KpnI*_b. Double digestion of OM15 DNA with *KpnI* and *HincII* yielded a 0.7-kilobase fragment hybridisable with OM_R as expected, as well as two DNA fragments of 6.0 and 1.1 kilobases that hybridised with OM_L as predicted from the map (Fig. 4). Thus, the *KpnI*_b site seems to be the one that is present towards the 5'-terminus of the structural ovomucoid gene (Fig. 1). Because this *KpnI* site is separated from the *HincII*_c site by a *HindIII* site in genomic DNA but not in the structural gene, there should be a second intervening sequence separating these sites in the natural ovomucoid gene.

The OM15 DNA was digested by *HaeIII* into a unique 0.7-kilobase DNA fragment hybridisable with OM_R and resistant to *HincII* digestion (Table 1). Hence, there is a *HaeIII* site (*HaeIII*_c) about 0.7 kilobases to the left of *EcoRI*_b, which could be one of the two *HaeIII* sites located near the unique *HincII* site in the structural gene (Fig. 1). As there are two *HaeIII* fragments of 1.0 and 0.5 kilobase that hybridised with OM_L, there should be a *HaeIII* site (*HaeIII*_a) 1.5 kilobases to the left of *HaeIII*_c. Furthermore, the 0.5-kilobase *HaeIII* fragment seem to be resistant to *HincII*, whereas the 1.0-kilobase fragment is cleaved by this enzyme to a 0.6-kilobase fragment hybridisable with OM_L (Table 1). Thus, the *HaeIII*_b site should be located 0.5 kilobases to the left of *HaeIII*_c. Otherwise, the 1.0-kilobase *HaeIII* DNA fragment would be resistant to *HincII* and the 0.5-kilobase fragment would be digested to a 0.4- and a 0.1-kilobase fragment. Furthermore, this *HaeIII*_b site may be the second *HaeIII* site present in the structural gene (Fig. 1), as both the 0.5- and 1.0-kilobase *HaeIII* DNA fragments hybridised with OM_L. By similar analysis, a *TaqI* site (*TaqI*_b) has been located between the *HaeIII*_b and *HaeIII*_c sites (Fig. 4). As the *HaeIII* sites in the structural gene are not separated by a *TaqI* site, there may be a third intervening sequence within the ovomucoid gene.

PstI digested the OM15 DNA into a unique 0.65-kilobase fragment hybridisable with OM_R, and resistant to further digestion by *HincII* (Table 1). Hence, a *PstI* site (*PstI*_c) should be located 0.65 kilobases to the left of *EcoRI*_b and could be the *PstI* site located 0.05 kilobases to the right of the unique *HincII* site present in the structural gene (Fig. 1). Although *PstI* digestion yielded only one 0.75-kilobase band when hybridised with OM_L (Table 1), there must be at least two DNA fragments of the same size because one of the fragments was digested by *HincII* into a 0.3-kilobase fragment and the other fragment remained resistant. Hence, there should be two *PstI* sites, 0.75 kilobases apart, to the left of *PstI*_c. Moreover, the *PstI*_b site may be the other *PstI* present in the structural gene (Fig. 1), because the 0.75-kilobase *PstI* fragment would have been digested to one of 0.45 kilobases instead of a 0.3-kilobase fragment if *PstI*_a were the site present in the structural gene instead. The finding that the *PstI*_b and *HaeIII*_b sites are separated by 0.3 kilobases in genomic DNA but by only about 0.07 kilobases in the structural gene (Fig. 1), suggests that there may be a fourth intervening sequence within the ovomucoid gene.

These results indicate that the structural ovomucoid gene sequences are not contiguous and are apparently separated into several portions by multiple intervening sequences within the chick genome. The existence of four intervening sequences as established by limited restriction analyses of the cloned OM15

DNA should be a minimum estimation of the number of intervening sequences.

Electron microscopic mapping of the ovomucoid gene

Although intervening sequences within structural genes can be detected by restriction enzyme mapping, a complete map requires other methods independent of the chance occurrence of restriction sites. Short structural gene regions may be missed due to the relative instability of the hybrids formed during hybridisation, resulting in weak signals on Southern filter analysis. In an attempt to characterise the structure of the ovomucoid gene more fully, hybrid molecules formed between ovomucoid mRNA and the cloned 15-kilobase ovomucoid DNA were examined by electron microscopy.

DNA was thermally denatured and incubated with ovomucoid mRNA in conditions that permit only RNA:DNA hybridisation but not DNA:DNA reassociation. Thus, homologous regions between the mRNA and the cloned DNA would appear as double-stranded structures and between the double-stranded regions, DNA sequences non-homologous with the mRNA would appear as single-stranded loops. Figure 5 shows such a hybrid molecule formed between ovomucoid mRNA and the cloned OM15 DNA. Six intervening DNA loops of various sizes are apparent in this molecule, all of which were present in 10 individual hybrid molecules examined. The relative positioning of these loops was established in a histogram as shown in Fig. 6. All the loop structures occurred at one DNA

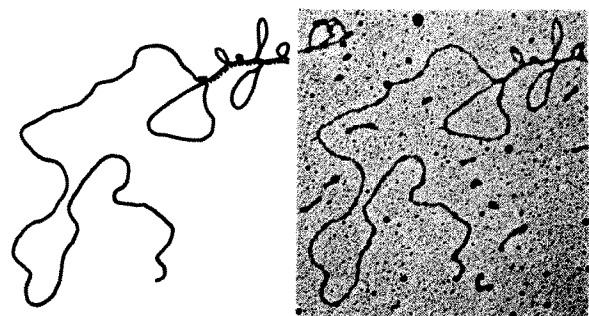


Fig. 5 Electron micrograph and line drawing of a hybrid molecule formed between the ovomucoid gene (OM15) and ovomucoid mRNA. Hybridisation was carried out using 10 $\mu\text{g ml}^{-1}$ of OM15 DNA and 20 $\mu\text{g ml}^{-1}$ of ovomucoid mRNA in 70% deionised formamide containing 100 mM Tris-HCl, pH 7.6, 10 mM sodium EDTA and 150 mM NaCl. The mixture was heated at 80 °C for 5 min to denature the DNA and hybridisation was carried out at 43 °C for 2 h. The samples were immediately prepared for electron microscopy as follows. The hybridisation mixture containing 0.1–0.5 μg of nucleic acids was diluted into 100 μl of a solution containing 70% formamide, 0.1 M Tris-HCl, pH 8.4, 0.01 M sodium EDTA and 100 $\mu\text{g ml}^{-1}$ of cytochrome *c*. The mixture was spread onto a hypophase of distilled water. Samples were collected on collodion-coated 300-mesh copper grids, stained with uranyl acetate, rotary shadowed with platinum-palladium, and examined at 80 kV on a Joel 100 C electron microscope. —, OM15 DNA; ----, mRNA_{om}.

terminus and occupied about one-third of the total length of the OM15 DNA molecule. This observation agrees with the restriction map as shown in Fig. 4. Two additional intervening sequences were detected in these studies. The short intervening sequences C and F were not detected by restriction mapping analysis.

Comments

We have reported the cloning of the chick ovomucoid gene in λ phage Charon 4A by *in vitro* packaging. The great efficiency of *in vitro* packaging has enabled us to limit purification of the gene to approximately 10-fold by preparative agarose gel electrophoresis and only nanogram quantities of DNA are required. *In vitro* packaging thus makes it possible to eliminate laborious DNA enrichment procedures before cloning.

Four intervening sequences were detected in the ovomucoid gene by limited restriction enzyme mapping. Electron microscopic analysis of RNA:DNA hybrids between ovomucoid mRNA and the 15-kilobase cloned ovomucoid gene fragment showed six loops corresponding to intervening sequences. Both methods have advantages in locating intervening sequences. Restriction mapping is more quantitative in that fragment sizes

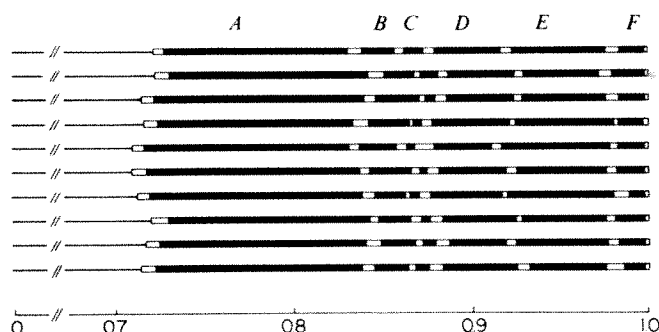


Fig. 6 Histogram of 10 individual hybrid molecules formed between OM15 DNA and ovomucoid mRNA in conditions described in the legend to Fig. 5. The molecules were measured using a map measure, and the sizes and positions of the loops were plotted on a linear scale of 0 to 1, with 1.0 representing 15 kilobases of DNA. Ovomucoid structural sequences are represented by $\square$, intervening sequences by $\blacksquare$, and flanking DNA sequences by —.

and position can be correlated with known distances in the structural sequence. However, in the absence of favourably placed restriction sites, some intervening sequences may be missed. Electron microscopy permits more direct analysis of intervening sequences; in our studies, it has detected two additional intervening sequences, but the overall structure was in good qualitative agreement with the limited restriction map.

There are at least six intervening sequences in the ovomucoid gene. This is, necessarily, a minimum number; nucleotide sequence data are required for a complete structural charac-

terisation. A short structural sequence at the 5'-end may not form a stable hybrid in the conditions used. A 5.5-kilobase potential RNA precursor has been detected in nuclei from stimulated oviducts (see accompanying paper²⁰). Preliminary RNA-excess hybridisation experiments with fragments of the cloned gene have indicated that an additional 1.5 kilobases of DNA to the left of the unique *Kpn*I site may be transcribed *in vivo*. These observations suggest that the difference in size between the gene (Fig. 4) and the high molecular weight RNA transcript may be due to an undetected intervening sequence at the 5'-end. The size of the ovomucoid gene then, including the structural sequence beyond the *Eco*RI site, is about 5.5 kilobases, which is in good agreement with the estimated size of the largest RNA precursor. As the *Eco*RI site in the structural gene is a few nucleotide pairs to the right of the termination codon, all six intervening sequences observed in OM15 DNA are situated within the peptide-coding portion of the ovomucoid gene.

Although intervening sequences have been observed in several other cloned eukaryotic genes^{10-14,21-29}, the frequency of occurrence of these sequences in the ovomucoid gene is striking. Even in the absence of additional intervening sequences in the 3'-untranslated region, there is a minimum of six such sequences within a structural gene only about 750 nucleotide pairs long. At 1 intervening sequence per 125 nucleotide pairs of structural gene sequence, it represents the highest frequency of intervening sequences observed among all eukaryotic genes examined to date.

With regard to the expression of the ovomucoid gene *in vivo*, the entire gene is apparently transcribed into a large RNA transcript which is subsequently spliced to yield the mature mRNA. The identification of high molecular weight ovomucoid RNA precursors by methylmercury hydroxide agarose gel electrophoresis followed by transfer of RNA to diazobenzyloxymethyl paper and hybridisation with labelled pOM100 is presented in the accompanying paper²⁰. In this regard, the ovomucoid gene is similar to other genes with intervening sequences, such as those coding for chick ovalbumin³⁰, mouse globins³¹, mRNAs of animal viruses^{32,33} and yeast tRNAs^{34,35}. Finally, studies of the structures and the expression of the ovalbumin and ovomucoid genes should further our understanding of the regulation of these chick oviduct genes.

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Note added in proof: Further electronmicroscopic mapping of the gene has indicated that there is a seventh intervening sequence. A short structural gene segment has been detected in intervening sequence A.

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Identification of potential ovomucoid mRNA precursors in chick oviduct nuclei

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Denaturing gel electrophoresis of chick oviduct nuclear RNA reveals multiple species of RNA that are 1.5–5 times larger than ovomucoid mRNA. By analogy with ovalbumin RNA processing, these data suggest that ovomucoid mRNA is derived from a primary transcript that contains intervening sequences.

ADMINISTRATION of oestrogen to immature chicks induces the accumulation of the mRNAs for the egg-white proteins ovalbumin^{1–7}, ovomucoid^{8,9}, conalbumin¹⁰ and lysozyme⁸ in oviduct target cells. Of these four mRNAs, that of ovalbumin is the most abundant and has therefore been most extensively studied. Several lines of evidence^{7,11,12} suggest that ovalbumin mRNA (mRNA_{ov}) is derived from a large nuclear precursor RNA which is a transcript of the chick ovalbumin gene which is interrupted by at least seven regions of intervening sequence DNA^{13,14}. RNA sequences complementary to the intervening sequence regions were detected in oviduct nuclei⁷, but not in oviduct polysomes¹¹, indicating that they are not transported from the nucleus. In addition, multiple species of nuclear RNA larger than ovalbumin mRNA have been fractionated by gel electrophoresis and have been shown to contain both ovalbumin structural and intervening sequences⁷. We report here that there are also multiple species of nuclear RNA larger than the mRNA for ovomucoid. The experiments have been made possible because we have recently cloned complementary DNA (cDNA) to ovomucoid mRNA (mRNA_{om}) and prepared a DNA fragment that contains the cDNA sequence¹⁵. Using this specific probe, we have analysed the distribution of ovomucoid mRNA sequences in oviduct nuclear RNA that has been fractionated by agarose gel electrophoresis in completely denaturing conditions.

Identification of multiple nuclear species of ovomucoid sequence-containing RNA

To search for species of ovomucoid RNA larger than mature mRNA_{om}, nuclear RNA from oviducts of oestrogen-stimulated chicks was subjected to agarose gel electrophoresis in the presence of the denaturing agent, methylmercury hydroxide. Following transfer of the RNA from the gel onto diazobenzyloxymethyl (DBM) paper and hybridisation to a ³²P-labelled ovomucoid cDNA probe, multiple discrete bands containing ovomucoid structural sequences were observed (Fig. 1, lane A). The band with the greatest mobility co-migrated with mature mRNA_{om} (Fig. 1, lane B). Five major bands were significantly larger than ovomucoid mRNA (1,100 nucleotides). In addition, two minor bands were detected in certain nuclear RNA preparations (Fig. 2, lane C). As shown in Table 1, the sizes of the seven species of high molecular weight (MW) ovomucoid RNA, which were labelled a–g, ranged from 1.5 to 5 times the size of ovomucoid mRNA. The mobilities of the prominent ovomucoid RNAs (bands a, b, d, e, g) were reproducibly observed in all preparations of oviduct nuclear RNA that were analysed. Based on its intensity, band g, which has 1,700 nucleotides, seems to be the most abundant species of high MW ovomucoid RNA. Based on the radioactivity associated with each band, as determined by liquid scintillation counting, we estimate that 20–45% of the nuclear ovomucoid sequences reside in the forms higher in MW than mRNA_{om}.

High MW species of RNA that contain ovomucoid sequences were not found in oviduct cytoplasm, where only mature mRNA_{om} was detected (Fig. 1, lane C). In addition, the presence of nuclear high MW ovomucoid RNA, as well as that of mature mRNA_{om}, was tissue specific. No ovomucoid bands were detected in the nuclear RNA from spleen or liver that was isolated from oestrogen-stimulated chicks (Fig. 1, lanes D, E). Thus, high MW ovomucoid RNA, as well as mRNA_{om}, is present in significant amounts only in those tissues that actively synthesise egg-white proteins.

Although the conditions used for electrophoresis were rigorously denaturing^{7,18}, an attempt was made to generate high MW species of ovomucoid RNA by mixing liver nuclei and oviduct cytoplasm (both prepared from chicks stimulated with diethylstilboestrol (DES)) and then extracting the RNA from the mixture. However, mRNA_{om} was the only species detected (Fig. 1, lane F). Thus, as was observed previously for ovalbumin RNA⁷, the high MW species of ovomucoid RNA do not seem to be due to aggregation of mRNA_{om} with itself or other RNA molecules.

Comparison of the nuclear species of ovalbumin and ovomucoid RNA

To permit an exact comparison of the bands of high MW ovalbumin and ovomucoid RNA, oviduct nuclear RNA that had been electrophoresed and transferred to DBM paper was first hybridised to a ³²P-labelled probe specific for ovalbumin RNA sequences (Fig. 2, lane A). Following autoradiography, the ³²P-ovalbumin probe was eluted (Fig. 2, lane B) and the same DBM paper rehybridised, except this time to the ³²P-ovomucoid probe (Fig. 2, lane C). Two of the species of ovalbumin RNA (Fig. 2a,b) had significantly higher MWs than the largest species of ovomucoid RNA. Of all the bands observed, only ovalbumin RNA f and ovomucoid RNA b shared the same electrophoretic mobility. As the intensity of the bands differed, however, it is unlikely that they represent the same species of RNA. Thus, ovalbumin and ovomucoid RNA do not seem to have any of the high MW species in common.

Table 1 Sizes of the ovomucoid sequence-containing RNA from chick oviduct nuclei

RNA _{om} species	Relative mobility (to mRNA _{om})	Length (nucleotides)
a	0.25	5450 ± 120
b	0.45	3100 ± 60
c	0.48	2750 ± 60
d	0.55	2500 ± 60
e	0.59	2300 ± 50
f	0.65	2000 ± 30
g	0.76	1700 ± 75
mRNA _{om}	1.0	1100 ± 110

Relative mobility was calculated as the average of 6 individual experiments. Length was calculated by comparison with the electrophoretic mobility of RNA and DNA standards. RNA standards were chicken 27S rRNA (4,530 nucleotides), chicken 18S rRNA (2,076 nucleotides), *Escherichia coli* 23S rRNA (3,129 nucleotides) and *E. coli* 16S rRNA (1,550 nucleotides). DNA standards were obtained by digestion of λ DNA with *EcoRI* (21,746, 7,524, 5,920, 5,523, 4,793 and 3,380 nucleotides) or with *HindIII* (23,222, 9,730, 6,460, 4,492, 2,301, 2,000 and 682 nucleotides). In the conditions of electrophoresis (in the presence of methylmercury hydroxide), DNA becomes single stranded and its mobility is similar to that of RNA. Error limits indicate the standard deviation.

Based on the relative intensities of the bands, the concentration of ovalbumin mRNA, as expected, is greater than that of ovomucoid mRNA. By contrast, the high MW species of ovomucoid RNA are apparently more abundant in oviduct nuclei than the high MW species of ovalbumin RNA. It is of further interest that a significant portion of the nuclear ovalbumin RNA sequences was smaller than mRNA_{om}. By contrast, no significant degradation of mRNA_{om} was observed.

Identification of ovomucoid intervening sequences in high MW nuclear RNA

Restriction endonuclease mapping and electron microscopic analysis of a cloned DNA fragment reveal that, like the ovalbumin gene, the natural chick ovomucoid gene has at least six regions of intervening sequence¹⁹. Using as a hybridisation probe a clone with a DNA insert that seems to contain all the ovomucoid structural and intervening sequences, it has been possible to determine that the nuclear ovomucoid RNA molecules larger than the mature mRNA_{om} contain sequences complementary to the intervening sequences of the ovomucoid gene. Apart from band *f*, all the bands of ovomucoid nuclear RNA, including mature mRNA_{om}, that hybridised to the ovomucoid cDNA probe were again detected when hybridised to the natural ovomucoid gene probe (Fig. 3, lane A). In addition, two bands (located between bands *a* and *b*) that were extremely faint after hybridisation to the ovomucoid cDNA probe were clearly detected by the natural ovomucoid gene probe. To detect only those bands that contained intervening sequences, it was necessary to hybridise in the presence of excess unlabelled ovomucoid mRNA to compete out the structural sequences in the natural gene. In these hybridisation conditions, detection of the band corresponding to mature mRNA_{om} was eliminated (Fig. 3, lane B). In contrast, all the bands of high MW ovomucoid RNA were still detected. This indicates that all the species of ovomucoid RNA larger than mature mRNA_{om} contain sequences complementary to the intervening sequences of the ovomucoid gene.

Oestrogen-induced accumulation of high MW species of ovomucoid RNA

The accumulation in the oviduct nucleus of species of ovomucoid RNA larger than mature mRNA_{om} was dependent on oestrogen. From 20 µg of oviduct nuclear RNA isolated from chicks that had been withdrawn from DES treatment for 14 d, neither high MW ovomucoid RNA nor mRNA_{om} was detected (Fig. 4, left, lane W). Thus, in agreement with our previous studies using *R₀* analysis⁹, the amount of ovomucoid RNA sequences in withdrawn oviduct nuclei is very low. When 100 µg of withdrawn oviduct nuclear RNA was analysed, a faint band of mRNA_{om} was observed (Fig. 4, right, lane W). Thus, the few ovomucoid RNA sequences that remain after hormone withdrawal seem to be present mainly in the form of mature mRNA_{om}.

As soon as 1 h after administration of DES to hormone-withdrawn chicks, both mature mRNA_{om} and high MW ovomucoid RNA species have accumulated. Analysis of 20 µg of nuclear RNA revealed the accumulation of the most abundant high MW species, band *g* (Fig. 4, left), whereas analysis of 100 µg of RNA revealed the accumulation of the three most predominant species larger than mRNA_{om}, bands *b*, *e* and *g*, (Fig. 3, right). Species *a*, *c*, *d* and *f* were not detected, probably because these are the least abundant of the high MW ovomucoid RNA molecules.

After short periods (1–2 h) of secondary oestrogen stimulation, the relative intensity of the ovomucoid RNA bands (Fig. 4, right) was similar to that observed after chronic oestrogen stimulation (Fig. 1, lane A; Fig. 2, lane C). The bands cor-

responding to species *g* and mRNA_{om} were of roughly equal intensity, whereas that of species *e* was of lesser intensity and that of species *b* even less. After longer periods (>4 h) of secondary stimulation, this pattern shifted. The concentration of mRNA_{om} increased at each time point so that 16 h after the DES injection a very intense mRNA_{om} band was observed. By contrast, the concentration of the most abundant high MW ovomucoid RNA species, band *g*, increased only slightly during this period. In addition, no significant increases in the concentration of ovomucoid species *e* were observed. Thus, 4–16 h after DES injection, mature mRNA_{om} was clearly more abundant than any of the high MW ovomucoid RNA species.

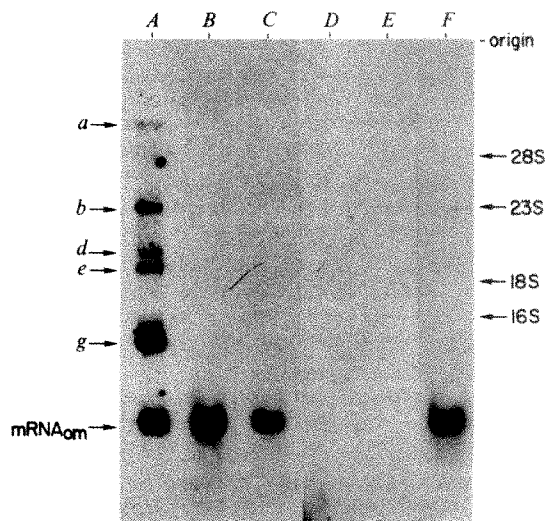


Fig. 1 Identification of ovomucoid mRNA sequences in high MW nuclear RNA. Nuclear or cytoplasmic RNA was isolated from the indicated tissues from White Leghorn chicks stimulated chronically with diethylstilboestrol (DES) as described previously⁷. Ovomucoid mRNA (~40% pure) was prepared from hen oviduct as previously described¹⁵. RNA was then subjected to agarose gel electrophoresis in the presence of 10 mM methylmercury hydroxide (Alfa) as described previously⁷. All procedures involving methylmercury hydroxide were carried out in a fume hood and all waste was disposed of as for hazardous chemicals. The RNA was transferred by blotting from the gel to diazobenzylxymethyl (DBM) paper by the method of Alwine *et al.*¹⁶. Following transfer the DBM paper (12×14 cm) was hybridised to the ³²P-labelled ovomucoid probe (3×10⁷ c.p.m., specific activity 5×10⁸ c.p.m. per µg) in the conditions used previously⁷. Following hybridisation, the DBM paper was washed 3 times in 200 ml of 0.5×SSC (75 mM NaCl, 7.5 mM Na citrate, pH 7.0) for 2 h at 65°C. This washing procedure significantly reduced the background during autoradiography. The DBM paper was then dried and autoradiographed for 16 h using a Dupont Cronex intensifying screen at -20°C. The probe specific for ovomucoid RNA sequences was prepared from a *Hha*I fragment of pOM100, a chimaeric plasmid which was previously constructed by our laboratory and contains an ovomucoid cDNA insert¹⁵. This fragment, which contained ~700 base pairs that correspond to the sequence of ovomucoid mRNA, was labelled by nick translation by a modification of the procedure used previously⁷. The reaction was carried out in a final volume of 10 µl which contained: 50 mM Tris, pH 7.8; 5 mM MgCl₂; 10 mM 2-mercaptoethanol; 0.5 µg of bovine serum albumin; 60 µM dATP, dGTP; 6 µM unlabelled dCTP, dTTP; 3 µM ³²P-dCTP, dTTP (2,000 Ci mmol⁻¹ each, Amersham) and 0.1 µg of DNA. These components were assembled on ice and then 28 pg of DNase (Worthington, DPFF) was added. The mixture was incubated at room temperature for 1 min and immediately cooled in an ice water bath. *E. coli* DNA polymerase (2 µl, 9 units, Boehringer Mannheim) was added and the mixture incubated at 14°C for 4 h. The reaction was stopped by the addition of 25 µl of 40 mM EDTA, 25 µl of *E. coli* DNA (4 mg ml⁻¹) and 3 µl of 20% SDS. Unincorporated dNTPs were separated from the labelled DNA by gel filtration on Sephadex G-50 in 10 mM Tris-HCl, pH 7.5, 100 mM NaCl. Radioactive fractions eluting in the void volume were pooled, made 0.2 M in Na acetate, pH 4.5, and precipitated by addition of two volumes of ethanol. The specific activity of the ³²P-DNA was 5–10×10⁸ c.p.m. per µg. A, Oviduct nuclear RNA, 20 µg. (High MW ovomucoid RNA species *c* and *f* were not detected in this preparation.) B, Ovomucoid mRNA purified to approximately 40%, 15 ng. C, Oviduct cytoplasmic RNA, 20 µg. D, Spleen nuclear RNA, 20 µg. E, Liver nuclear RNA, 20 µg. F, RNA isolated after mixing liver nuclei with oviduct cytoplasm, 20 µg. The position of 28S, 23S, 18S and 16S ribosomal RNA markers is indicated.

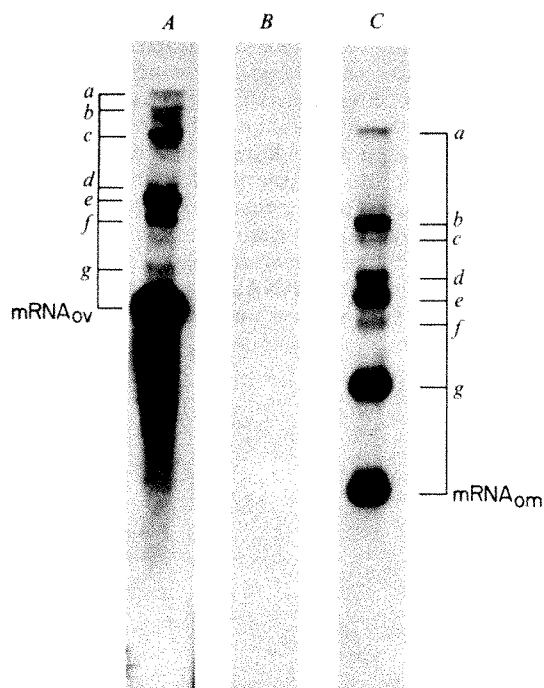


Fig. 2 Comparison of ovomucoid and ovalbumin nuclear RNA. Oviduct nuclear RNA from chronically DES-stimulated chicks, 20 μ g, was electrophoresed and transferred to DBM paper (2×14 cm), hybridised to the ^{32} P-ovalbumin probe (5×10^6 c.p.m., specific activity 5×10^8 c.p.m. per μ g) and autoradiographed (lane A). The probe specific for ovalbumin sequences was prepared from a *Hha*I fragment of a nearly full-length ovalbumin cDNA clone, pOV230, as described previously⁷. Following autoradiography, the ^{32} P-ovalbumin probe that was hybridised to the RNA bound to the DBM paper was eluted by incubation in 200 ml of 99% formamide, $0.1 \times$ SSC (15 mM NaCl, 1.5 mM Na citrate, pH 7.0) for 45 min at 65 °C. The completeness of the elution was checked by autoradiography (lane B). The DBM paper was then hybridised to the ^{32} P-ovomucoid probe (5×10^6 c.p.m., specific activity 5×10^8 c.p.m. per μ g) and autoradiographed (lane C).

Comments

We have demonstrated the existence in chick oviduct of multiple species of ovomucoid RNA larger than mature mRNA_{om}. These high MW forms of ovomucoid RNA are found in oviduct nuclei, but not in spleen or liver nuclei, nor in oviduct cytoplasm. They do not result from aggregation of mRNA_{om}. The high MW forms contain sequences complementary to the intervening sequences as well as to the structural sequences of the ovomucoid gene. Their accumulation in oviduct nuclei is regulated by oestrogen. These results suggest that ovomucoid mRNA may be synthesised by the processing of a large primary gene transcript and that the multiple bands of ovomucoid RNA larger than mRNA_{om} may represent transcripts at different stages of processing.

To date, mRNA precursors have been carefully characterised only for β -globin mRNA. A 15S globin RNA molecule has been demonstrated to be a precursor to mRNA by pulse-label and chase experiments²⁰⁻²⁴. Electron microscopic analysis of globin RNA-DNA hybrids has shown that this 15S molecule consists of a continuous transcript of the three structural and two intervening sequence regions of the β -globin gene^{25,26}. In addition, a smaller precursor has been identified that probably contains only one of the intervening sequences of the globin gene^{22,23}. These results suggest that discrete species of RNA may exist for each combination of intervening sequences. Thus, the observation that the species of ovomucoid RNA larger than mRNA_{om} are comprised of both structural and intervening sequences of the ovomucoid gene strongly suggests that the high MW species represent mRNA_{om} precursors that lose intervening sequences as they are processed.

The kinetics of accumulation in oviduct nuclei of ovomucoid RNA sequences after secondary oestrogen stimulation is also consistent with the hypothesis that the high MW forms are precursors of mRNA_{om}. First, the prominent high MW species of ovomucoid RNA were detected after only 1 h of secondary stimulation. Second, at early times (1-2 h) after oestrogen administration, the concentration of species g seems to be the same as that of mRNA_{om}. After longer periods (up to 16 h), the concentration of species g increased only slightly, whereas that of mRNA_{om} increased to much higher levels. Although other explanations are possible, this differential accumulation is consistent with the hypothesis that the high MW species of ovomucoid RNA are efficiently processed to form mature mRNA_{om}. If they are precursors, this species should be detected before mRNA_{om}. Although this was not observed, it is possible that accumulation of high MW forms of ovomucoid RNA before that of mRNA_{om} might be observed if oviduct nuclear RNA is analysed within <1 h of DES injection. However, these detailed kinetic studies are difficult to control with intact animals and are perhaps better approached using an organ culture system where newly synthesised RNA may be radioactively labelled.

As the largest species of ovomucoid RNA is fivefold larger than mRNA_{om}, at least 80% of the ovomucoid gene may be intervening sequence DNA. In comparison, we estimate the ovalbumin gene to contain about four times more DNA than required to encode mRNA_{ov}. Thus, the overall organisation and structure of the ovomucoid gene is similar in complexity to that previously described for the ovalbumin gene^{11,12}. However, studies of the ovomucoid RNAs may offer advantages over those of the ovalbumin RNAs in the elucidation of precursor-product relationships. First, the fraction of nuclear ovomucoid RNA that is in the form of high MW species seems to be greater than that of nuclear ovalbumin RNA. Second, the ovomucoid

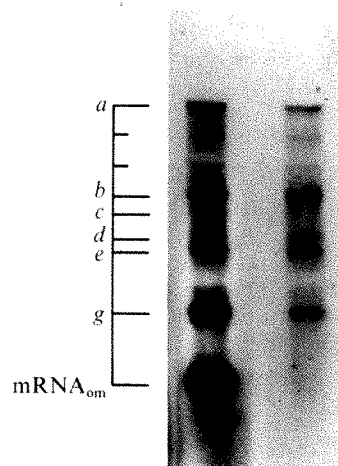


Fig. 3 Identification of ovomucoid intervening sequences in high MW ovomucoid nuclear RNA. For these experiments a hybridisation probe was initially prepared from a clone, selected from a library of chicken DNA fragments that were cloned in Charon 4A phage containing a 15-kilobase ovomucoid natural gene insert. This clone, which will be described in detail elsewhere²⁷, seems to contain all the structural and intervening sequences of the ovomucoid gene. Total DNA was isolated from the cloned phage, labelled with ^{32} P by nick translation and used as a hybridisation probe. Subsequent experiments in which the cloned ovomucoid fragment described in the accompanying paper¹⁹ was used as a hybridisation probe gave identical results. Oviduct nuclear RNA from chronically stimulated chicks, 20 μ g, was electrophoresed, transferred to DBM paper (2×14 cm) and allowed to hybridise to the ^{32} P-labelled natural ovomucoid gene probe (10^7 c.p.m., specific activity 6×10^8 c.p.m. per μ g) in the absence (lane A) or presence (lane B) of unlabelled ovomucoid mRNA as a competitor. The conditions for competition were as follows: ovomucoid mRNA (20 μ g, ~20% pure) was preincubated with the ^{32} P-labelled probe in 1.0 ml of hybridisation buffer at 42 °C for 1 h. The mixture was diluted to 5.0 ml with hybridisation buffer and then hybridised to the DBM paper containing the bound nuclear RNA. Autoradiography of both samples was for the same length of time (about 1 week).

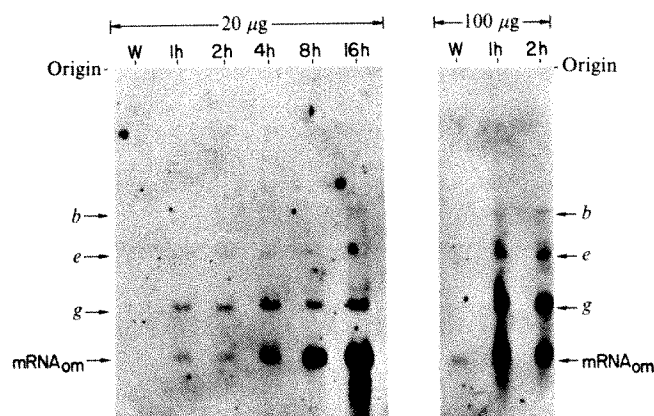


Fig. 4 Induction of high MW ovomucoid RNA by secondary oestrogen stimulation. Oviduct nuclear RNA was isolated from chicks that had received daily subcutaneous injections of 2.5 mg of DES for 14 d and subsequently withdrawn from hormone for 14 d (W). In addition, hormone-withdrawn chicks were injected with a single dose of 2.5 mg of DES and the oviduct nuclear RNA was isolated after 1, 2, 4, 8 and 16 h. The RNA was electrophoresed, transferred to DBM paper (15 × 15 cm) and hybridised to the ^{32}P -ovomucoid probe (3.3×10^7 c.p.m., specific activity 8×10^8 c.p.m. per μg). Left panel, 20 μg of nuclear RNA was analysed; right panel, 100 μg of nuclear RNA was analysed.

RNA bands are well separated from each other on the gel, whereas the ovalbumin RNA bands are more closely spaced. These two features should enhance the assessment of planned kinetic labelling studies as well as the purification of individual species of high MW nuclear RNA.

R_{ot} analysis has previously shown that the concentration of total ovomucoid RNA sequences in oviduct nuclei gradually increased from approximately 1 part per 10^6 to 30 parts per 10^6 after 16 h of secondary oestrogen stimulation⁹. These results are consistent with the accumulation of individual nuclear ovomucoid RNA species shown in Fig. 3. Both analyses clearly show that the low concentration of mRNA_{om} in withdrawn oviduct polysomes⁸ is not the result of nuclear sequestration of immature or mature forms of mRNA_{om} . In addition, by hybridising nuclear radiolabelled mRNA_{om} to an excess of cloned

ovomucoid DNA, we have been able to estimate the rate of mRNA_{om} synthesis and have shown it to depend on oestrogen⁹. Taken together, these results are consistent with regulation of the ovomucoid gene by oestrogen mainly through control of its transcription.

The library of chicken DNA fragments which were cloned in Charon 4A phage was provided by Tom Maniatis and Richard Axel. We thank Ms Melanie Vinion and Mr William Tate for technical assistance. This work was supported by NIH grant HD-8188, the Baylor Center for Population Research and Reproductive Biology. J.L.N. is the recipient of USPHS Postdoctoral Fellowship GM 06893-01. All cloned DNA was prepared using a P3 facility in accordance with the NIH guidelines.

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letters

A model for the unpulsed light from the Crab pulsar

THE recent detection¹ of a significant unpulsed fraction of the light from the Crab Nebula pulsar invites comment regarding models for the beaming of the radiation. One such model is the relativistic beaming model²⁻⁴ in which the radiation comes from a region near the speed-of-light cylinder of a neutron star magnetosphere. Here the predictions of this model are investigated, using model parameters found by Ferguson *et al.*⁵ from fits to the polarisation characteristics of the Crab pulsar, and which were subsequently found⁶ to agree with the change of light curve with colour of the secondary pulse.

The parameters are β , the fraction of the light cylinder radius at which the radiation is emitted; T , the angle between the rotation equator and the line of sight; and ϵ , the spectral index. It is assumed that in the frame of reference of the emission regions co-rotating with the neutron star the radiation is unbeamed, and

only becomes pulsed to an outside observer because of the Doppler effect and the aberration of light.

In the following, quantities in the corotating frame are primed, and those to an outside observer unprimed. Subscript 1 refers to the main pulse emission and 2 to the secondary pulse emission. Subscripts max and min refer to the maximum and minimum of the quantities observed by an outsider. In the relativistic beaming theory, the intensity

$$I = RI$$

where $R = (1 - \beta \cos \theta \cos T)^{-3-\epsilon}$, θ is the angle in the emission region orbit between the emission region and the point of minimum radial velocity, and ϵ is the spectral index from $I \propto \nu^{-\epsilon}$. $\beta_1 = 0.65$, $\beta_2 = 0.50$, $T = 25^\circ$ (from ref. 5), and $\epsilon = 0.2$ (from ref. 7) Then,

$$I_{1,\text{peak}} \approx I_{1,\text{max}} + I_2(\theta_2) \quad (\theta_1 = 0)$$

$$I_{2,\text{peak}} \approx I_{2,\text{max}} + I_1(\theta) \quad (\theta_2 = 0)$$

and $I_{\min} \approx I_2(\theta_{2m}) + I_1(\theta_{1m})$

Here, $I_{\min}$ is the minimum of the observed intensity, taken to occur midway between the secondary and main pulses, θ_{1m} is the value for emission region 1 at that time, and θ_{2m} is the corresponding value for emission region 2. θ_1 is the value of θ for emission region 1 at the time of the secondary pulse peak and θ_2 is the value for emission region 2 at the time of the main pulse peak.

To compute the values of θ , we must relate the observed pulse longitudes to phases in the emission region orbits through the relationship⁸

$$\theta_{\text{obs}} = (\theta - \beta \sin \theta \cos T)$$

Taking the separation of the main and secondary pulses to be 146° , we have

$$146^\circ = -(\theta_2 - \beta_2 \sin \theta_2 \cos T) = (\theta_1 - \beta_1 \sin \theta_1 \cos T)$$

and at the midpoint of their greatest separation,

$$\begin{aligned} \frac{(360 - 146^\circ)}{2} &= (\theta_{2m} - \beta_2 \sin \theta_{2m} \cos T) \\ &= -(\theta_{1m} - \beta_1 \sin \theta_{1m} \cos T) \end{aligned}$$

Solving for the various values of θ and substituting, we find

$$I_{1,\text{peak}} \approx 17.22I'_1 + 0.329I'_2,$$

$$I_{2,\text{peak}} \approx 6.90I'_2 + 0.247I'_1$$

and

$$I_{\min} \approx 0.345I'_1 + 0.458I'_2$$

Now from the tabulated light curves with $I_{\min}$ subtracted⁶ we find

$$(I_{2,\text{peak}} - I_{\min}) = 0.270(I_{1,\text{peak}} - I_{\min})$$

Solving this for I'_2 , we find $I'_2 = 0.719I'_1$, which shows that the intensities of both pulses are roughly equal in their emission frames. Finally, on substituting we have $I_{\min}/I_{1,\text{peak}} \approx 0.039$, or the minimum in observed intensity should be $\sim 3.9\%$ of the main pulse peak.

The observed values of Peterson *et al.*¹ are $3.6\% \pm 0.7$ and $3.7\% \pm 0.8$, using two independent techniques. The agreement between the theoretical and observed values is remarkable. The simplistic model predicts an underlying component of emission almost identical to that observed, and does this with no free parameters. Of course, to explain the Crab pulsar's asymmetrical pulse shapes, Cocke and Ferguson⁶ postulated a certain amount of intrinsic beaming to supplement the relativistic beaming. Also, the recent resolution of the Crab pulsar optical main peak⁹ indicates a width consistent with $\beta \approx 0.9$. If this pulse width is indicative of the true value of β for the main peak, the unpulsed light would need to come mainly from the secondary pulse emission region, which has a much lower value of β . It is not clear whether the inclusion of these effects would worsen the numerical agreement arrived at by the above analysis. However, the fact that this simple version of the model agrees even roughly with the observations, using the same model parameters necessary to fit the polarisation and change of light curve with colour, is further evidence of the power of the relativistic beaming model for the Crab Nebula pulsar.

With the new information about the unpulsed emission, it would be worthwhile to reanalyse the old Crab polarisation data in the hope of correcting the percentages and angles near the polarisation minima, which are most affected by errors in the subtracted noise level, and to see how the polarisation behaves in the regions between pulses. If the model given here is correct, the between-pulse polarisation should be high and smoothly vary from one emission region to the next.

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Ion-induced dipole H_n^- clusters

THERE has long been an interest in the study of ion-induced dipole clusters of the type H_n^+ (n , odd integer). These positive clusters may be present in gaseous nebulae^{1,2}, and Clappitt and Gowland³ have discovered in mass spectrometer experiments such H_n^+ ions for $3 \leq n(\text{odd}) \leq 99$. The ions are very weakly bound because of the nature of the ion-induced dipole forces involved, and thus must be produced at low temperatures. In these experiments a layer of hydrogen gas is deposited on a metal surface maintained at 3 K in a baked vacuum apparatus operating at $\sim 10^{-10}$ torr. The surface is swept by a 23 eV electron beam and the ions thus created are focused into a quadrupole filter and detected. The surface mechanism for production of the ions is not understood but the presence of grains in the interstellar medium might provide surfaces which could play an analogous role in similar conditions of low pressure and temperature. To study the binding energy and geometry of such clusters we performed *ab initio* Hartree-Fock-Roothaan calculations⁴, using a contracted s-type Gaussian orbital basis. Our previous use of this type of calculation⁴⁻⁷ for ion molecule complexes which dissociate into closed shell fragments, indicated that reliable results can be obtained. The results shown in Fig. 1 and Table 1 are all based on this type of calculation implemented with the Gaussian 70 program^{8,9} using four gaussians per hydrogenic s-orbital. As expected, we found the positive cluster to be quite weakly bound, that is the binding energy was of the order of 1 kcal mol^{-1} against dissociation according to



(the largest and smallest binding energies found were $6.0 \text{ kcal mol}^{-1}$ for H_3^+ and $0.3 \text{ kcal mol}^{-1}$ for H_{15}^+). In our calculations the geometries of the positive clusters were dominated by the strongly bound triangular nucleating centre H_3^+ . Each member of the series H_n^+ , $5 \leq n(\text{odd}) \leq 15$ is formed by the addition of one hydrogen molecule to the basic structure of the previous member in the series. We report here calculated binding energies and geometries for the negative ion induced dipole clusters H_n^- , $5 \leq n(\text{odd}) \leq 13$.

These ions have not apparently been previously investigated theoretically or experimentally although a search for such clusters was suggested by Clappitt and Gowland³. It would be interesting to investigate whether the binding energies are of the same order of magnitude as in the analogue clusters H_n^+ , as it has been established that in this case the clusters may be experimentally produced in favourable conditions². As in the case of

Table 1 Calculated binding energies and geometrical parameters for hydrogen clusters H_n^-

Molecule	Binding energy (kcal mol)	Geometric parameters		
		$a(\text{\AA})$	$b(\text{\AA})$	$c(\text{\AA})$
H_3^-	8.91	2.05	0.74	
H_5^-	7.30	2.10	0.74	
H_7^-	6.52	2.12	0.74	
H_9^-	5.59	2.15	0.74	
H_{11}^-	4.00	2.21	0.74	2.27
H_{13}^-	3.97	2.34	0.74	

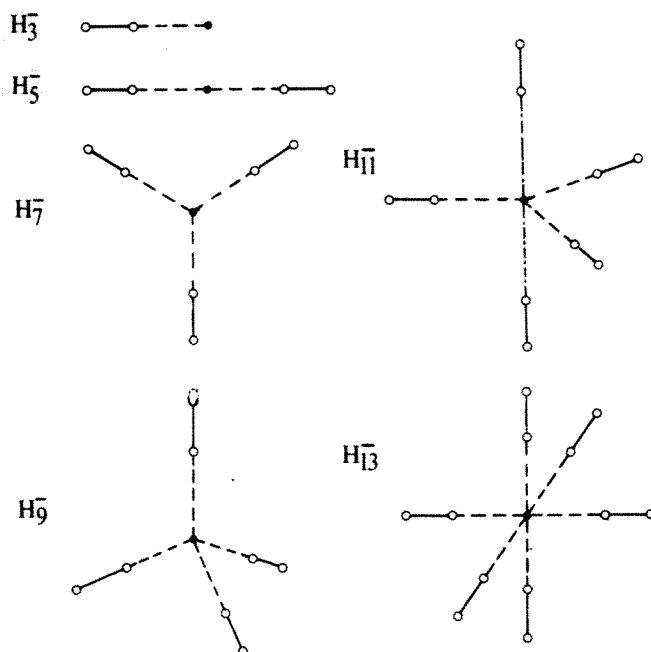
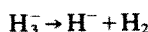


Fig. 1 Geometry of H_n^- clusters. See Table 1 for magnitudes of bond distances: a , —; b , ---; c , - · - ·.

the positive clusters⁴, the conditions in gaseous nebulae may be suitable for weakly bound negative clusters to exist (some of the conditions that have been laid down¹⁰ for a possible molecular source of the diffuse interstellar absorption lines are in fact met by the H_n^- and H_n^+). We expected that the geometries for the negative ions would be quite different than for the positive ions as both the charge sign and the geometry of the nucleating centre differ. In the latter, the tightly bound triangular geometry of the nucleating centre H_3^+ governs the geometries of the clusters of increasing mass. It is energetically favourable for a positive charge to be approached by a hydrogen molecule along a line perpendicular to its bond. In the former, the nucleating centre is a H^- ion which presents an essentially spherical static electric field to H_2 molecules in the clusters. It is energetically favourable for a negative charge to be approached by a hydrogen molecule along a line through its bond. The molecular ion H_3^- dissociates in ordinary conditions according to the reaction

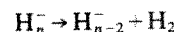


although it^{11,12} exhibits a shallow potential minimum (about 0.8 kcal mol⁻¹ for a linear geometry with H^- about 2.41 Å to the nearest atom in H_2) capable of supporting discrete vibrational and rotational states in favourable experimental conditions. Our calculations also reproduce the shallow potential minimum for H_3^- in a linear geometry, found by previous workers. At the geometry of minimum energy we find the nucleating centre H^- at a distance of 2.05 Å from the nearest atom in the H_2 fragment. The H_2 fragment has a bond distance equal to 0.74 Å, the same as for the free H_2 molecule.

Our new results are summarised in Fig. 1 and Table 1. All these results correspond to calculations in which the total energy of the system has been optimised with respect to all geometrical parameters (bond lengths and bond angles) within the system.

For each molecule the energy surface displayed a potential well whose minimum was sufficiently deep enough to support a few discrete vibrational and rotational states and which geometrically correspond to ion-induced dipole bonds. All the clusters contain polarised H_2 fragments (having a bond distance corresponding to the free H_2 molecule) attached to the negative ion nucleating centre H^- . In fact the geometries adopted by the various clusters under energy optimisation correspond to Gillespie's rules^{13,14} of molecular geometry based on minimisation of repulsion of electron pairs.

Table 1 shows that all of the clusters displayed are bound by a few kilocalories per mole against the dissociation



a result which is analogous to the previous calculation for the experimentally known H_n^+ molecular ions.

The binding energy of the H_5^- ion is 7.3 kcal mol⁻¹, and has a linear geometry ($C_{\infty v}$) with the nucleating centre 2.10 Å from either H_2 fragment. H_7^- has a binding energy of 6.52 kcal mol⁻¹. The geometry of the ion is planar and trigonal (D_{3h}) with an H_2 fragment to nucleating centre distance of 2.12 Å. H_9^- is bound by 5.59 kcal mol⁻¹ with a tetrahedral geometry (T_d) and fragment to centre distance 2.15 Å. The ion H_{11}^- decreases in bonding energy to 4.00 kcal mol⁻¹ and has a geometry which is trigonal bipyramidal (D_{3h}) with fragment to centre distances 2.21 Å and 2.27 Å as shown in Fig. 1. Finally H_{13}^- is found in its minimum energy configuration to be bound by 3.97 kcal mol⁻¹ in an octahedral geometry (O_h) with fragment to centre distance 2.34 Å. This last result answers the question raised by Clappitt and Gowland³ on whether H_{13}^- might be the most stable of the negative clusters. We find that it is not. In fact Table 1 shows that for the negative ion clusters studied there is a continual decrease of binding energy as the size of the cluster grows.

In conclusion our results indicate that the negative ion clusters H_n^- (n is odd) are bound by a few kilocalories per mole, the same order of magnitude as found earlier for binding energy of the positive ion clusters H_n^+ (n is odd) which are experimentally known. The negative ions may form in favourable conditions.

We thank Barbara Rubenson for constructing Fig. 1.

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Polonium haloes in mica

GENTRY¹ has reported that some anomalous haloes found in ancient mica seem to arise from the α -decay of polonium isotopes only, without any observable long-lived β -emitting precursors. Fremlin² suggested that these might occur as a result of the diffusion of β -emitting radiogenic lead isotopes from relatively distant or widely dispersed uranium or thorium inclusions, and the absorption of the lead ions by small pre-existing inclusions of foreign material. As Gentry³ pointed out,

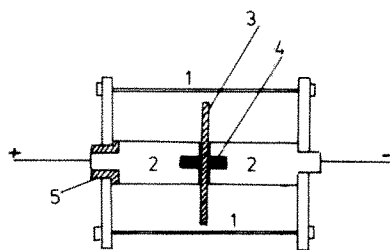


Fig. 1 Apparatus used to introduce lead into mica at elevated temperatures and under an applied electrical field. The muscovite mica sheet ($\sim 3 \times 2.4 \text{ cm}^2$, $200 \mu\text{m}$ thick) is held tightly, with the help of screws, between two stainless-steel cylinders (diameter 8 mm, height 2 cm). Central holes (diameter 3 mm, depth 6 mm) in these cylinders are filled with lead. The electrical field ($\sim 10^5 \text{ V cm}^{-1}$) is applied across the sample through insulated wires, and the whole assembly is placed inside a furnace. 1, Screw; 2, cylinder; 3, mica; 4, lead; 5, insulator.

this would involve abnormally high diffusion rates for lead. We have carried out experiments that demonstrate the possibility of such high rates. Here we also discuss the reasons for the absence of an excess of observable tracks left in mica by the recoil of the lead daughter when α -decay takes place in the polonium isotopes.

The method used in this work involved two major techniques: (1) introducing lead into mica and establishing conditions for diffusion; and (2) radioactivating to transform some of the lead nuclei to radioactive products, and the detection of the radioactivities by autoradiography using plastic nuclear track detectors. These two stages are considered below.

To study the diffusion of lead in mica, we first need to introduce an adequate number of lead atoms into the samples. This has been done by using the apparatus shown in Fig. 1. This

apparatus comprises two identical steel cylinders of diameter 8 mm and height 2 cm. Each cylinder has a coaxial hole of diameter 3 mm and depth 6 mm. These holes were filled with lead, and the mica sample ($\sim 3 \times 2.4 \text{ cm}^2$ in area) was clamped between the cylinders by the screws shown. The cylinders were connected to a power supply by heat-resistant insulated wires. The apparatus was then placed in a furnace at the desired temperature for periods of up to 48 h.

An electrical field ($\sim 10^5 \text{ V cm}^{-1}$) was applied at right angles to the cleavage planes of the mica. Any field-induced diffusion of lead was parallel to the field and between the areas in contact with lead (within a circle of diameter 3 mm), or at most between the areas over which the potential difference was applied (within a circle of diameter 8 mm). Any diffusion outside this region would be independent of the applied electric field.

A variety of activation methods have previously been used for lead analysis. For determination of the spatial distribution of lead in mica the reactions producing γ and/or β emitters are not suitable, as the interference from other γ and/or β emitters produced in the matrix is always very high.

We used ^3He and ^4He bombardments to produce α -particle-emitting isotopes of polonium according to reactions of the type $\text{Pb}(\text{He}, n)\text{Po}$. α -particles from the polonium could then be detected using cellulose nitrate films (Kodak LR-115, type II; Kodak-Pathé).

Rectangular pieces of muscovite mica ($\sim 3 \times 2.4 \text{ cm}^2$) of thickness $\sim 200 \mu\text{m}$ were placed between the two cylinders of the apparatus shown in Fig. 1. The assembly was placed in a furnace at a desired temperature and an electrical field of $\sim 10^5 \text{ V cm}^{-1}$ was applied between the cylinders. After several hours of diffusion (across and along the cleavage planes of mica sample), the sample was removed from the apparatus and a few layers of mica were peeled away from each face of the sample to remove any surface contamination. The remaining part of the sample was irradiated with ^3He or ^4He ions, of energy 30 MeV or 40 MeV respectively, and with a current of $2 \mu\text{A}$ for a period of 2 h on each face. To avoid the possibility of the disturbance of the lead distribution on the surfaces of mica during the irradiation, a very thin layer of mica was again peeled off from each face of the sample. Cellulose nitrate films (LR-115 type II) of the same size as the sample were then placed in close contact with

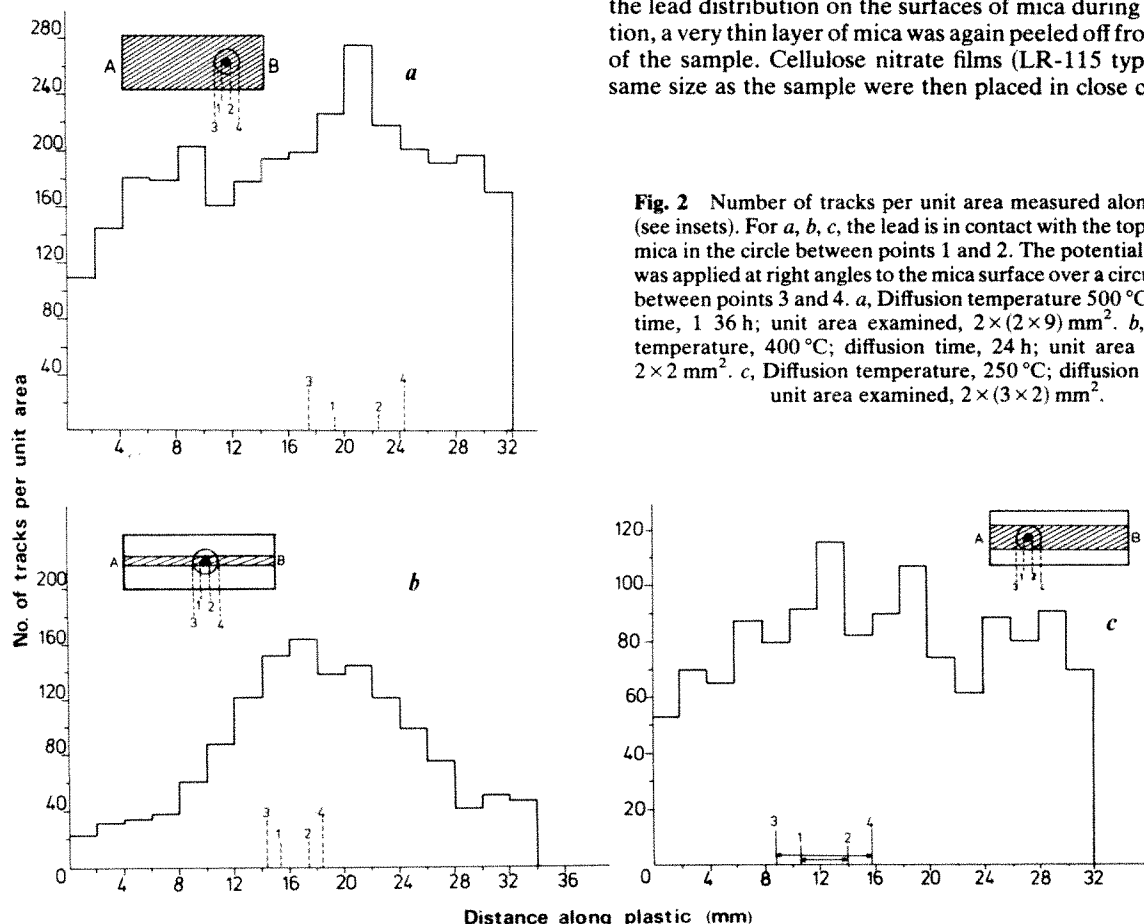


Fig. 2 Number of tracks per unit area measured along axis AB (see insets). For *a*, *b*, *c*, the lead is in contact with the top surface of mica in the circle between points 1 and 2. The potential difference was applied at right angles to the mica surface over a circular region between points 3 and 4. *a*, Diffusion temperature 500°C ; diffusion time, 36 h; unit area examined, $2 \times (2 \times 9) \text{ mm}^2$. *b*, Diffusion temperature, 400°C ; diffusion time, 24 h; unit area examined, $2 \times 2 \text{ mm}^2$. *c*, Diffusion temperature, 250°C ; diffusion time, 48 h; unit area examined, $2 \times (3 \times 2) \text{ mm}^2$.

each surface of the sample. After several days of exposure, the plastics were etched in a 25% solution of sodium hydroxide at a temperature of 55 °C for 2 h. The resulting α -tracks were counted under an optical microscope, using an overall magnification of $\times 40$.

This diffusion experiment was carried out at temperatures of 600, 500, 400 and 250 °C for different lengths of time. Figure 2a, b, c shows some of the results.

Although none of the results shown in Fig. 2a, b, c can provide an accurate numerical value for the migration velocity of lead atoms in mica, they show that diffusion of lead in mica is a fairly rapid process and that the traversal of at least 1 cm was possible in 24 h over a considerable range of temperatures.

It may be true, as assumed by Gentry³, that the diffusion coefficient of lead is only of the order of $10^{-18} \text{ cm}^2 \text{ s}^{-1}$ at 20 °C in the case of field-free diffusion across the cleavage plane. Our observations suggest that, even at 500 °C and with an applied field of 10^5 V cm^{-1} , the diffusion coefficient D across the planes is $< 6 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$. But Fig. 2a, b, c indicates that, at elevated temperatures, it must be much higher along the cleavage planes than the above-mentioned value: for example, $D > 3 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ at 250 °C in the field-free area.

Gentry³ has pointed out that if active lead or polonium isotopes do diffuse through mica, recoil tracks should be observable from nuclei, which had undergone α decay during their diffusion through the mica. Such tracks should have appeared as 'excess' α -recoil tracks because during diffusion, each of the daughters recoiling from its α decay would have produced a separate track, whereas in the case of stationary U, Th nuclei the daughters of which did not diffuse away, the whole decay chain would have looked like a single, local recoil. No such excess recoil tracks have, in fact, been observed. We explain this apparent anomaly as follows.

Recoil tracks produced by α -decaying nuclei (α -RT) have an etchable range in mica of $\sim 0.01 \mu\text{m}$ (a combined fission-fragment etchable range of $\sim 20 \mu\text{m}$). The much shorter α -recoil tracks would fade more easily than the longer, more energetic, fission tracks, when subjected to thermal annealing in nature (see refs 4, 5; this conclusion is also supported by our unpublished data). The work of Nagpaul *et al.*⁶ confirms this fact.

They have shown that, in a biotite sample (from the Nellore mica belt of India), the length reduction of fossil fission tracks due to natural annealing at normal temperatures ($\sim 20^\circ\text{C}$) was $\sim 12\%$, which corresponds to 6.5% reduction in the apparent fossil-fission track density. This phase had been preceded by a high-temperature period lasting for about 190 Myr, during which the average temperature of the region must have been about 150 °C. This earlier period of geological annealing at 150 °C resulted in a reduction of 71% in fission track length or 65% reduction in apparent fission track density.

As α -recoil tracks fade more easily than fission tracks, the reduction in the density of the former during both geological phases must have been high. Such severe losses in the density of α -RT could easily reduce the recoil/fission track ratio sufficiently to create the impression that the expected 'excess' recoil tracks during the diffusion of lead or polonium isotopes had never occurred.

Our experimental results show that the diffusion of lead in mica along the cleavage planes is a rapid process. Hence, if our explanation of the lack of observable excess α -recoil tracks is accepted, the diffusion of lead isotopes from decay chains could well cause some of the polonium haloes. In that case ^{210}Pb is available (β^- , $T_{1/2}$ 22 yr), in one uranium series (^{238}U); and ^{211}Pb (β^- , $T_{1/2} = 38 \text{ min}$) in the other (^{235}U). Furthermore, the ^{232}Th series, which eventually produces ^{208}Pb , has ^{212}Pb with a half life of 19.6 h as a carrier.

We do not rule out the possibility that the diffusion of polonium itself may be a contributory—or even in some cases the main—cause. Experiments on this are in progress. Note that the three most commonly used minerals for halo investigations, biotite, fluorite and cordierite, all have cleavage properties; although, in the case of the cordierite, cleavage is not perfect. In

such materials, fast ion-conduction parallel to the cleavage planes could be expected and would probably be associated with high mobilities at elevated temperatures.

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Secondary batteries powered by forced ionisation

SECONDARY batteries normally store energy by accumulation of electrode products formed by electron transfer, that is, production of a reducing agent at one electrode and an oxidising agent at the other electrode. I have demonstrated a storage battery concept that depends on the energy associated with ionisation of slightly dissociated compounds, for example, forcing the equilibrium $\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$ to the right stores energy. A cell for recovery of this energy as electricity is described here. De Kőrös and Zeigerson have mentioned the possibility of a primary battery based on these principles¹.

The key element of such a cell must allow union of the ions at a fixed phase boundary. Removal of H^+ from one solution and OH^- from the other produces a potential which is related to the equilibrium constant for the reaction. The phases may be separated by a bipolar ion exchange membrane or a bipolar depolarising electrode. The cell configuration for each type of separator is shown in Figs 1 and 2. The flow of ions indicated is for the charging cycle and flow is reversed for discharge. The net process is: $\text{H}_2\text{O} + \text{MX} \rightleftharpoons \text{MOH} + \text{HX}$.

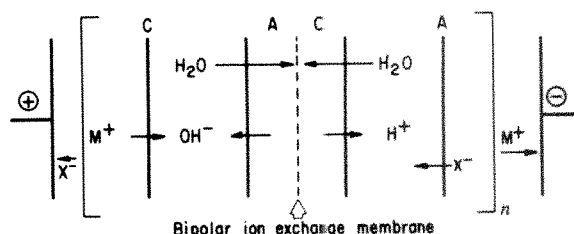


Fig. 1 Schematic for repeating cell in a forced ionisation secondary battery using a bipolar ion exchange membrane.

Bipolar ion exchange membranes have been made by lightly fusing a cation exchange membrane to an anion exchange membrane². The alternative procedure of de Kőrös and Zeigerson¹ forms both the anion and cation exchange sites on opposite sides of a sheet of sulphochlorinated polyethylene. The use of bipolar ion exchange membranes for production of acid and base has recently been discussed in considerable detail^{3,4}. During this cycle, that is charging, inefficiency is associated with migration of either hydrogen or hydroxyl ions across the ion exchange barrier.

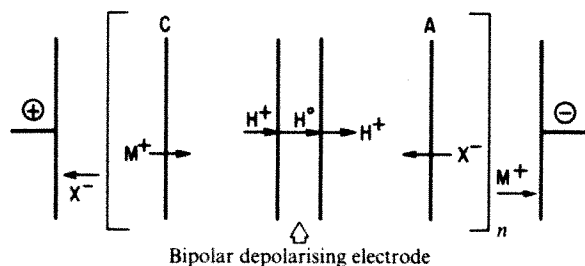


Fig. 2 Schematic for repeating cell in a forced ionisation secondary battery using a bipolar depolarising electrode. A, anion exchange membrane; C, cation exchange membrane.

A depolarising bipolar electrode has been used in this laboratory for production of an acid in a base. The same type of cell has now been used to produce electricity by neutralisation of acid and base. The cell is shown in Fig. 3. Hydrogen electrodes are used to convert the potential produced by migration of cations across the cation exchange membrane. The migration of hydroxyl ions to the acidic solution is a source of inefficiency as the potential is immediately nullified by the free migration of a sodium ion in the same direction.

The production of electrical energy from acid and base was carried out as follows. The entire system was placed under a positive pressure of hydrogen and the solution compartments filled with aqueous sodium phosphate. The central palladium foil was made cathodic and the end foils anodic⁵. This caused a net transport of hydrogen into the central palladium foil to speed equilibration with the external hydrogen. After equilibration acidic and basic solutions were passed in. The current-voltage relationships obtained are shown in Fig. 4.

These experiments demonstrate the use of two working fluids (water and methanol). As shown by methanol, compounds with lower ionic dissociation give higher cell voltage. The theoretical cell voltage associated with a number of equilibrium systems is calculated in Table 1.

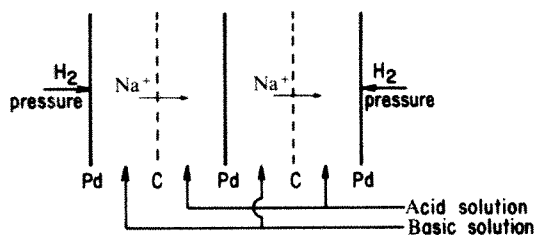
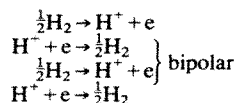


Fig. 3 Schematic for cell with net reaction $\text{H}^+ + \text{OH}^- \rightarrow \text{H}_2\text{O}$. Electrode reactions, left to right:



Pd, activated palladium foil; C, Na ion cation exchange membrane.

The higher voltage systems place limits on the counter ions associated with the above ions of the charged state. These ions must move readily through ion exchange membranes and form ionic compounds with highly active ions. The alkali metal ions and the counter ions of superacids would be required in the most energetic systems. In addition, the salt formed by these ions must be sufficiently soluble to give good electrical conductivity. It is apparent that this scheme requires a bipolar member stable to extremely strong acids and bases. In these circumstances, the bipolar depolarising electrode may be more suitable than the bipolar ion exchange membrane. The latter are generally subject to degradation on the anion exchange side at extremely basic conditions.

The cells shown in Fig. 1 also require an anion exchange and a cation exchange membrane for each repeating unit. The anion exchanger operates between a salt solution and a strongly acidic solution. As a result, high efficiency for hydrogen ion rejection is necessary. However, chemical resistance will be less demanding than that in the corresponding part of the bipolar ion exchange membrane. Similar considerations apply to the cation exchange portions.

Table 1 Theoretical repeating cell e.m.f. for several working fluids as a function of the equilibrium shown

	$-\log$ Equilibrium constant	cell e.m.f.
$\text{HF} = \text{H}_2\text{F}^+ + \text{F}^-$	3	0.18
$\text{H}_2\text{O} = \text{H}_3\text{O}^+ + \text{OH}^-$	14	0.83
$\text{NH}_3 = \text{NH}_4^+ + \text{NH}_2^-$	35 (6)	2.06
$\text{CH}_4 = \text{CH}_5^+ + \text{CH}_3^-$	~ 60	~ 3.5

The high mobility of hydrogen and hydroxyl ions make high rejections of these ions particularly difficult. The use of any other working fluid with less mobile ions would be desirable from this point of view. However, such ions will add to the internal resistance of the bipolar ion exchange membrane.

The systems discussed all involve transport of H^+ or H^0 , but this is not essential. Any of a large number of liquids which yield ions under high electric field might be suitable. For example, the equilibria



are reported.

Such working fluids could greatly reduce the demands on the anion exchange component of the bipolar membrane. At the same time, the character of the cation exchange portion becomes more critical.

The metal foil depolarising bipolar electrode is obviously not suitable for such working fluids. The basic requirements for this component of the system are the same as those for gas depolarised electrodes—electronically conductive, liquid impermeable, but gas permeable. The surface should also be catalytic for the desired reaction, for example, $\text{Cl}^- \rightarrow \text{Cl}^0 \rightarrow \text{Cl}^+$. Such electrodes are readily built but any practical system requires a balance of mechanical strength and rapid gas permeability which is difficult to achieve.

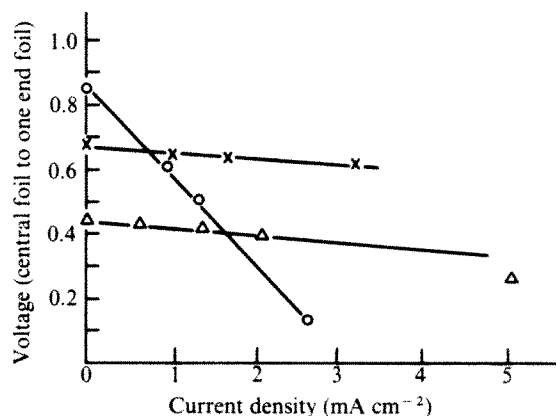


Fig. 4 Current-voltage plot for cells powered by the net reaction $\text{H}^+ + \text{OH}^- \rightarrow \text{H}_2\text{O}$. $\times$, 17% aqueous phosphoric acid, 20% aqueous sodium hydroxide; Δ , 20% aqueous sodium dihydrogen phosphate, 20% aqueous sodium hydroxide; $\circ$, Phosphorus pentoxide in methanol 25 gl^{-1} , sodium methoxide in methanol 25 gl^{-1} . Current density was established by a variable external resistance.

At the ends of any assembly of cells of this type, electrodes which convert ionic charge to electronic charge are required. The hydrogen electrode was used in the above experiment. Electrodes such as those used in secondary batteries may be suitable. For example, nickel electrodes may be suitable for an aqueous cell provided they are placed in strongly alkaline compartments. The electrodes would operate on the reversible $\text{Ni}^{2+} \rightleftharpoons \text{Ni}^{3+}$ cycle with no net contribution to energy storage.

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Evidence for a higher natural uranium content in world rivers

α -SPECTROMETRIC uranium isotopes analyses on groundwater samples from the highly fertilised Handschuhsheimer Feld area in Heidelberg suggest that the uranium content of the groundwater is primarily of natural origin; uranium derived from phosphate fertilisers is adsorbed on the uppermost soil layers and the uranium content of the water is correlated to the HCO_3^- content. A similar relationship between uranium and HCO_3^- also applies for world rivers and lakes. We show here that this indicates that a low uranium content is due to low HCO_3^- contents and not to lower contamination levels. On the basis of this correlation the average natural uranium content of world rivers, for 35% of the water input to the ocean, was calculated to be $0.3 \mu\text{g l}^{-1}$.

The uranium content of river waters varies by about two orders of magnitude, from $0.04 \mu\text{g l}^{-1}$ for the Amazonas, to a few $\mu\text{g l}^{-1}$ (refs 1-5). This higher uranium content is, however, suspected to be partly of anthropogenic origin due to uranium derived from phosphate fertilisers⁵. In fact, compared with normal soil and rock material with a few p.p.m. uranium, phosphate fertilisers have up to 200 p.p.m. uranium. This fertil-

iser uranium is thought to contaminate the ground and eventually the river-water. Thus the question arises of whether the present-day estimates for the average uranium input by rivers to world oceans are representative of all geological time and therefore applicable to steady-state model calculations^{4,6}. However, several factors seem to contradict the assumption of a high uranium-pollution level of river water: a clear-cut relationship between the uranium and phosphate content of the water has not been found. Rivers show a significant ^{234}U excess, that is, the activity ratio $\text{AU} = ^{234}\text{U}/^{238}\text{U} > 1$ (ref. 7), whereas fertiliser uranium has an $\text{AU} \sim 1$, as in the case of soil and rock material. In addition, high uranium contents were also measured for some Indian rivers with catchment areas of low fertilisation⁸. Moreover, the uranium concentration in natural waters is assumed to be mainly controlled by the redox potential of the environment and by CO_2 (ref. 7).

To estimate the uranium contribution to groundwater by fertilisers and hence to evaluate the natural average uranium content of rivers, we have carried out uranium-isotope analyses on five groundwater samples from the highly fertilised area of the Handschuhsheimer Feld, Heidelberg, which has been used for horticulture for several decades (Table 1). Results are also presented here for three groundwater samples from an unfertilised forest area some 15 km out of Heidelberg, for the commercially available fertilisers, as commonly applied in this area (Table 2), and for a number of major German rivers, including the Neckar, that receives a groundwater contribution from the studied area (Table 1).

A 10-l sample was spiked with ^{232}U (in weak HCl solution), left at least 2 h for equilibration of uranium isotopes, then filtered (paper filter) and passed through a column containing 3 g of Hyphan-resin⁹ ($\text{pH} > 5$, flow-rate 40 ml min^{-1}). Back-extraction was achieved with 50 ml 2 M HCl. The pH of this solution was then adjusted to ~ 5 with NH_4OH , uranium extracted into 20 ml TTA (0.1 M in benzene) and back-extracted into 20 ml 1 M HCl. The TTA extraction and back-extraction steps were then repeated. Adding concentrated HCl, the final aqueous solution was made 8 M and passed through a Dowex I resin to separate uranium from thorium isotopes. The following electroplating step and measurement of the uranium isotopes have been described elsewhere¹⁰. Counting times around 3,000 min made the counting errors smaller than 5%, even if the uranium content was as low as $0.1 \mu\text{g l}^{-1}$.

In this area of the Rheinebene, groundwater recharge of about 200 mm yr^{-1} has been estimated from the downward

Table 1 U, HCO_3^- , nitrate, phosphate, tritium content and activity ratio of the water samples from the Handschuhsheimer Feld area, the Südkreis Mannheim area, and for some major German rivers

Sample	U content ($\mu\text{g l}^{-1}$)	AU	HCO_3^- (mg l^{-1})	Depth (m)	Nitrate (mg l^{-1})	Phosphate (mg l^{-1})	Tritium (TU)
Entensee I	0.84 ± 0.06	1.62 ± 0.07	108.9	20	126	ND	187
Wiesenweg III	1.05 ± 0.04	1.97 ± 0.06	129.8	26	125	ND	113
Wiesenweg I	0.92 ± 0.04	1.52 ± 0.05	134.2	36	110	ND	—
Wiesenweg II	1.59 ± 0.05	1.57 ± 0.05	138.6	43	120	ND	—
Entensee II	—	—	—	60	—	ND	94
Entensee III	1.84 ± 0.07	1.59 ± 0.05	107.8	108	56	ND	54
Südkreis Mannheim							
Brunnen I	0.23 ± 0.01	1.28 ± 0.04	92	24	6	ND	—
Brunnen II	0.30 ± 0.01	1.00 ± 0.08	92	24	6	ND	—
Brunnen III	0.49 ± 0.01	1.10 ± 0.05	92	24	6	ND	—
Neckar (Heidelberg)	1.46 ± 0.04	1.49 ± 0.04	152.5	—	—	—	—
German rivers							
Rhein	2.48 ± 0.12	1.29 ± 0.07	222.65	—	—	—	—
Lahn	0.71 ± 0.04	1.91 ± 0.10	146.4	—	—	—	—
Mosel	1.80 ± 0.20	1.40 ± 0.10	213.5	—	—	—	—
Maas	1.20 ± 0.20	1.60 ± 0.20	176.9	—	—	—	—
Nahe	3.50 ± 0.20	1.60 ± 0.10	190.6	—	—	—	—

ND, not determined.

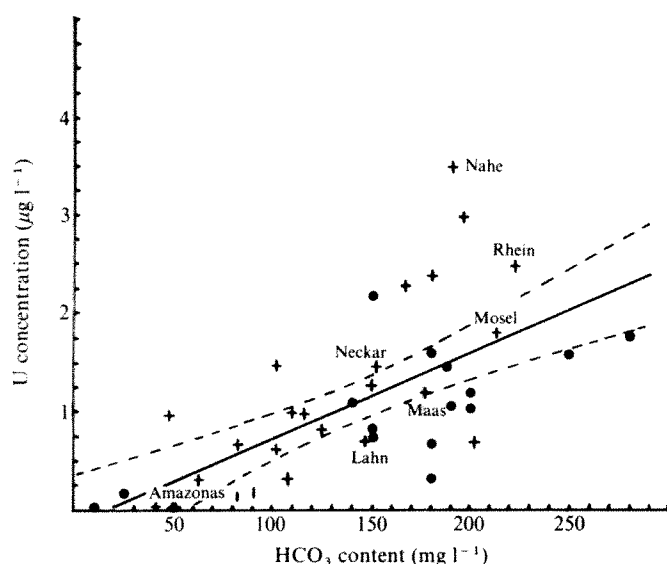


Fig. 1 Plot of the uranium content against HCO_3^- content for all world rivers and lakes, where both data are available. The circles indicate Texas rivers. The straight line is a linear fit for all values, the dotted lines show the 1σ deviation range. Apart from our values, U and HCO_3^- were not measured contemporarily (refs for the uranium contents are 1, 3–6 and 19–23, for the HCO_3^- values are 24, 26). The uncertainty in the HCO_3^- values exceeds 30% in some cases.

movement of precipitation water in the unsaturated zone, determined by tritium tracer experiments (sandy soil near Sandhausen-Heidelberg)¹¹. In addition a groundwater recharge of 190 mm yr^{-1} has been calculated from the bomb tritium vertical distribution in the shallow groundwater body of Sandhausen¹². In the Handschusheimer Feld, groundwater recharge by local rainfall is assumed to be about 113 mm yr^{-1} (data from Wasserwirtschaftsamt Heidelberg), as estimated using conventional hydrogeological methods. Both estimates agree with the bomb tritium standing crop in the shallow groundwater (see Table 1). At a fertilisation rate of $100 \text{ g m}^{-2} \text{ yr}^{-1}$, as commonly applied, some 12 g of nitrogen are annually incorporated to the soil. With an average groundwater

supply of 113 mm one can determine that some 25% of the fertiliser nitrate goes into the shallow groundwater, raising the natural nitrate concentration by some mg l^{-1} (as in the Südkreis Mannheim area) to values as high as 126 mg l^{-1} . (Water is used for irrigation only.) Although fertilisers have a high uranium content (Table 2) the uranium content of the shallow groundwater is even lower than that of the deeper groundwater, having lower nitrate and tritium contents. At the fertilisation rate of $100 \text{ g m}^{-2} \text{ yr}^{-1}$, taking into account an average uranium content of fertilisers of 50 p.p.m., the contamination should be as high as $40 \mu\text{g l}^{-1}$, if all uranium was transported in the newly formed groundwater.

The conclusion must be that more than 95% (2σ) of uranium supplied with the fertilisers are retained in the uppermost soil layers. This conclusion is corroborated by the fact that the activity ratio of the uranium isotopes $^{234}\text{U}/^{238}\text{U}$ of the groundwater lies around 1.6, and is similar to that of the Neckar, whereas uranium in fertilisers has an $\text{AU} = 1$; less than 0.01% of the phosphate goes into groundwater. Assuming a similar transport mechanism for uranium the contamination would make less than $0.004 \mu\text{g l}^{-1}$. Although uranium is apparently released in only negligible amounts to groundwater, or river water, the uranium enrichment in the ploughed and therefore well mixed upper 20–30 cm soil layer should be no higher than 1 p.p.m. and therefore almost undetectable. The lithological profile of a length of 205 m consists of a sequence of water 2–15 m deep bearing gravel horizons interbedded with clays 5–10 m thick acting as aquicludes (data from Wasserwirtschaftsamt Heidelberg). Uranium analyses on samples from the borehole core Entensee showed normal uranium contents, ranging between 1 and 4 p.p.m.

In our groundwater samples the uranium content is fairly well correlated with the HCO_3^- content. A correlation between uranium and HCO_3^- has been reported for groundwaters from a small area of the UK¹³, for the uranium content in a salt lake in California¹⁴ and for mineral waters in the Taunus area¹⁵. This correlation should presumably be attributed to the water-soluble $\text{UO}_2(\text{CO}_3)_3^{4-}$ complex with a high formation constant in a range of $\text{EH} > -0.3$, $\text{pH} > 5$ and $\log P \text{CO}_2 > -4$ (ref. 16). Most rivers meet these conditions and in fact a broad correlation also results for world rivers and lakes, including Texas rivers where a high contribution of fertiliser uranium was suggested

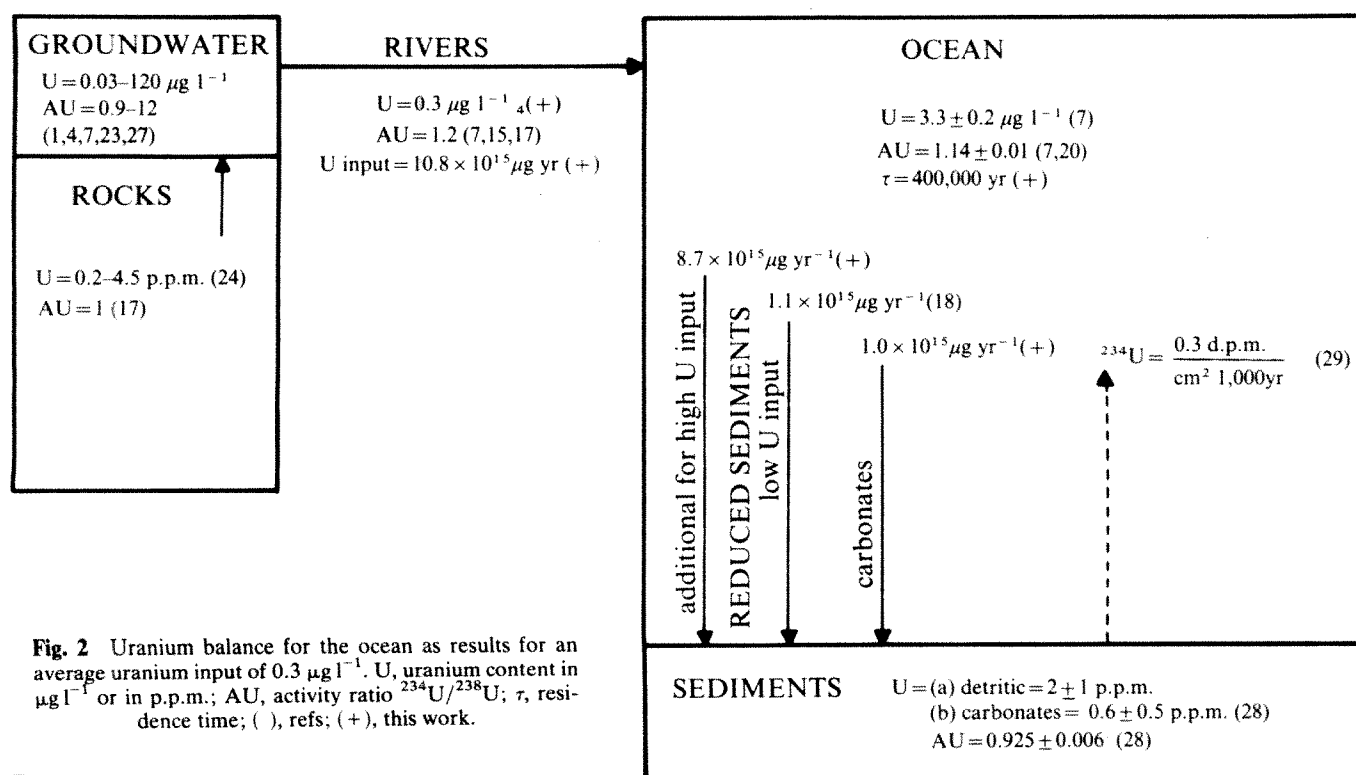


Fig. 2 Uranium balance for the ocean as results for an average uranium input of $0.3 \mu\text{g l}^{-1}$. U, uranium content in $\mu\text{g l}^{-1}$ or in p.p.m.; AU, activity ratio $^{234}\text{U}/^{238}\text{U}$; τ , residence time; (), refs; (+), this work.

Table 2 U content and AU of commercially available fertilisers

Fertiliser	Phosphate content	U content (p.p.m.)	AU
Kalksalpeter	Very low	0.3 ± 0.03	0.91 ± 0.1
Thomaskali	12%	3.3 ± 0.3	0.95 ± 0.1
Orgamin	6%	10.8 ± 2.0	1.05 ± 0.15
Nitrophoska-Blau	12%	48.8 ± 2.0	1.00 ± 0.04
Nitrophoska	12%	50.3 ± 2.0	1.01 ± 0.04

Only the nitrophoska fertilisers are usually applied in the Handschusheimer Feld area.

(Fig. 1). Thus the uranium content largely depends on the available HCO_3^- in the water, giving evidence that the recent uranium content in the rivers is primarily of natural origin. Differences in the bedrock material from which uranium is being leached and uranium derived from fertilisers are obviously of less importance. They might be responsible for the large scattering in Fig. 1, together with the large uncertainty of the HCO_3^- values, not contemporaneously measured with uranium. In the light of these results it seems that the low uranium content of the Amazonas is due to its unrepresentatively low HCO_3^- content and not to a lower level of anthropogenic contamination.

On the basis of this correlation we estimate the natural uranium input to the ocean, for some 35% of the supplied water, to be $0.3 \mu\text{g l}^{-1}$, in agreement with Turekian's previous estimate³. The average activity ratio of the supplied water is ~ 1.2 (refs 7, 15, 17). Applying these values to a steady-state model we calculate a residence time for uranium of 400,000 yr and an annual removal to the sediments of $10.8 \times 10^{15} \mu\text{g}$, a value some 10 times higher than when the Amazonas content is taken as the average. A roughly calculable part of uranium is deposited with the marine carbonates. The rest should then be incorporated into anaerobic sediments, which are deposited mainly on the shelf. We calculate a uranium deposition of $500 \mu\text{g cm}^{-2}$ per 1,000 yr for the entire, and $30 \mu\text{g cm}^{-2}$ per 1,000 yr, for some 6% of the shelf area. The latter is similar to the uranium-deposition rate given by Veeh for the Walfish Bay area, South-west Africa¹⁸.

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Distribution of rare earth elements in some North Atlantic Kimmeridgian black shales

RARE earth element (REE) patterns of Upper Jurassic, Kimmeridgian, shales from various localities are shown here to have only minor variation in spite of deriving from very different source rocks. The formation of such uniform patterns indicate that these sediments are mature, well mixed and formed in similar depositional environments. An extension of the North Sea Kimmeridgian petroleum source rocks is indicated. Minor differences in REE patterns can be related to variations in the sand/silt content.

The REEs from La to Lu ($Z = 57–71$), have been the basis of many geochemical studies. Most of these have considered hardrock problems, but several studies have also been carried out on sediments and sedimentary rocks. Sedimentary deposits often seem to have REE distributions reflecting the different source rocks, sedimentary processes and grain size variations^{1,2}, and some of the REE distributions have been related to REE fixation in marine depositional environments^{3,4}. Generally the

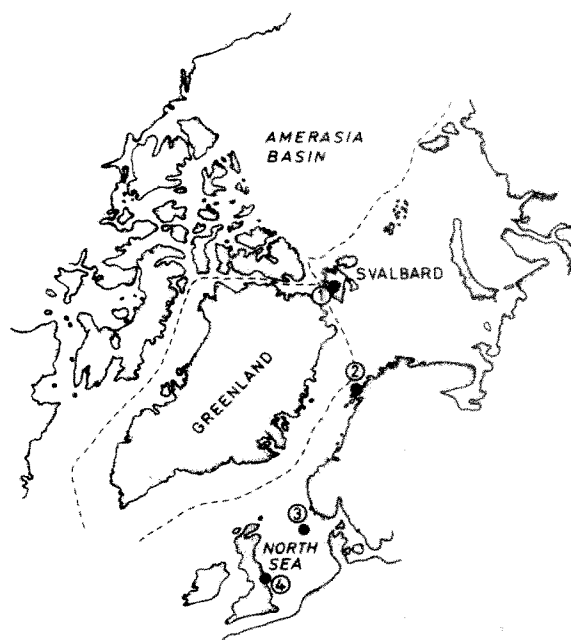


Fig. 1 Reconstruction of the Arctic and adjacent areas before the lower Tertiary opening of the Norwegian Sea. Stippled lines indicate Tertiary plate boundaries. (The compilation is taken from refs 14–17). ① Wimannfjellet, Svalbard; ② Andøya, Northern Norway; ③ Well 17/11-1, North Sea; ④ Yorkshire, UK.

REEs have rather short residence time in the oceans and relative enrichment in the heavier REE (HREE) is common^{3,5}. Because HREE form complexes with higher stability than the lighter REE (LREE), LREE have a shorter residence time in seawater and may be preferentially adsorbed on clay minerals⁴. Alteration and fractionation of the REE patterns may occur in weathering horizons⁶ in addition to variation due to fixation of REE from seawater. Weathered rocks are generally enriched in total REE compared with the original unweathered rocks⁷. Of the main constituents of sediments and sedimentary rocks, the clay fraction (clay minerals and colloids mainly) contains more REE than the sand/silt fraction (rich in quartz and feldspars)⁸. Separations of different sedimentary fractions, either by varying depositional conditions or by weathering reactions may lead to fractionation of the REEs.

REE analyses are normally presented in 'comparison diagrams' (ref. 9), where the elements analysed are all related to a reference distribution. Sedimentary and metamorphic rocks are generally compared with proposed upper crustal average, based on composite sample of 40 North American Shales (40 NAS)^{2,10}. The samples analysed here have been correlated

with this composite sample. The individual element/element ratios have been plotted on a logarithmic scale against atomic number (Fig. 2).

The present work was designed to study how different source rocks affect the REE compositions of the sediments of shallow marine depositional regimes. Radiochemical neutron activation analyses (RNAA) were carried out on 22 samples from the following localities (Fig. 1): Svalbard, Wimannfjellet; Northern Norway, Andøya; North Sea, Well 17/11-1, Norwegian sector; UK, Yorkshire. The method used is based on USGS standards G2 and BCR 1, using the recommended values of Flanagan¹².

In Upper Jurassic Kimmeridgian times similar depositional regimes existed in these four areas (Fig. 1). The four sections represent original different positions in the rather shallow epicontinental Kimmeridgian sea. However, different source rocks were exposed in the adjacent areas. The sediments deposited were generally dark, marine, organic-rich clays, although those on Andøya were somewhat more sandy/silty than in the other localities.

Eight (La, Ce, Nd, Sm, Eu, Tb, Yb, Lu) of the REEs have been determined (Table 1), distribution patterns are shown in

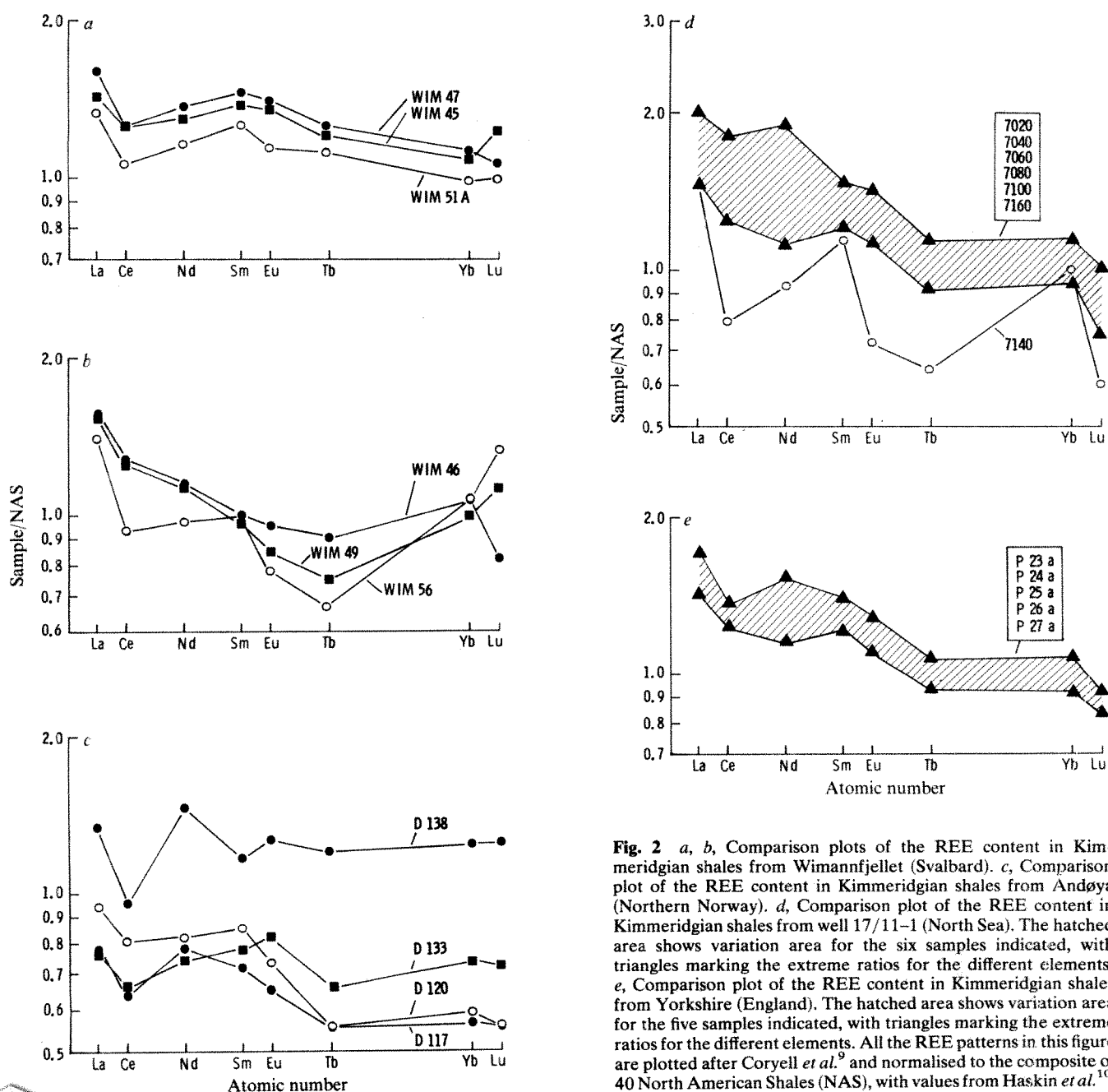


Fig. 2 *a, b*, Comparison plots of the REE content in Kimmeridgian shales from Wimannfjellet (Svalbard). *c*, Comparison plot of the REE content in Kimmeridgian shales from Andøya (Northern Norway). *d*, Comparison plot of the REE content in Kimmeridgian shales from well 17/11-1 (North Sea). The hatched area shows variation area for the six samples indicated, with triangles marking the extreme ratios for the different elements. *e*, Comparison plot of the REE content in Kimmeridgian shales from Yorkshire (England). The hatched area shows variation area for the five samples indicated, with triangles marking the extreme ratios for the different elements. All the REE patterns in this figure are plotted after Coryell *et al.*⁹ and normalised to the composite of 40 North American Shales (NAS), with values from Haskin *et al.*¹⁰.

Table 1 REE (p.p.m.) of Kimmeridgian shales from the North Atlantic region.

	Sample	La	Ce	Nd	Sm	Eu	Tb	Yb	Lu
Svalbard	WiM 45	45.8	93	42.8	7.81	1.69	1.03	3.41	0.60
	WiM 46	51.0	93	38.3	5.70	1.18	0.77	2.88	0.58
	WiM 47	51.1	94	45.8	8.37	1.75	1.08	3.47	0.57
	WiM 49	49.2	88	37.0	5.55	1.05	0.63	3.10	0.55
	WiM 51A	43.2	78	38.2	7.27	1.41	0.94	3.08	0.48
	WiM 56	44.9	67	32.0	5.71	0.98	0.57	3.38	0.64
Andøya	D 133	24.6	48	24.6	4.43	1.03	0.56	2.29	0.35
	D 117	25.1	47	25.6	4.16	0.81	0.47	1.74	0.26
	D 120	30.1	59	26.9	4.83	0.91	0.48	1.83	0.27
	D 138	43.2	70	48.4	6.74	1.59	1.02	3.82	0.60
North Sea	7020	46.9	96	37.2	7.40	1.41	0.81	3.24	0.46
	7040	50.7	102	53.2	6.92	1.51	0.91	2.91	0.43
	7060	64.1	121	62.7	8.54	1.75	0.96	3.12	0.45
	7080	50.1	91	44.2	6.98	1.42	0.78	2.65	0.36
	7100	52.8	98	42.1	8.28	1.51	0.88	3.53	0.43
	7140	46.8	59	30.7	6.63	0.89	0.56	3.00	0.29
	7160	52.5	100	47.4	7.68	1.47	0.90	3.47	0.49
Yorkshire	P 23a	54.3	95	50.2	7.98	1.61	0.91	3.24	0.44
	P 24a	48.3	94	42.2	7.47	1.45	0.82	2.95	0.43
	P 25a	49.4	91	40.6	7.72	1.57	0.84	2.88	0.42
	P 26a	47.8	100	49.2	7.02	1.37	0.79	2.84	0.44
	P 27a	45.1	94	38.3	6.90	1.45	0.85	3.33	0.40
	NAS	32	73	33	5.7	1.24	0.85	3.1	0.48

NAS is a composite of 40 North American Shales values from Haskin *et al.*¹⁰.

Fig. 2. The patterns of the different samples are very similar. In spite of deposition at different localities and derivation from different sources, no major variations in the REE distributions are found (Fig. 2). This probably reflects rather extensive weathering, repeated reworking and homogenisation of sediments before final deposition as fine-grained silts and clays. The sample sets from the four different localities generally show minor enrichments in LREE. This may be due to minor selective adsorption of LREE on weathered clay minerals in the seawater.

In detail the samples from Yorkshire (Fig. 2e) and the North Sea (Fig. 2d) have higher total REE contents and show enrichment of LREE compared with samples from Andøya (Fig. 2c) and Svalbard (Fig. 2a, b). This correlates with certain mineralogical differences—the sediments in Yorkshire and the North Sea being generally fine grained, containing less quartz and feldspar. Higher sand/silt concentrations in the Andøya samples have served to dilute the samples which have lower REE concentrations.

The Svalbard samples analysed are, based on their REE distribution patterns, divided into two different populations (Fig. 2a, b). A selective leaching of the middle REEs due to diagenetic processes is the only explanation we can propose for this observation.

In Svalbard, Andøya and North Sea areas the Upper Jurassic transgression¹³ is reflected in a progressive increase in finer material towards the top of the Kimmeridgian. These developments are partly seen in an upwards increase in total REE and in lower LREE/HREE ratios, reflecting influx and deposition of finer-grained material with lower percentage of sand and silt. However, the similar REE patterns in the different localities clearly indicate rather persistent depositional conditions. Only sample 7140 in the North Sea sample set and sample D 138 from the Andøya sample set seem not to conform. These variations may be explained by occurrences of dolomite in D 138 which is not detected in the other Andøya samples, while sample 7140 from the North Sea have higher sand/silt and lower carbonate contents than other samples from that locality.

Strong weathering of source rocks and later reworking of weathering products resulted in deposition of marine clays with rather similar REE distribution patterns in the different localities, in spite of derivation from different source rocks. This

shows rather similar and persistent depositional regimes in the huge Kimmeridgian epicontinental sea and may indicate possibilities for Kimmeridgian petroleum source rocks north of the North Sea. The generally weak LREE enrichments point towards selective LREE adsorption on clay minerals in seawater. Minor variations in the REE concentrations may be due to grain size variation which in turn reflect minor differences in sedimentation conditions in the Kimmeridgian epicontinental sea.

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Losses of nitrous oxide dissolved in drainage water from agricultural land

DISCUSSIONS on the fate of nitrogen in soil usually assume that the gases produced by denitrification—nitrous oxide and molecular nitrogen—are lost only by movement in the gaseous phase towards the soil surface, where they escape to the atmosphere. As far as we know, there are no published reports of the transport of these gases dissolved in water draining from agricultural land. However, the anaerobic conditions necessary for denitrification develop to an appreciable extent in drained agricultural soils when the soil water potential is zero or positive¹⁻⁶; there is then likely to be drainage of water through the soil. In the soils of southern England, nitrous oxide can be found for extended periods during the wetter months^{7,8}, where there is on average ~250 mm rainfall in excess of evapo-transpiration and soil storage⁹. As nitrous oxide is fairly soluble in water (1.0 ml gas per ml water at 5°C) we have examined the possibility that significant quantities are transported from the soil in drainage water. We report here for the first time observations that nitrous oxide can be lost from the soil by leaching in amounts comparable with those lost in the gas phase from the soil surface.

Water issuing from agricultural drains in heavy clay soils (Table 1, site 2 Lawford (Rowsham) series¹⁰, sites 3 and 4 Denchworth series¹¹) contained much more nitrous oxide than was present in rainfall. Appreciable concentrations of nitrous oxide were also found in water draining from lysimeters containing intact columns (80 cm diameter and 135 cm deep) of two other fine textured soils (Salop¹¹ and Bromyard¹² series). Laboratory incubation experiments showed that the denitrifying activity of drainage water was negligible, even when nitrate and

glucose were added in anaerobic conditions (unpublished results). The nitrous oxide in drainage water had, therefore, come from the soil and not from biological activity in water as it passed down the drain. In addition to water from agricultural drains, appreciable concentrations of nitrous oxide were found in samples from streams, a lake and domestic water supplies (Table 1), but some of the nitrous oxide contained in these sources may possibly have been produced after the water had emerged from the drains (for example, as a result of microbial activity in bottom sediments¹³).

A preliminary estimate of the quantity of nitrous oxide that might be contained in drainage from our experimental fields can be made by assuming that all the excess of rainfall over evapo-transpiration during the winter months (November to March inclusive), on average about 250 mm of water, was removed from the soil by the tile drainage system, and that also this drainage contained a concentration of 100 $\mu\text{g N}_2\text{O-N l}^{-1}$; the amount of nitrogen leached as nitrous oxide would then be about 0.25 kg ha^{-1} , but greater rates of loss may be expected during short periods of high rates of denitrification and drain flow. In comparison the fluxes of nitrous oxide gas from the soil surface in winter, measured at the same sites and during the same period as those in drainage were equivalent to 0.15–0.9 kg N ha^{-1} (ref. 14). Losses as molecular nitrogen in the gas phase from the soil surface may be considerably greater than this, as it is commonly believed¹⁵ that the ratio of $\text{N}_2:\text{N}_2\text{O}$ is ~16:1. However, leaching losses of molecular nitrogen greater than that of nitrous oxide are unlikely because the solubility of nitrogen in water is 50 times less than nitrous oxide (0.02 ml gas per ml water at 5°C) and it would therefore require an unusually high $\text{N}_2:\text{N}_2\text{O}$ ratio of 50:1 or more to achieve comparable rates of loss. For comparison, the concentration of nitrate-N in drainage water always exceeded the concentration of dissolved nitrous oxide-N by 1–3 orders of magnitude (Fig. 1).

Water samples collected from an open drain outlet, as in the present work, provide an inaccurate basis for estimating the amounts of nitrogenous gases leached from the soil, as an

Table 1 Nitrous oxide content of water from various sources

Source of water	Site*	Sampling period	No. of sampling occasions	Concentrations observed	
				Mean ($\mu\text{g N}_2\text{O-N l}^{-1}$)	Range
Agricultural drains	1	Dec 1977–May 1978	41	8.1	2–25
	2	Feb 1977–Apr 1977	7	21.4	4–56
		Nov 1977–May 1978	60	21.4	4–132
	3	Dec 1977–Jan 1978	2	8.7	1–27
Lysimeter drainage	4	Dec 1977–May 1978	20	108.2	0.7–415
Soil solution	1	Nov 1977–May 1978	17	15.1	0.3–544
	2	Oct 1975–Apr 1976	25	1.2	0.3–28
		Dec 1976–Jul 1977	18	277.3	0.5–9984
		Nov 1977–Mar 1978	17	92.8	0.3–1405
Bore-hole in chalk	5	June 1978	2	32.2	4–56
Domestic supplies	4	June 1978	2	53.5	24–83
	5	June 1978	2	20.6	7–61
Surface waters	4 (Lake)	June 1978	1	9.4	–
	4 (Stream)	June 1978	1	7.1	–
	1	Jan 1978	1	4.3	–
	2	Jan 1978	1	3.6	–
Rainfall	4	June 1978	1	0.3	–

Water was sampled using 5-ml glass syringes sealed with 3-way plastic valves. Samples were analysed using an equilibration technique¹⁶ on a Pye-Unicam GCV gas chromatograph equipped with an electron capture detector (350°C) and a column (2 mm wide, 100 cm long) of Carbosieve B at 110°C.

* Sites: 1, Northfield, near Stanford-in-the-Vale, Oxfordshire; 2, Compton Beauchamp, Uffington, Oxfordshire; 3, MAFF Experimental Husbandry Farm, Drayton, near Stratford-upon-Avon, Warwickshire; 4, ARC Letcombe Laboratory, Wantage, Oxfordshire; 5, Mill Farm, Marten, Wiltshire.

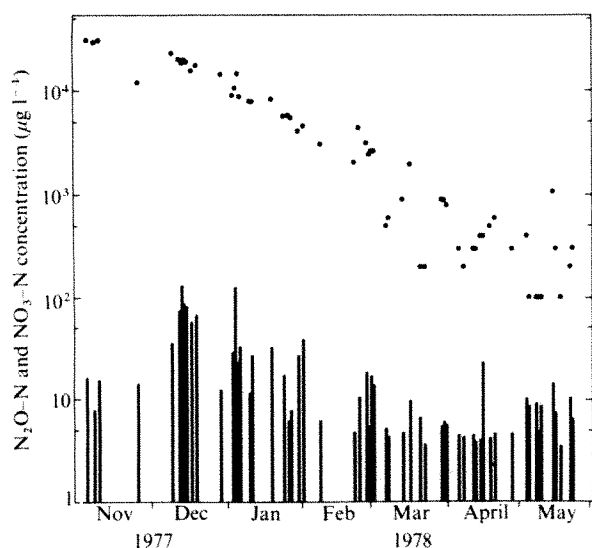


Fig. 1 Nitrous oxide (solid bars) and nitrate (points) content of water draining from an arable field during the winter of 1977–78 at Compton Beauchamp, Uffington, Oxfordshire. The field was sown with winter wheat in October 1977; no nitrogen was used until April 1978 when 80 kg N ha⁻¹ as ammonium nitrate was applied. The rainfall between November 1977 and May 1978 was 440 mm (the long term average for this period is 405 mm).

unknown amount of gas diffuses from the water or soil into the atmosphere of the drain. Air drawn from drains contained nitrous oxide concentrations of up to 100-fold that in the terrestrial atmosphere, but neither measurements nor estimates of loss by gas movement in drains have been made. However, improved estimates of leaching losses of nitrous oxide made during a number of contrasting winters may be obtained from a long term field experiment, begun in 1978, where the equipment for the continuous monitoring of water is designed to prevent the gas phase transport of nitrous oxide from the drain.

Although in our preliminary experiments the loss of nitrogen from the soil profile by nitrous oxide dissolved in drainage water seemed a minor component of the soil nitrogen balance, in other conditions losses by this route could be larger, particularly where high rates of denitrification and drainage of water are experienced. However, the loss of nitrous oxide in drainage from agricultural land is an important new factor to be considered in assessments of the contribution made by denitrification to the nitrous oxide content of the atmosphere.

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Developmental fate of the cyanogenic glucoside linamarin in Costa Rican wild lima bean seeds

WHEN a seed germinates, we expect the defensive secondary compounds it contains to be transferred intact to the growing seedling, or variously decomposed to produce resources for the growing seedling. The seeds of wild indigenous Costa Rican lima beans (*Phaseolus lunatus* L.) contain about 3.45% fresh weight of linamarin¹, a cyanogenic glucoside that can enzymatically decompose to produce 0.37% fresh weight hydrocyanic acid (HCN). We report here that, after germination, the amount of linamarin in the total seedling (roots, cotyledons, and shoots) remains essentially constant during a 26-day experimental period and moreover corresponds to the amount of linamarin present in the seed before germination (Fig. 1).

To determine the fate of this defensive secondary compound in young seedlings, we germinated seeds and monitored the linamarin content of the seedlings for 26 days. While the amount of linamarin per seedling remained constant, the distribution of the glucoside present initially only in the seed changed dramatically with time. For example, after 6 days 42% of the total linamarin in the seedling was contained in the cotyledons, 27% was in the new leaves and shoot tip, 20% was in the stem and 11% was in the roots. After 14 days, 69% of the linamarin was in the leaves, and after 16 days the cotyledons were devoid of linamarin and dropped off. The change in fresh weight of the different plant parts, expressed as per cent of the plant's total weight, paralleled the change in linamarin for each different part. That is, the cotyledons decreased in weight each day by an

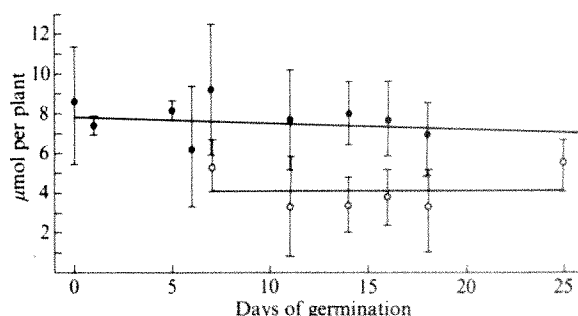


Fig. 1 The linamarin content of individual seeds or seedlings of wild lima beans (*Phaseolus lunatus*). Seeds were germinated by pricking the thin seed coat with a pin and soaking overnight in distilled water. Seedlings were grown in vermiculite at 24 °C with a 14-h photoperiod. On the 7th day of growth the cotyledons were carefully removed from 15 seedlings (○) to permit comparison with untreated seedlings (●) whose cotyledons remained on the plant for ~2 weeks. Linamarin content was determined by grinding weighed fresh seeds or seedlings in liquid nitrogen with a mortar and pestle. The ground sample was immediately transferred to 2 ml of 0.1 M phosphate buffer (pH 6.8) containing the enzymes emulsin and linamarase in the outside compartment of a centre well flask; the centre well contained 1 ml of 1 M NaOH. After 24 h in a shaking water bath at 37 °C, the contents of the centre well were assayed for HCN, a product of the enzymatic decomposition of linamarin. The method used⁶ detects HCN in the range 0.01–0.15 µmol. Qualitative examination of 80% (v/v) ethanolic extracts by gas liquid chromatography⁷ established that linamarin was the only cyanogenic glycoside present either in seed or seedlings. Seeds were hand-shelled from mature dry pods from a large wild plant in March, 1976 at Finca La Parca, Cañas, Guanacaste Province, Costa Rica. An average of 62 mg (s.d. 13 mg, n = 25). Seeds from this morphic; 60% were black and 30% brown. The black seeds were used in the

amount corresponding to 5% of the total weight of the seedling and lost 4.4% of the total linamarin each day. Roots gained 2% in weight and 0.5% in linamarin each day while stems lost 0.9% in weight and 0.4% in linamarin each day.

The data displayed in Fig. 1 suggest that the plant either transfers its linamarin intact from the seed (cotyledons) or breaks it down and resynthesises it in equal amounts during new growth of the seedling. To determine what happens, the cotyledons were removed from 15 plants after 7 days of growth. The excised cotyledons contained approximately 4.5 μmol linamarin, and the glucoside content of the seedlings dropped to about 4.0 μmol per plant. Although the treated seedlings continued to grow and develop, their linamarin content remained constant throughout the experiment. This could be explained by assuming that the plant uses only the breakdown products of the linamarin for resynthesis in the growing seedling. However, the breakdown products of the aglycone of linamarin are acetone and HCN, compounds which are not readily converted to valine, the demonstrated primary precursor of linamarin in other linamarin-containing species¹. We therefore prefer the more parsimonious explanation that the linamarin is transferred intact from the cotyledons to the growing seedling.

The degree to which plants synthesise secondary compounds as they change and develop, as opposed to shifting the location of compounds already synthesised, should be an important consideration in the pattern and budget of chemical defenses against herbivores. In *Heteromeles arbutifolia*, a chaparral evergreen shrub in California, the cyanogenic glucoside in the immature fruit is transferred to the ripening seed, and thus the same chemicals probably serve as a defence in consecutive developmental stages². On the other hand, seeds of *Sorghum vulgare* do not contain a cyanogenic glucoside but the seedlings synthesise large amounts of it (dhurrin) in the first 5 days of growth³. Some kinds of secondary compounds are probably removed from deciduous tree foliage before the leaves are shed; presumably they can be used in other parts of the plant or in leaf production in later seasons.

Seeds contain a large variety of secondary compounds which are undoubtedly defensive for the seed⁴, amongst other possible functions. While it is easy to postulate that such secondary compounds are stored as resources for developing seedlings, this has never been demonstrated. In the present study we have demonstrated that they may also be defense resources for the developing seedling. In short, if the seed is a container whose volume or weight is to be minimised (for dispersal purposes), the parent plant produces a higher quality package for the seedling if it puts the intact, defensive molecule into the seed than if it puts in the raw materials for synthesis of that molecule.

With this view in mind, there are at least three selective forces acting on the quantity of a given kind of secondary compound to be found in a seed. First, there are the levels that the parent plant can donate considering the total number of seeds it is producing and its own needs. Second, there are the levels necessary to deter the relevant herbivores from eating the seeds. Third, there are the levels needed to give enough defensive compounds to the seedling to meet its herbivore challenges. The first selective force will act to minimise the compound level and the second two to maximise it; however, the second two need not necessarily have the same optimal level. For example, co-evolutionary selection by a bruchid beetle known to feed on lima bean seeds (D.H.J., unpublished) may drive the concentration of linamarin in the seed up until it is higher than that which is needed by the seedling. At this point, the levels of linamarin in the seedling may have increased to a point well over that which is needed to deter an insect that eats seedling leaves. Conversely, strong selection on the seedling for increased linamarin content by a defoliator may drive the linamarin content of the seed to a high enough level that the bruchid larvae can no longer survive on the seed. This would be a real-life example of the theoretical model in which herbivores competitively interact through the resource budget of the plant in evolutionary

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Somatic fusion between cell wall mutants of *Chlamydomonas reinhardtii*

SOMATIC FUSION has recently been used to isolate hybrids from auxotrophic mutants of fungi^{1,2}, yeast³ and mosses^{4,5}. These fusions were performed by using polyethylene glycol (PEG) and/or CaCl_2 solutions on protoplasts which were prepared from cells of tissues treated with enzyme mixtures to dissolve the cell wall. In the unicellular green alga *Chlamydomonas* mutant strains unable to make a cell wall have been isolated^{6,7}. Such mutants, similar to plant protoplasts prepared by enzymatic digestion, can be easily grown in defined liquid or agar media⁸. However, they differ from true protoplasts in that they do not burst in hypotonic medium or even in distilled water, indicating that some mechanism of osmoregulation is present⁶. It was thus of interest to determine whether these cell wall mutants could be used as plant protoplasts in fusion experiments. We report here the isolation of stable fusion products

Table 1 Comparison of four fusion products from 303 $\times$ 308 with the wild-type haploid strain and a diploid *arg-7* $\times$ *arg-7-3* obtained by sexual mating

Strain	Mating type	Cell volume* (μm^3)	Nuclear volume† (μm^3)	Relative DNA content§		Chloroplast DNA (% of total DNA)
				1	2	
Haploid (WT)	+ or -	30 (± 5)	2.8 (± 0.5)	1.0	1.0	14.1
Diploid (<i>arg-7</i> $\times$ <i>arg-7-3</i>)	-	58 (± 10)	6.9 (± 1.9)	1.9	1.9	11.1
303 $\times$ 308/1	+	58 (± 10)	7.1 (± 1.2)	1.8	2.0	7.3
303 $\times$ 308/2	+	56 (± 10)	-	-	-	-
303 $\times$ 308/3	+	56 (± 10)	7.6 (± 1.4)	-	-	-
303 $\times$ 308/4	+	60 (± 10)	-	-	-	-

* Cells grown on agar medium were suspended in distilled water and incubated overnight in the dark before being measured. The volumes were determined with a Coulter electronic counter model ZF (orifice 100 μm).

† Cells grown on agar medium were fixed and stained for nuclei (Feulgen reaction). The nuclear volumes were determined from diameter measurements of 20 nuclei after photographic magnification, assuming that nuclei were spherical.

§ Cells were grown in 300 ml of liquid medium and collected during exponential growth. 1. The DNA was estimated by the diphenylamine reaction (modification of the Burton's method). Values are means of two experiments. 2. Cells were incubated overnight in distilled water then fixed in alcohol. They were treated with RNase for 1 h (1 mg ml⁻¹ at 37 °C) and labelled with propidium iodide. The DNA content was measured by flow microfluorimetry.

|| The cells were grown in 1 l of liquid medium. They were lysed with SDS (10% final concentration) and then DNA were extracted by successive digestions with pronase, RNase and proteinase K, interspersed with alcohol precipitations¹³. The DNA were further purified on agarose¹⁴ and ultracentrifuged in CsCl gradients. Nuclear ($d = 1.724 \text{ g cm}^{-3}$) and chloroplast ($d = 1.695 \text{ g cm}^{-3}$) were clearly separated and analysed by UV spectrophotometry.

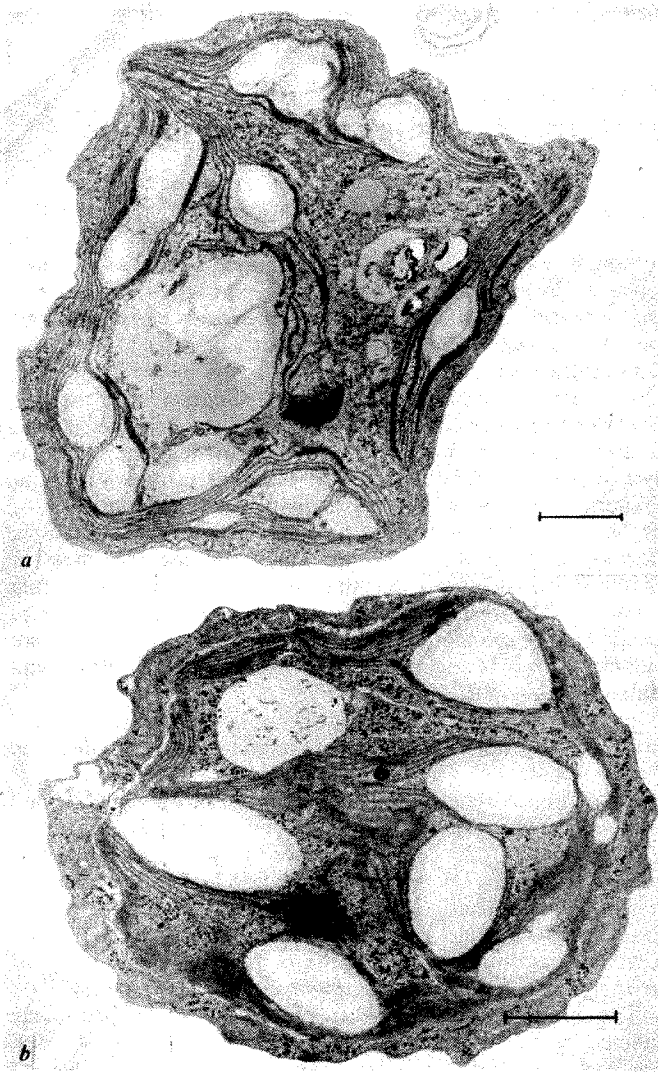


Fig. 1 Ultra-thin sections through cells of 303 (a) and 308 (b) strains lacking a cell wall. The cells were fixed with 4% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.0, for 1 h, post-fixed in 2% osmic acid for 2 h. The samples were dehydrated through an alcohol series and embedded in Epon. Sections were stained with uranyl and lead. Scale bar, 1 μ m.

obtained from cell wall haploid mutants of *Chlamydomonas reinhardtii* having the same mating type (mt^+) and bearing genetic markers of auxotrophy.

The two following strains, isolated after crosses between mutants available in our laboratory, were used in the fusion experiments: strain 303, mating-type plus (mt^+), absence of cell wall ($CW15$)⁶, auxotrophy for arginine ($arg-7$); strain 308, mating-type plus (mt^+), Cw_d (a putative cell wall strain defective for four phosphatases and called $P_2P_dPD_dPD_d$ by Loppes and Deltour⁷), auxotrophy for arginine ($arg-7-3$). The $arg-7$ and $arg-7-3$ mutants, both defective for the same enzyme and mapping in the same cistron are able to complement and restore the wild phenotype in diploids⁹.

Analysis of the strains 303 and 308 under the light microscope revealed that both were deprived of a cell wall. The absence of wall was confirmed in electron microscopy (Fig. 1).

A complementation test made between the strain 308 mt^+ and a strain identical to 303 but mt^- led to recover prototrophic diploid cells with a normal cell wall. This indicates that $CW15$ and Cw_d mutations do complement and are probably located in different genes.

The mt^+ cells used in the fusion experiments were routinely grown on agar mineral medium deprived of ammonia and enriched with yeast extract (M-N + YE¹⁰), containing enough arginine to allow growth of the auxotrophs. Several assays were

conducted by treating the cells in PEG solutions for 30 min, then washing them and plating on minimal agar medium. Positive results were obtained in two experiments and negative results in several others. We finally obtained reproducible positive results by using the following simple method. About $4-7 \times 10^7$ cells of each strain were sampled with a platinum loop and transferred onto a minimal agar medium¹¹ in Petri dishes (9 cm diameter). The cells were mixed with a loop, then 0.1 ml of the following fusion solution was added: 50 mM PEG 6000 Merck, 7.5 mM $CaCl_2$, 5 mM glycine, NaOH to make pH 8.0. This solution was sterilised by filtration through a Millipore membrane (0.45 μ m). The cells were carefully mixed in the fusion solution then spread on the agar surface. Two types of control were made: (1) each strain was separately treated with the fusion solution; and (2) a mixture of the two strains was treated with distilled water. After an eight-day growth at 25°C 8,000 lux, 30-100 colonies ranging between 0.2-1.2 mm diameter were observed in the plates corresponding to the fusion treatment. No colony was observed in the controls.

Four colonies were isolated and subcultured for further analysis on medium deprived of arginine. A microscopical observation of the four isolates revealed the presence of a cell wall in all of them. This observation was confirmed by electron microscopical analysis (Fig. 2). The fusion products form a wall as do the diploids obtained by sexual mating.

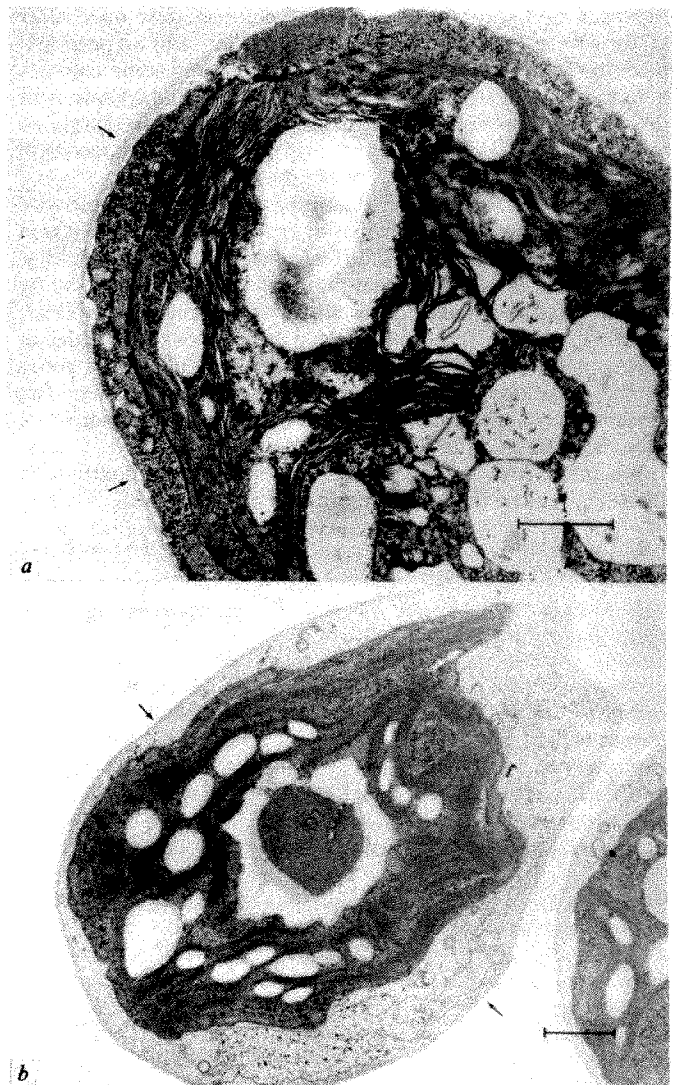


Fig. 2 Ultra-thin sections through cells of a fusion product 303 $\times$ 308/1 (a) and a diploid $arg-7 \times arg-7-3$ (obtained by sexual mating) (b). The cell wall is present (arrow). The samples were prepared for electron microscopy as described in the legend to Fig. 1. Scale bar, 1 μ m.

The fusion products were compared to the wild-type haploid strain and to a diploid strain (*arg-7* × *arg-7-3*) obtained by sexual mating: they were analysed for their cell volume, nuclear volume, total DNA content and chloroplast DNA: total DNA ratio. The results (Table 1) indicate that the fusion products are very similar to diploids as far as cellular volume, nuclear volume and DNA content per cell are concerned. The fusion product 303 × 308/1 (the only one analysed for that criterion) has however a lower chloroplast DNA content (7.3% of total DNA) than that of the haploid or diploid strains (14.1 and 11.1%). Chiang¹⁵ and Lane and Sager¹⁶ found that vegetative haploid cells contain about 14% chloroplast DNA whereas gametes contain only 7%. We do not know whether the low value found for the isolate studied here corresponds to a peculiarity of the strain or if it is a common feature of the fusion products. This point will be further analysed.

The fusion products were, moreover, found to be mating type plus (Table 1): they differ in that respect from diploids obtained by sexual mating which are always mating type minus (*mt*⁻ gene is dominant over *mt*⁺). The four strains were crossed with gametes of haploid wild type *mt*⁻. Zygotes were visualised in all crosses, indicating that the four strains were fertile. The zygotes issued from two crosses were plated on minimal medium containing 4% agar and matured in the dark⁴⁷. The unmated cells were killed by exposure to chloroform vapour and after germination of the zygotes the meiotic products were plated on a rich agar medium (M-N+YE). The colonies developed after 10 days were replicated on rich (M-N+YE) and on poor (M) medium. In both crosses, wild type colonies and some colonies able to grow on rich medium only were detected. Analysis of these auxotrophs showed that they specifically required arginine for growth. This indicates that the two strains analysed were still bearing *arg*⁻ markers.

These results demonstrate that somatic fusion is possible with cell wall mutants of the same mating type (*mt*⁺) in the green alga *Chlamydomonas reinhardtii*. The fusion products are very similar to diploids obtained by sexual mating but differ from them by their mating type and perhaps also by their chloroplast DNA content. They constitute new tools to improve our knowledge of problems such as the maternal inheritance of chloroplast genes or the gene dosage effects in mating-type expression. The present results also open new possibilities for the isolation of hybrids between different algae species.

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Genetic analysis of maternal information in *Drosophila*

EMBRYONIC development is controlled by the information carried by the gametes of the zygote's parents. However, although the chromosomal information is similar in both gametes the oocyte also provides a structural frame (determining embryonic axes), determinants in the cortex and a metabolic pool to support early embryonic events (see ref. 1). The active transcription of thousands of DNA loops in the lampbrush chromosomes of amphibian oocytes suggests that the egg contains transcripts of a large fraction of the genome². In sea urchin oocytes the mRNA complexity might account for the synthesis of several thousands of different protein species^{3,4}. Genetic analysis should help to establish the functional meaning of the information present in the oocytes for embryonic development. In this study we have analysed the effect of chromosome deletions on the development of *Drosophila* embryos. The results indicate that the products of a large proportion of the maternal genome, present in the oocyte, are sufficient and necessary for epidermal morphogenesis and differentiation in the embryo.

In *Drosophila* about 90% of the genes have lethal alleles⁵, and about 30% of those lethals die in the homozygous condition in embryonic stages⁶. The lethal effective phase (LEP) of most of these mutants is late—after completion of morphogenesis and sometimes even after completion of epidermal differentiation⁷. Lethal mutations caused by chromosome deletions may show earlier LEPs, possibly because they have a higher probability of including null-alleles of early acting genes^{7,8}. However, it is not known how far the LEP of the embryos is due to the actual genetic constitution of the zygote or to insufficient supply of gene products from the heterozygous mother to the oocyte.

The chromosome deletions used in this study were previously defined as being homozygous cell lethal in clones of adult epidermal cells resulting from mitotic recombination⁹. This cell lethality implies that products of the genes contained in the deletion are necessary for cell division and/or cuticular differentiation. These deletions might therefore be expected to prevent cell proliferation and cuticular differentiation in homozygous embryos. In this study we analyse the lethality of embryos from parents both of which are heterozygous for chromosome deletions (HM cross) compared with that of embryos from heterozygous mothers but wild-type fathers (♀ HT cross) or from heterozygous fathers but wild-type mothers (♂ HT cross). Whereas the first cross gives rise to homozygous (25%) and heterozygous embryos (50%) the second and third cross yields only heterozygous embryos (50%). As all the deletions studied are recessive lethals, heterozygous embryos are expected to show normal morphogenesis and differentiation.

To avoid lethality due to deleterious factors present in the rest of the genome the experimental crosses were made with F₁ flies from an outcross of the deletion-carrying stock to a wild-type (Vallecas) stock. Females were maintained with an excess of males for 3-4 d in rich medium and afterwards transferred every day to an egg deposition chamber for 3-4 h. Eggs were counted after removal of the parents. After 24 to 26 h (embryonic development takes 21-22 h) the unhatched eggs were counted, dechorionated in sodium hypochlorite (7%) and observed under paraffin oil under a compound microscope. Samples of these eggs were mounted in Faure's medium. We have classified the LEPs of dead embryos as before any visible cuticular differentiation (before stage 9 of Bownes¹⁰) or with clear segmentation and cuticular differentiation of mandibles, tracheae, dorsal and ventral bands of hooks (stage 14). Dead embryos between stages 9 and 14 occurred in less than 10% of all crosses studied. Samples of eggs from each cross were studied 4-6 h after laying to check that all had initiated development. We have made no

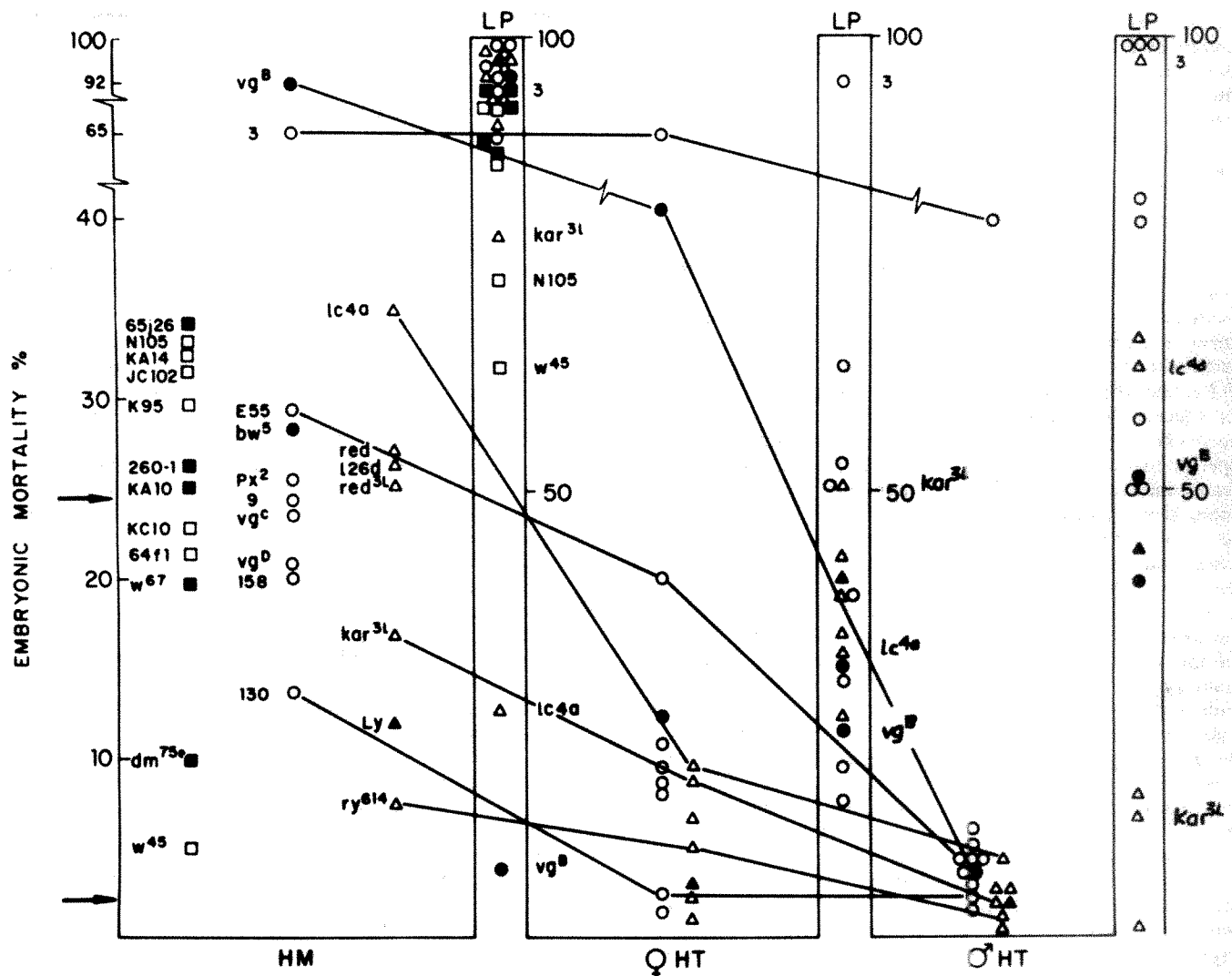


Fig. 1 Per cent of lethal embryos and embryonic lethality phase (LP) in crosses of flies carrying chromosome deletions. LP, percentage of lethal embryos which reach stage 14 (ref. 10). Deletions of X(□), 2nd (○) and 3rd (△) chromosome (deletions described in ref. 9). Open symbols indicate deletions which are not viable in clones of epidermal cells; full symbols viable ones⁹. HM, cross of heterozygous mothers to heterozygous fathers (for deletions in X, males are wild-type). ♀ HT and ♂ HT, crosses where either the female or the male were deletion heterozygotes, crossed to wild-type flies. Percentages of lethal embryos based on 5–10 laying periods and 400–1,200 eggs. Arrows, expected lethal fraction in the HM cross and in wild-type control flies. Connecting lines between representative deletions in the three crosses.

attempt to determine the phenocritical phase⁶ (earliest mutant effects) of these deletions (see below) nor how they affect morphogenesis of internal organs.

For the various crosses the numbers of lethal embryos and their LEPs are presented in Fig. 1. In the HM cross the proportion of lethal embryos varies around the expected 25%. Lower values indicate that some of the homozygous embryos are capable of hatching as larvae and should die in subsequent developmental stages. Higher values indicate that not only homozygous embryos but also other sibling embryos (heterozygotes or even wild type) die. Surprisingly, in most of the deficiencies studied the embryos reach stage 14 (Fig. 1). They show normal cuticular segmentation and differentiation as well as malpighian tubules and even active movements. These findings indicate that gene products which are essential for epidermal cell proliferation and for cuticular differentiation in imaginal disks must either have been available from the oocyte or are not required for embryonic development.

The following data indicate that maternal gene products are necessary for early development. In 8/17 ♀ HT crosses embryonic lethality is significantly higher than in wild-type controls (2%) (Fig. 1). This lethality could result from the genetic insufficiency of deletion heterozygous embryos. However, the lethality found in the ♂ HT cross, which gives rise to the same

heterozygous embryos is, in 8/17 of the deletions studied, lower than in the ♀ HT cross (Fig. 1). Thus, the lethality in embryos from deletion heterozygous mothers must be due to the haplo-insufficiency of the genes included in the deletion chromosome in the oocyte. Lethality phase data for embryos from the ♀ HT cross support that inference: these embryos die mainly in the early stages of development. This suggests that, for some of the zygotes, the chromosomal information is not expressed in time to rescue the oocyte insufficiency. Embryonic lethals usually show several lethal phases⁷, so it is possible that the different LEPs found in embryos of the HM cross actually correspond to the effect of either maternal or zygotic insufficiency.

We have studied 29 deletions in the major chromosome arms, extending from 2 to 30 salivary chromosome bands⁹. The total number of deleted bands studied is 342, of which 283 are not overlapping. The latter figure corresponds to about 6% of the total number of bands (5,000) in the polytene chromosomes. If the chromosome regions analysed are considered as a representative sample of the genome, several general conclusions can be drawn. There is a poor correlation between the size of the chromosome deletions and embryonic lethality in the HM cross ($R=0.35$) or in the ♀ HT cross ($R=0.32$), supporting the conclusion^{7,8} that lethality does not result from additive effects of the deleted genes. More than 50% of the homozygous

embryos of all the deletions studied reach advanced stages of embryogenesis, including normal segmentation and cuticular differentiation. To that group four *minute* loci studied and the *nullo-IV* embryos¹¹ should be added. Thus, for epidermal derivatives it seems that embryonic morphogenesis and differentiation can proceed in the absence of many genes including those necessary for the viability of epidermal cells in imaginal anlagen. Both developing systems, embryo and imaginal anlagen, differ in that whereas the imaginal anlagen undergoes extensive cell proliferation during the larval period, cell proliferation in the embryo following blastoderm is minimal^{12,13}. Thus enough maternal gene products (mRNAs or proteins) may perdure in embryonic cells to allow normal segmentation and cuticular differentiation. If this interpretation is correct then there are two important corollaries.

Lethals with phenocritical phases⁶ in post-embryonic stages or affecting particular tissues cannot be used to indicate the existence of specific genes for larval or imaginal development or specific tissues. It is possible that a large fraction of genes are transcribed in the oocyte and active in embryonic as well as in other developmental stages and in several tissues, but have different perdurance periods. The difficulty of ascertaining phase and tissue specificity of genes is increased when we know only their leaky mutant alleles.

Mutant alleles with phenotypes in the progeny of homozygous mothers (maternal-effect mutants) are known in a small fraction of the genetic loci^{14,15}. Some were shown to be allelic to homozygous visible mutants, others to express the phenotype only at restrictive temperatures. The study of their null-alleles will help to distinguish maternal information genes proper from genes with pleiotropic effects during development and oogenesis.

If embryonic morphogenesis, at least for cuticular segmentation and differentiation, can proceed without the zygotic contribution of an important fraction of the genome, how is it achieved? Systematic study of deletions in lethal embryos may show which genes are responsible for structural elements and which are involved in the specification of segments¹⁶, germ layers and tissues. The haplo-insufficiency shown by some deletions in heterozygous mothers should help to distinguish morphogenetic effects assignable to the female gamete from those which result from the zygotic constitution and only appear in homozygous embryos.

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Meiotic crossing over exchange in the female mouse visualised by BUdR substitution

ALTHOUGH chiasmata have long been thought to be sites of crossing over exchange between chromosomes during meiosis, in keeping with the partial chiasmatype hypothesis^{1,2}, there has been no convincing visual evidence of this despite many attempts (summarised in refs 3-6). Taylor⁷ confirmed by autoradiography that meiotic crossing over occurs by exchange involving breakage and reunion of chromatids. However, because of the poor resolution of autoradiography, requiring study of meiotic chromosomes after 1st metaphase, and because of interference by sister chromatid exchanges, a one-to-one correspondence between chiasmata and exchanges of labelled chromatid segments could not be established^{8,9}. The difficulty was alleviated by correlating chiasmata and exchanges with temperature variation¹⁰, or by selecting a species with localised chiasmata and thus with predictable points of exchange¹¹. 5-Bromodeoxyuridine (BUdR) substitution with differential labelling and consequent differential staining of mitotic chromosomes¹²⁻¹⁵ offer the potential for studying chromosomes at 1st meiotic metaphase, giving an unequivocal picture and separating sister chromatid exchanges from chiasma exchange points between homologues. Tease¹⁶ has reported success with male grasshopper meiotic chromosomes, but an attempt with the male mouse showed only sister chromatid exchanges in the sex chromosomes, and the autosomal bivalents at 1st meiotic metaphase were not differentially stained¹⁷. In the male Armenian hamster the sex chromosomes showed differential staining and an apparent exchange of segments but again the autosomes were not critically differentially stained¹⁸. This may be partly due to the make-up of the male meiotic chromosomes. We have overcome these problems by using an *in vitro/in vivo* technique¹⁹ which enables us to expose the differentiating germ cells of embryonic ovaries to BUdR *in vitro* and to collect and

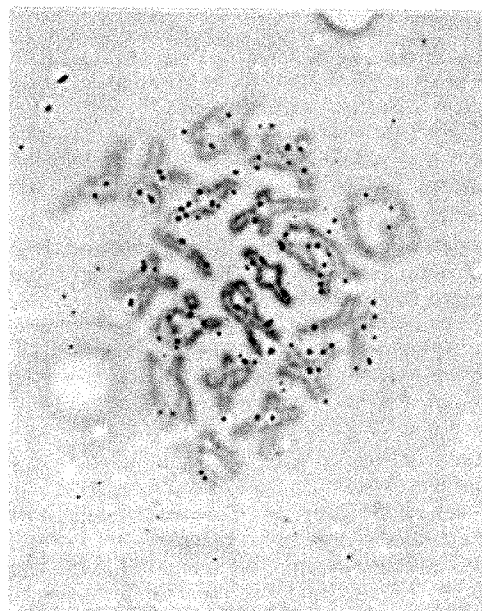


Fig. 1 14-Day (plug = 1) embryonic ovaries were set up in culture on agar strips in Ham F10 (Gibco Biocult) plus penicillin/streptomycin, L-glutamine, 20% calf serum and 3 μ M deoxycytidine, with ³H-BUdR, 0.1 μ Ci ml⁻¹ (0.5 Ci mmol⁻¹). After 48 h they were transferred to basic medium without ³H-BUdR, and transplanted on day 8 of culture (setting up day 0), with minimum light throughout. Meiotic preparations from ova were collected on day 19 (transplant day 0). Autoradiography was carried out after stripping film application and 6 weeks development (photographed Kodak 5460, linear magnification $\times 800$).

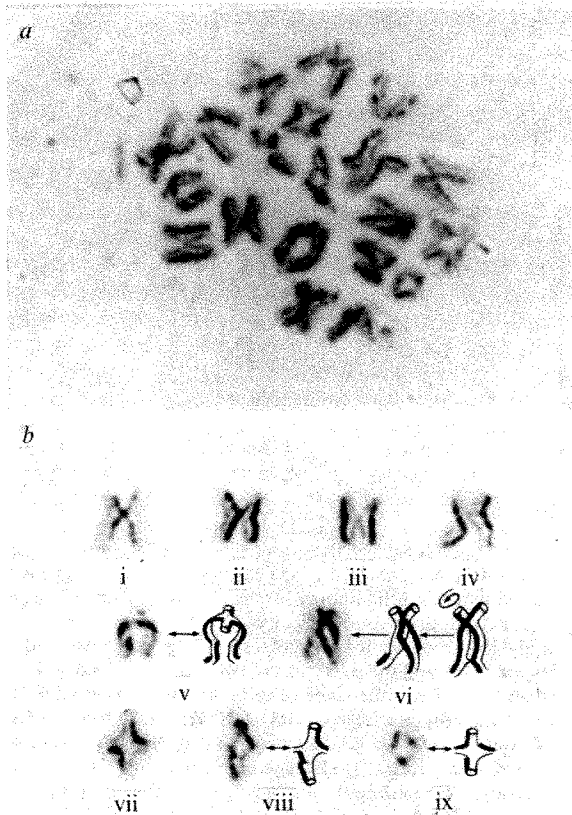


Fig. 2 14-Day embryonic ovaries were cultured in basic medium with cold BUdR, $2.0 \mu\text{g ml}^{-1}$. After 24 h this was changed to basic medium without BUdR. They were transplanted on day 8 and ova were collected on day 19. The cells were stained with Hoechst fluorochrome and examined by differential fluorescence in a UV light microscope. They were then exposed to daylight for 3 d, stained with 10% Giemsa (Gurr) in phosphate buffer, pH 6.8, and mounted in Xam (for details see legend to Fig. 1; linear magnification $\times 800$). *a*, 1st meiotic metaphase with differentially labelled chromatids. *b*, Meiotic configuration selected from *a*, with diagrams (see text). Heavy and light lines reflect the pattern of BUdR substitution; circles represent centromere positions. Note that in *v* an exchange between homologues has occurred very near the centromere.

display the chromosomes²⁰ of the mature oocytes some time after transplantation into young spayed adults. We report our results here.

We used A Strong female fetuses as donors and CBA/H-T6T6 females as recipients, and were completely successful, but in general it is known that even in conditions similar to ours ovarian isografts fare better than allografts²¹. By chase-labelling germ cells *in vitro* with ^3H -thymidine, followed by autoradiographic scoring at pachynema/diplonema 4 d later, we established the optimum time for BUdR labelling in our system and strain.

Using ^3H -BUdR we were able autoradiographically to ascertain its presence in most 1st metaphase chromosomes (Fig. 1). Subsequent use of the minimal concentration of BUdR which gave differential staining of mitotic chromosomes, enabled us to obtain such staining of the meiotic chromosomes in our system. Because of the distinctive differential staining of the chromatids (light chromatids have more BUdR than dark ones) label segregation can provide critical information even at 1st meiotic metaphase (Fig. 2*a*), thus obviating some of the problems with autoradiography. All three types of side-by-side chiasma formation are observed (Fig. 2*b*, dark to dark: i; dark to light: ii, etc.). Two configurations (Fig. 2*b*, *v*, *ix*) of the displayed cell show the points of crossing over and one of these also shows an interstitial sister chromatid exchange (Fig. 2*b*, *v*). In two of the three 'crosses' (Fig. 2*b*, *vii*, *viii*, the latter of which is undergoing separation of homologues) the point of exchange corresponding

to the single chiasma is not detectable, and these crosses can be interpreted as being derived from exchanges between equally labelled homologous chromatids (Fig. 2*b*, *i* or *iii* and *iv*). It is also interesting that one of the configurations (Fig. 2*b*, *vi*) shows a three-strand double involving proximally two dark chromatids and distally a dark and a light one. The latter we interpret as a resolved chiasma; we can therefore detect the point of distal exchange in the two homologous chromatids. In an unlabelled metaphase preparation the latter crossover point would have been undetected.

Our data do not indicate whether or not chiasmata are terminalised in the mouse, or at least in the female of the species. Tease¹⁶ found that there is no terminalisation in the male desert locust. The issue of terminalisation⁵ is relevant both theoretically (assessment of chiasma and crossover frequency) and practically (alleged factor in maternal-age-dependent non-disjunction in mammals²²).

In conclusion, this is the first demonstration of partial chiasmamtypy with crossover exchange, and its unequivocal relation to chiasmata, in a female mammal, by an *in vitro/in vivo* technique which is very versatile in the investigation of other problems of mammalian female meiosis.

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Movement of microorganisms in viscous environments

SOME microorganisms swim well in solutions containing viscous agents (molecules with long unbranched chains, such as methylcellulose)¹⁻⁷. *Leptospira*, a slender helical bacterium, swims more rapidly in such an environment than it does in water³, even at viscosities of several hundred centipoise ($1 \text{ cP} = 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1}$). This behaviour is baffling until one realises that solutions of viscous agents are highly structured (gel-like). The solute forms a loose quasi-rigid network easily penetrated by particles of microscopic size. The network can exert forces

normal to a segment of the body of a slender cell even when that segment does not possess a component of velocity in the normal direction; hydrodynamic treatments of the motion of microorganisms (or of cilia and flagella) do not apply. Solutions containing highly branched polymers, for example, Ficoll, are much more homogeneous. Here, we will review existing evidence for the gel-like character of viscous agents and describe experiments in which the motion of *Leptospira* or *Escherichia coli* are compared in solutions of methylcellulose and Ficoll of the same apparent viscosity. Our data show that solutions of methylcellulose are gel-like even when quite dilute, when the bulk viscosity is as small as 2 cP.

The viscosity of a liquid, η , is defined in terms of the force, F , required to maintain a velocity gradient, dv/dx , between adjacent planes of area A ; thus, $F = \eta A dv/dx$. In a true liquid, η is independent of the size of the velocity gradient (of the rate of shear), and F is parallel to v . In a non-newtonian liquid, η depends on the rate of shear; an apparent viscosity is defined as a function of the rate of shear. If the medium is quasi-rigid, F may not be parallel to v .

A macroscopic determination of η can be made by dropping a ball through the liquid and measuring its rate of fall. The ball is subjected to a viscous force, $F = 6\pi\eta av$, where a is its radius and v its velocity (Stoke's law). η can be determined microscopically by using a sphere of microscopic size and measuring its sedimentation rate or, alternatively, its diffusion constant. The diffusion constant, D , and the frictional drag coefficient, $f = F/v$, are related by the Einstein-Smoluchowski relation, $D = kT/f$, where k is Boltzmann's constant and T is the absolute temperature. For a sphere, $D = kT/6\pi\eta a$. For water and aqueous solutions of substances of low molecular weight (MW), measurements of rates of fall, sedimentation rates and diffusion constants all lead to similar values for η , even with particles as small as globular proteins.

The situation is radically different for solutions of unbranched polymers. Their macroscopic viscosities are high, their microscopic viscosities low. A 1% solution can have an apparent viscosity thousands of times greater than that of the solvent (depending on the rate of shear and the ionic strength: the viscosity of solutions of cellulose decrease markedly at high rates of shear⁸; methylcellulose fails to increase the viscosity of 4M NaCl, K. W. Foster, personal communication). However, the sedimentation rates and diffusion constants of small particles change relatively little. Their motion depends more on the viscosity of the solvent than on the viscosity of the solution; the solution acts as a molecular sieve. For example, the diffusion constant of bovine serum albumin in 0.3% (w/v) rooster-comb hyaluronic acid (weight-average MW 1.7×10^6 , a solution of apparent viscosity about 30 times that of the solvent⁹) was found to be only 20% smaller than that in the solvent¹⁰. A similar value was observed in a 1.2% Ionagar gel¹¹. The rotational diffusion constant of serum albumin was not measurably affected by hyaluronic acid¹². In general, the larger the particle or the higher the concentration of viscous agent (or gel), the more restricted the movement^{10,11}. Methylcellulose behaves similarly¹³.

Given this porosity, it is not surprising that a bacterium that can swim in water (by whatever mechanism) can move through a solution of a viscous agent, provided that its body (protoplasmic cylinder) is sufficiently thin. Indeed, some bacteria known to swim in such solutions have been isolated on the basis of their ability to pass through cellulose ester filters (pore size 0.3 or 0.45 μm) and to form spreading subsurface colonies in 1% nutrient agar¹⁴⁻¹⁸. Other bacteria that swim in viscous media, for example, *Leptospira*, have a similar colonial morphology¹⁹. Most of these organisms are helical, but some are not^{4,18}; most are less than 0.4 μm in diameter.

When a helical bacterium swims, it spins about its helical axis. In water, more than one revolution is required to carry the cell forward a distance equal to the pitch: the helix slips circumferentially. In a solution of a viscous agent, on the other hand, the helix moves without slip, like a corkscrew through a cork. *Spirillum volutans* behaves this way in 5% gelatin²⁰. *Spirochaeta*

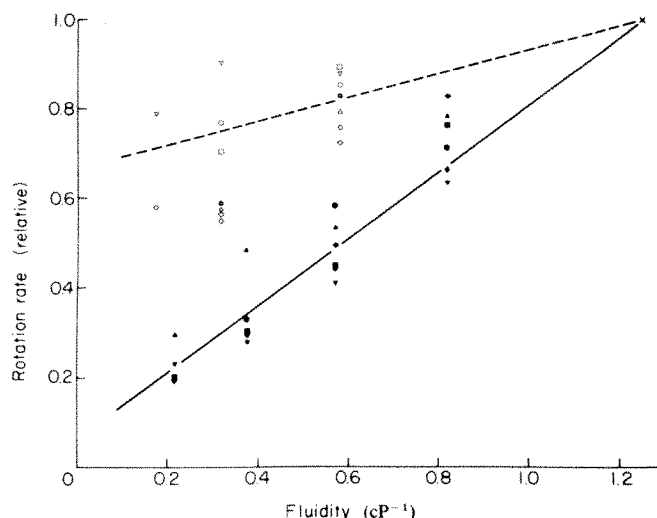


Fig. 1 Relative change in the rotation rates of tethered *E. coli* (strain AW405) as a function of the fluidity ($1/\eta$) of solutions of Ficoll (solid symbols, solid line) or Methocel (open symbols, dashed line). Clockwise and counterclockwise rates were normalised separately (by dividing by the corresponding rate observed in motility medium) and the mean of the two values was plotted; the two values were about the same. The mean rates observed in the absence of the polymer were 8.1 (●), 8.2 (■), 11.2 (◆), 11.4 (▲), 12.9 (▼) and 13.0 (⬢) Hz, and 6.8 (○), 6.8 (□), 7.0 (◇), 7.6 (△), 9.4 (▽), 12.6 (⋈) and 13.0 (⋈) Hz. The concentrations (% w/v) and bulk viscosities (cP) of the solutions were, respectively: for Ficoll 0, 0.802; 2.5, 1.22; 5.0, 1.76; 7.5, 2.68; 10.0, 4.61; for Methocel 0, 0.802; 0.1, 1.72; 0.2, 3.16; 0.3, 5.74. All measurements were made at 32.0 °C. The lines were drawn from the point at fluidity 1.25 through the mean values of the clusters near fluidity 0.6.

aurantia does so in 1% methylcellulose (the helical axes of the best swimmers are straight; our observation on var. *aurantia* strain J1, unpublished), so does *Leptospira interrogans* serotype *illini*²¹ (the helical axis is bent into a spiral at the front of the cell, a hook at the rear; the ends of the cell gyrate, and the protoplasmic cylinder moves without slip along the local helical axis; see also refs 7, 22-24).

This behaviour implies that solutions of viscous agents are quasi-rigid. Circumferential slip is essential if a helical object is to develop thrust in a true liquid, that is, if it is to propel itself using viscous forces; hydrodynamics demands it^{25,26}. However, it is not required in a rigid environment, that is, in one that can push on an object in a direction normal to its surface. Gray illustrated this point in a discussion of undulatory propulsion by comparing the waving motion of the tail of a spermatozoon to the serpentine movement of a snake²⁵. The resistances offered by the media through which (or over which) these organisms move (water for the spermatozoon, stones for the snake) are fundamentally different.

The helical bacteria act like passive rigid screws driven by an external torque. In *Spirillum* the torque is provided by the rotation of the polar flagella²⁰, in *Spirochaeta* by the roll (or flow) of the external sheath²⁷, and in *Leptospira* by the gyrations of the ends of the cell²¹. In a gel-like medium, the cells thread or force their way through the spaces between the molecules of solute. *Leptospira* swims efficiently in such an environment³; the gyrations of the ends of the cell are hindered, and the roll of the protoplasmic cylinder is enhanced²¹.

Leptospira behaves in a different manner in solutions of methylcellulose or Ficoll of the same apparent viscosity. It does not swim efficiently in solutions of Ficoll, which are less structured. Methylcellulose (Methocel 90 HG, 4,000 cP, Dow, a hydroxypropyl methylcellulose product) is an unbranched polymer of MW 143,000; its solutions are newtonian at concentrations of $\leq 0.5\%$ (w/v), non-newtonian at higher concentrations²⁸. A stock solution (1% w/v) was prepared in a motility medium containing 0.067 M NaCl, 0.01 M potassium phosphate (pH 7.0) and 10^{-4} M EDTA (by stirring overnight at room

temperature, 21 °C). Solutions at lower concentrations were prepared by dilution in the same medium. Ficoll (Ficoll 400, Pharmacia) is a highly branched polymer of sucrose and epichlorohydrin of MW ~400,000; its molecules are roughly spherical²⁹, its solutions newtonian³⁰. Solutions of various concentrations were prepared directly in motility medium (by stirring at room temperature). All solutions used in the experiments (of both Methocel and Ficoll) were sterilised by passage through cellulose ester filters (Millipore, pore size 0.45 µm) and stored at room temperature. Their viscosities were determined in Cannon-Ubbelohde viscometers (at the temperatures specified; flow times 1–6 min). *Leptospira interrogans* serotype *illini* was grown at 30 °C on a Tween-80 albumin medium³¹ (less glycerol) to an optical density of about 0.3 (by eye). Samples of this suspension (0.02 ml) were mixed with aliquots of solutions of Methocel or Ficoll (1 ml) and examined by dark-field microscopy (at room temperature, between a coverslip and a slide separated by a ring of Apiezon L with a Nikon S-Ke microscope: 30 W tungsten or 100 W tungsten-halogen lamp, ultra dark-field condenser, Fl 40× plan objective, 15× oculars).

Serotype *illini* translated rapidly in 1% Methocel ($\eta = 95$ cP at 32 °C, our measurements; 140 cP at 25 °C and zero rate of shear, ref. 28). It swam in a serpentine fashion in the hook-spiral configuration, spiral-end first, the protoplasmic cylinder moving without slip^{21–24}. Cells with only one hook (or spiral) translated at about the same speed, spiral-end first or hook-end last²¹. Translational velocities of more than $20 \mu\text{m s}^{-1}$ have been reported for other strains in similar media³. In contrast, serotype *illini* failed to translate in 35% Ficoll (92 cP at 21 °C). The ends of the cell gyrated slowly; the body remained nearly stationary. The cells had a tendency to wind up on one another.

Differences between the motion of serotype *illini* in solutions of Methocel or Ficoll were still marked at concentrations at which both media are thought to be newtonian^{28,30}. The cells translated well in 0.25% Methocel (4.3 cP at 32 °C) but not as well as in 1% Methocel. Some slip of the protoplasmic cylinder was apparent. Cells with only one hook (or spiral) moved more rapidly spiral-end first than hook-end last. In contrast, the motion in 10% Ficoll (4.6 cP at 32 °C) was hard to distinguish from that in motility medium. The cells gyrated and rolled rapidly but translated slowly. Cells with only one hook (or spiral) moved spiral-end first but not hook-end last²¹.

A more quantitative idea of the difference in behaviour between solutions of Methocel and Ficoll was obtained by comparing the rotation rates of tethered *E. coli* in the two media. Cells were grown and tethered as described previously³² and observed at 32 °C, either with the tracking microscope³³ or with a version of the linear-graded filter apparatus³⁴ in which an image from the microscope is analysed directly. The same cell was studied in Ficoll or Methocel at various concentrations. With Ficoll the rotation rates were nearly inversely proportional to the viscosity, as expected if the flagellar motors run at a constant torque; with Methocel they were not (Fig. 1). Solutions of Methocel perturbed the motion of *E. coli* less than did solutions of Ficoll of the same apparent viscosity; evidently, the cells were able to push the chains of methylcellulose out of the way and to move more as they would in pure solvent. Note that all the solutions used in these experiments were passed through Millipore filters (see above); they were homogeneous both by eye and by phase-contrast microscopy. However, differences were apparent to *E. coli*, even at the smallest concentrations tested (Ficoll 2.5% w/v, Methocel 0.1% w/v).

Many kinds of bacteria swim more rapidly in dilute solutions of viscous agents (viscosities of the order of 2 cP) than they do in ordinary media^{1,2,6}. The efficiency of flagellar propulsion is probably enhanced by the gel-like structure of these solutions. Dextran, a branched polysaccharide, failed to show the effect¹; Ficoll has not been tested. Flagellar bundles are more easily seen in solutions of viscous agents than in ordinary media^{35–38}. Is this also due to a qualitative change in the structure of the medium? Does Ficoll show this effect? Is there a better agent that could be used for experiments of this kind? The choice is limited, as most

bacteria fail to swim in solutions of high ionic strength or high osmolarity. If one's purpose is to slow the motion of a micro-organism (or a cilium or flagellum) while keeping the hydrodynamics the same, Ficoll is a better choice than methylcellulose or probably any other unbranched polymer.

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Growth of human B-lymphocyte colonies *in vitro*

RECENTLY developed *in vitro* culture systems that support clonal formation of lymphoid cells have promoted remarkable advances in our understanding of lymphocyte proliferation and the nature of the proliferating cells. Human T lymphocytes from venous blood, bone marrow and lymphoid organs and murine T and B lymphocytes from lymphoid tissues have been grown into colonies of T and B cells in semisolid culture. In these systems, T-lymphocyte colony formation requires that the seeded cells undergo T-mitogen stimulation, that is, by phytohaemagglutinin (PHA) or concanavalin A, and B-lymphocyte colony growth necessitates sensitisation by mercaptoethanol, a humoral thymic factor, or a polyclonal B-cell activator, such as lipopolysaccharide, purified protein derivative or dextran sulphate^{1–7,17}. Previous attempts to achieve colony formation of human B cells were unsuccessful, even in the presence of known polyclonal B-cell activators. However, in certain experimental conditions, the plant lectins, pokeweed mitogen (PWM) and PHA, stimulate both human B and T lymphocytes into transformation and

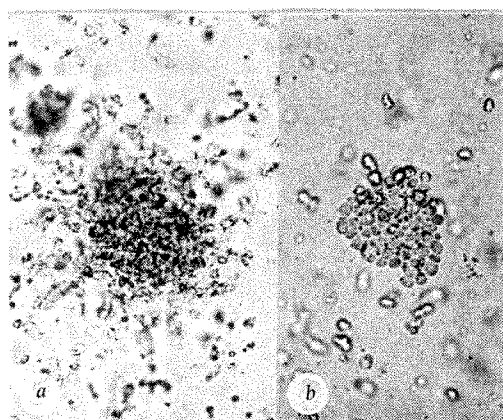


Fig. 1 Viewed under a light microscope at low magnification, unstained colonies grown in agar medium from human, venous blood, B-lymphocyte-enriched population stimulated with PHA. *a*, Large colonies (type I) containing 250–500 cells which developed within the upper agar layer 5 days after seeding; *b*, small, flat colonies (type II) of 50–100 cells which grew on the surface of the upper agar layer 7 days after plating.

proliferation in cell culture with a synergistic effect between the sensitised T and B cells^{8–13}. We report here that, based on this information and earlier techniques for clonal formation of human lymphocytes *in vitro*, we have evolved a two-layer soft agar culture technique in which the mitogens, PHA and PWM, were able to generate the appropriate signal for triggering B-lymphocyte precursors of venous and umbilical cord blood, tonsil, adenoid and chronic lymphatic leukaemia (CLL) origin into colony-forming and immunoglobulin-producing cells.

Lymphoid cells, obtained from human adenoids and tonsils excised in sterile conditions, were suspended in Dulbecco's modified Eagle's medium (MEM). The cells were incubated for 20 min at 37 °C in MEM containing 50 µg ml⁻¹ erythromycin and 200 µg ml⁻¹ kanamycin, then washed twice and resuspended in MEM. Heparinised blood (10 IU ml⁻¹) was obtained from umbilical cord, healthy adult donors and patients in whom

CLL had been diagnosed but who had not received therapeutic treatment. Lymphocytes were isolated by Lymphoprep (sodium metrizoate/Ficoll) gradient (D 1.077)¹⁴, washed twice with MEM containing penicillin and streptomycin (100 U ml⁻¹ and 100 µg ml⁻¹, respectively) and resuspended at 10⁶ cells per ml of the same medium supplemented with 12.5% of inactivated pooled human serum.

B-lymphocyte-enriched populations were obtained from mononuclear cells by separating E-rosette-forming cells by density sedimentation through Lymphoprep. The T-cell-depleted interphase fraction contained at least 70% B cells when normal blood was processed, 65% B cells for cord blood, 70% for tonsils and 80% for CLL. B cells were quantitated by direct immunofluorescent staining of surface membrane immunoglobulins (SmIg) with fluorescein isothiocyanate-conjugated anti-µ chain or polyvalent rabbit antiserum to human immunoglobulins¹⁵. The B-cell-enriched population contained <4% E-rosette-forming cells. For sensitising the cells with mitogen in liquid phase, suspensions of B-cell-enriched populations or mononuclear cells (10⁶ cells per ml MEM containing 12.5% human serum) were incubated with PHA-M (Difco, 0.0125 ml ml⁻¹) or PWM (Sigma 2.5 µg ml⁻¹) for 72 h at 37 °C in a water-saturated atmosphere of 7.5–9% CO₂ in air (Table 1). The sensitised cells were then washed, resuspended and seeded in the upper soft agar layer (0.32% agar), at 5 × 10⁵ cells per

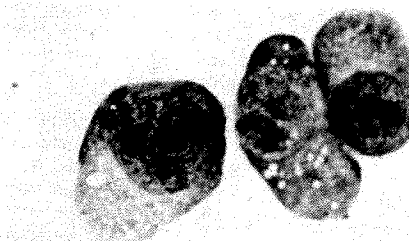


Fig. 3 Colony cells from PWM-stimulated, cord blood B-cell-enriched population, 6 days after plating, stained with May-Grünwald Giemsa. All cells had the morphological features of transformed lymphoblastoid and small or large plasmoid cells.

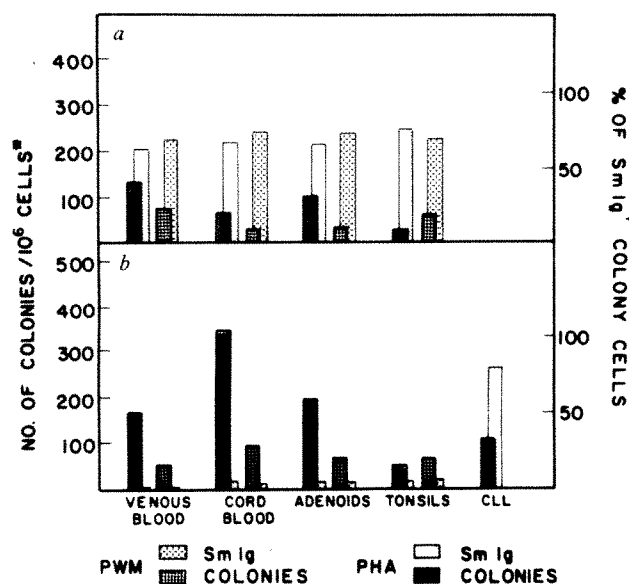


Fig. 2 Lymphocyte colony number and their cell SmIg, grown in Bacto agar from mitogen-stimulated lymphoid cells. All B-cell-enriched populations and CLL cells (80% B lymphocytes) developed into B-cell colonies with a characteristically high content of SmIg⁺ colony cells. Colonies grown from unfractionated lymphocytes from normal human venous blood were uniformly SmIg⁻ and those stemming from normal human lymphoid tissue had few SmIg⁺ cells. The data represent the average of 3 or more experiments (2–4 plates per culture). *a*, B-cell-enriched population; *b*, mononuclear cells.

30-mm Petri dish. The lower agar layer (0.5% agar) contained either 0.0125 ml PHA per ml medium or 2.5 µg PWM per ml medium. The Petri dishes were incubated at 37 °C in a fully humidified atmosphere at 7.5–9% CO₂ in air. In some experiments cell-free lymphocyte culture fluid (LCF) obtained from a 3-day culture of cord blood lymphocytes (1.5 × 10⁶ cells per ml MEM containing PHA 0.0125 ml ml⁻¹ or PWM 2.5 µg ml⁻¹) replaced the MEM of the lower agar layer¹⁶. For controls we used cultures which did not contain mitogen in the lower agar layer or in which the lymphocytes seeded in the upper layer had not been presensitised with mitogen.

The formation of the colonies and their morphology were observed under an inverted microscope. Colonies of 50 or more cells developed from PHA-stimulated cells 3–5 days after seeding and from PWM-treated cells after 5–7 days. Seeded PWM-sensitised, B-cell-enriched populations proliferated and formed lymphocyte colonies only when the soft agar culture had been supplemented with LCF. Two types of colonies formed: type I, large colonies containing 50–500 cells appeared within the upper layer, and type II, small, flat colonies containing 50–150 cells developed above the primary colonies, on the surface of the upper agar layer (Fig. 1). A stationary period of 2–3 days (PHA) or 4–5 days (PWM) followed colony development; the colonies began to degenerate on days 7–9, with cell disintegration and lysis. As distinct from the large primary colonies, the number of flat colonies varied greatly. We decided to use the score of the large primary colonies as a parameter for evaluating the effect of stimulation and growth conditions on lymphoid cells. When cord blood cells were seeded, a new kind of colony, composed of small macrophages, formed 12–13 days after culture.

To characterise the nature of the colony cells, 4–6-day colonies were collected, and their cells pooled and washed.

Table 1 Surface markers on mononuclear cells and B-cell-enriched populations before and after mitogen sensitisation

Source of cells	Cell population	% E rosettes	Liquid phase		
			0 h % SmIg ⁺	72 h, PHA* % SmLg ⁺	72 h, PWM* % SmIg ⁺
Peripheral blood	B cell	3.3±0.6	71.7±0.9	73.3±6.6	71.7±0.9
	Mononuclear	75.0±4.1	10.7±0.4	10.7±4.1	12.0±4.2
Cord blood	B cell	3.7±1.1	65.0±4.1	73.0±8.7	77.3±6.2
	Mononuclear	45.0±4.1	11.3±1.3	11.0±2.9	15.3±1.1
Adenoids	B cell	4.0±0.5	71.5±1.5	59.0±9.0	66.0±1.0
	Mononuclear	60.0±0.5	32.5±2.5	21.0±1.0	24.0±0.0
Tonsil	B cell	3.5±0.2	75.0±3.0	68.0±4.0	67.0±3.0
	Mononuclear	39.0±1.0	50.0±0.0	22.5±10.5	25.0±5.0
Peripheral blood (CLL)	Mononuclear	9.7±5.3	79.7±1.3	86.0±2.9	

'B cell' refers to the B-lymphocyte-enriched population, 'Mononuclear' to unfractionated mononuclear cells. It was found that in mitogen-sensitised lymphocyte populations, spontaneous E-rosette formation was no longer specific for T cells.

*After 72 h stimulation with mitogen, the cells were plated in the two-layer soft agar system.

After agar residues had been removed by passing the cell suspension several times through a tuberculin syringe and needle, SmIg were assayed. Detection of SmIg was used as the membrane marker for characterising colony cells. B-cell-enriched populations gave rise to B-cell colonies, as manifested by the large number of colony cells having SmIg⁺, and mononuclear cells evolved T-cell colonies, with SmIg⁺ colony cells (Fig. 2). Also, B-lymphocyte colony growth was consistently obtained from blood and lymphoid tissue B-cell-enriched populations sensitised with PHA or PWM and from CLL (80% B cells) treated with PHA. Mononuclear cells from normal peripheral blood gave rise to colonies whose cells bore no SmIg; among those from cord blood or lymphoid tissue, where the ratio of B to T cells is high, 1–4% SmIg⁺ cells were seen.

Morphological examination of the large B-cell colonies that developed from PHA-induced cells showed that these had compact centres whereas those that grew from PWM-treated cells had a loose, diffuse structure. From a microscopic study of colony cells, we found that both type I and type II colonies contained many ellipsoid or spherically shaped, large mononuclear cells with a blast-like appearance, that is, a round nucleus and basophilic cytoplasm, and plasmoid cells having the

characteristic eccentric nucleus and bulky basophilic cytoplasm (Fig. 3). In CLL colonies, many cells resembling small or medium-sized lymphocytes were also seen. The progeny cells generated by PWM-stimulated B lymphocytes were more differentiated than those of the PHA-treated cells. Electron microscope studies of the cells in B colonies that developed from PHA- and PWM-stimulated populations showed that these had the morphological features of either blast-like or plasmoid cells. Many strands of well developed rough-surfaced endoplasmic reticulum with cisternal dilations were observed, particularly in the PWM-treated cells (Fig. 4).

Differences in the number and morphology of PHA- and PWM-induced colonies and their component cells, dissimilar colony development times and distinct culture conditions (the need for lymphocyte culture fluid for colony formation of PWM-induced cells) indicate that the two mitogens may act either on different B-cell subpopulations or on a B-cell population at different stages of maturation.

This technique for cloning B cells from normal and leukaemic B lymphocytes in soft agar conditions should be a major breakthrough in investigations concerned with the mechanism of the immune response of normal and leukaemic lymphoid populations. It should be particularly helpful for analysing functional B-cell populations, and the role of monocytes and T cells in the activation of B lymphocytes and regulation of their colony growth.

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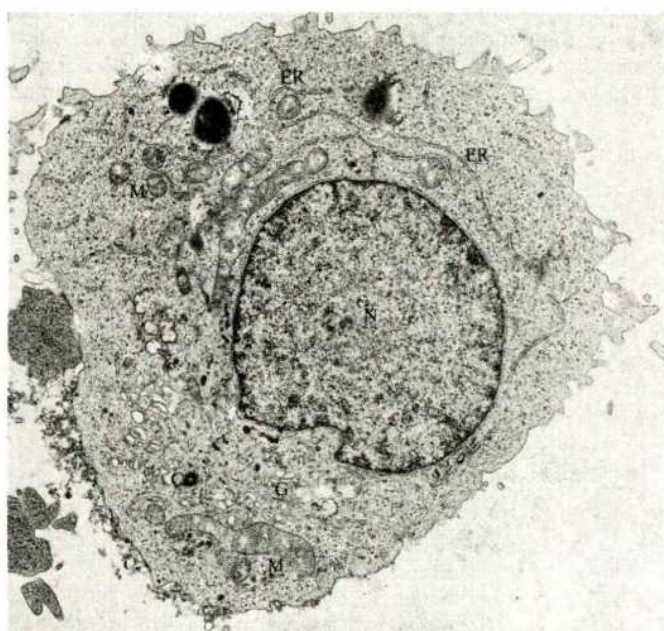


Fig. 4 Electron micrograph of a cell from a colony developed from PHA-sensitized normal venous blood, B-cell-enriched population 4 days after plating. Note that the eccentric nucleus (N) contains increased euchromatin. In the cytoplasm there are large and short rough-surfaced endoplasmic reticulum profiles (ER), many polyribosomes, a centriole (C), the Golgi apparatus (G) and different-sized mitochondria (M). ($\times 7,000$.)

Cooperation between antigen-reactive T cells and anti-idiotypic B cells in the anti-idiotypic response to antigen-antibody complexes

THE variable regions of antibody molecules carry unique antigenic determinants (idiotypes), which have been shown to be isoimmunogenic, that is, immunogenic in syngeneic animals¹⁻³. Jerne and others^{4,5} have proposed that autoimmune responses to self-idiotypes might form the basis of an immunoregulatory network, and evidence in favour of this hypothesis has been reported from several laboratories⁶⁻⁸. Preformed antigen-antibody complexes of dinitrophenylated keyhole limpet haemocyanin (DNP-KLH) and the DNP-binding myeloma protein M315 are very effective in inducing iso-anti-idiotypic antibodies to the M315 idiotypic in BALB/c mice⁹, and formation of immune complexes during an antibody response could therefore represent the mechanism whereby self-idiotypes become autoimmunogenic. As such anti-idiotypic antibody responses are T-cell-dependent, one must assume that if animals are immunised with purified antibody then anti-idiotypic T (helper) cells cooperate with anti-idiotypic B cells. The existence of iso-anti-idiotypic T cells has been formally demonstrated^{10,11}. When an antibody is administered as an immune complex, a similar cooperative event may occur; but it is also possible that antigen-specific T cells cooperate with idiotypic-specific B cells through the immune complex, as the latter can prime animals for immunity to the antigen¹² and to idiotypic determinants on the antibody (see below). Here I present evidence that this occurs.

Table 1 Cooperation between KLH-primed spleen cells and anti-M315 B cells

Group*	B cells primed with†:	KLH-primed spleen cells‡	Boost §	IBC ($\mu\text{g ml}^{-1}$)¶
1	DNP-BSA-M315	+	DNP-KLH-M315	43.0 ± 11
2	DNP-BSA-M315	+	M315	0.4 ± 0.2
3	DNP-BSA-M315	-	DNP-KLH-M315	2.4 ± 1.0
4	Nothing	+	DNP-KLH-M315	1.7 ± 0.5

*Irradiated (500 R) BALB/c mice ($n = 4$).

†Spleen cells from BALB/c mice given 10 μg (wt of DNP₅₀BSA) DNP₅₀BSA-M315 (1:10 w/w ratio) complexes 3 weeks previously. These cells were treated with anti-Thy 1 antiserum and rabbit complement (45 min for 37°C) and each mouse received 20×10^6 viable cells.

‡ 20×10^6 spleen cells from mice given 100 μg alum-precipitated KLH plus 2×10^9 *Bordetella pertussis* organisms 1 month previously.

§10 μg DNP-KLH-M315 complexes (1:5 w/w ratio) or 50 μg M315 given intravenously.

¶Idiotypic-binding-capacity (determined by double antibody radioimmunoassay using ¹²⁵I-labelled M315 (refs 9, 16) of sera taken 10 d post-transfer.

The first indication for this mechanism emerged from an experiment where mice were immunised with DNP-KLH-M315 immune precipitates, as described previously⁹. Three weeks later, some were boosted with M315 and others with complexes. Those boosted with M315 had similar anti-M315 antibody titres on day 31 to unboosted mice, whereas those boosted with DNP-KLH-M315 gave a true secondary response (data not shown).

In the experiment shown in Fig. 1 BALB/c mice were given DNP-KLH-M315 complexes at w/w ratios of antigen:antibody of 1:10 (antibody excess) or 1:1 (antigen excess)⁹, with or without alum-precipitated carrier, that is, KLH. Administration of KLH increased anti-M315 antibody titres, but only after boosting with immune complexes. The effect was most marked after an optimal (10 μg DNP-KLH) priming dose of DNP-KLH-M315 (1:10) complexes (fourfold increase in antibody),

and less with a 1- μg dose of complexes. Complexes prepared at a 1:1 ratio elicit poor anti-M315 antibody titres, and KLH priming did not enhance their immunogenicity. Priming with KLH also enhanced the anti-DNP antibody response in those mice given 10 μg DNP-KLH-M315 (1:10) (data not shown). Complexes in antigen excess and in antibody excess both induced anti-DNP antibodies (not shown), but only those in antibody excess induced substantial anti-M315 antibody titres.

The helper effect of KLH priming could have been due to the induction of KLH-specific T-helper cells, or to the enhanced production of anti-KLH (or anti-DNP) antibodies¹³. In subsequent experiments groups of mice were immunised with 1:10 complexes of DNP-bovine serum albumin (DNP-BSA) and M315. Three weeks later B cells from these mice were transferred with spleen cells from KLH-immunised mice (as a source of T-helper cells) to irradiated recipients, which were boosted with either DNP-KLH-M315 or M315 (Table 1). Mice given DNP-BSA-M315-primed B cells plus KLH-primed cells gave a marked anti-M315 response, but only when boosted with DNP-KLH-M315, and not when boosted with M315. DNP-BSA-M315-primed B cells alone gave a very weak response, comparable to that given by unprimed B cells plus KLH-primed cells.

In a further experiment irradiated mice were given B cells from mice primed with purified M315 in adjuvant³, with or without purified KLH-primed T cells (Table 2). Again, those given helper cells produced substantial anti-M315 antibody titres, but only when boosted with DNP-KLH-M315 complexes. The idiotype specificity of the cooperation is indicated by the low antibody titres of the group boosted with DNP-KLH-M460 complexes. M460 is a DNP-binding IgA myeloma protein with a different idiotypic from M315. The binding of the antibodies in group 1 to M315 was almost completely inhibited by 10^{-3}M DNP-lysine (not shown), indicating that they were primarily directed against the combining site of the antibody.

These experiments therefore suggest that in mice immunised with antigen-antibody complexes, antigen-specific helper cells can cooperate with anti-idiotypic B cells to generate an anti-idiotypic antibody response. Indeed, the available data suggest that this form of cooperation is the most important in anti-idiotypic immunity to immune complexes. It is unlikely that KLH priming induces anti-idiotypic T cells, since mice do not respond to purified M315 when given KLH-primed T cells and M315-primed B cells (Tables 1, 2). In addition, T cells from mice

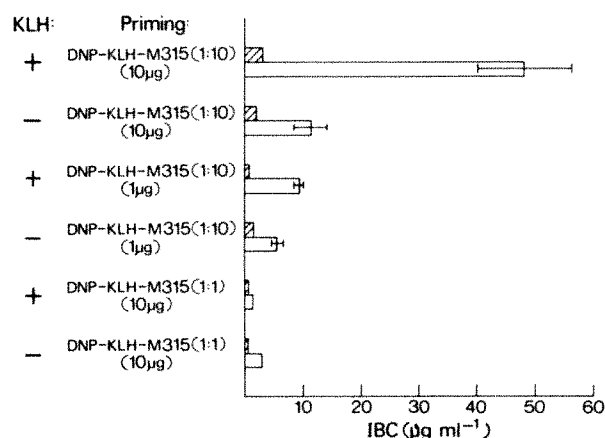


Fig. 1 Effects of carrier (KLH) priming on the anti-idiotypic response to DNP-KLH-M315 complexes. Groups ($n = 4$) of BALB/c mice were given 10 or 1 μg (wt of DNP-KLH) DNP₁₇₂KLH-M315 complexes (w/w antigen: antibody ratios 1:10 or 1:1 (ref. 9) intravenously on day 0. Some also received 100 μg alum-precipitated KLH intraperitoneally on the same day. The mice were bled on day 21 (■), boosted with 10 μg DNP-KLH-M315 and finally bled again on day 32 (□). Anti-M315 antibody titres were determined by double-antibody radioimmunoassay^{9,16}.

IBC, idiotype-binding capacity.

Table 2 Cooperation between KLH-primed T cells and anti-M315 B cells

Group*	T cells primed with:†	Boost‡	IBC ($\mu\text{g ml}^{-1}$)§
Exp a			
1	KLH	DNP-KLH-M315	89.2 ± 3.0
2	KLH	M315	3.5 ± 1.0
3	KLH	DNP-KLH-M460	2.4 ± 0.7
4	None	DNP-KLH-M315	3.9 ± 0.9
Exp b			
1	KLH	DNP-KLH-M315	15.3 ± 1.6
2	BGG	DNP-KLH-M315	5.0 ± 2.3
3	None	DNP-KLH-M315	2.5 ± 0.5

*Irradiated (500 R) BALB/c mice ($n = 4$); all received 10^7 B cells (treated as in Table 1) from mice primed with 100 μg M315 in Freund's adjuvant, followed by boosts of M315 in incomplete adjuvant and saline³.

† 10×10^6 nylon wool purified T cells¹⁵ from mice given 100 μg alum-precipitated bovine gamma globulin (BGG) or KLH plus *B. pertussis* 3 weeks previously.

‡10 μg DNP-KLH-M315 or DNP-KLH-M460 complexes (1:5 w/w ratios) or 50 μg M315.

§Idiotypic-binding capacity determined on sera taken 10 d post-transfer.

given bovine gamma globulin do not provide help in this system, thus confirming the antigen specificity of the helper effect (Table 2).

The results shown in Fig. 1 suggest that in the conditions used here DNP-KLH-M315 complexes do not prime KLH-specific T-helper cells optimally (see also ref. 12). Perhaps antigen-antibody complexes do not prime anti-idiotypic T-helper cells efficiently either, or alternatively they may induce anti-idiotypic suppressor cells. The co-existence of anti-DNP and anti-idiotypic antibodies in mice immunised with DNP-KLH-M315 complexes suggests that the M315 idiotype is not a prominent component of the anti-DNP antibody repertoire of the BALB/c mouse. As there is evidence that these mice do make the M315 idiotype in response to DNP¹⁴, another possibility is that administration of M315 complexes suppresses the expansion of the B-cell clone making this idiotype.

In the context of network theories of immunoregulation⁴, the present results suggest that if the generation of immune complexes during an antibody response represents the physiological mechanism for inducing autoimmunity to idiotypes, then these complexes need only prime anti-idiotypic B cells to give an anti-antibody response. These cells could then cooperate with pre-existing antigen-specific T-helper cells, thereby bypassing the requirement for anti-idiotypic T-helper cells.

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Micropuncture of lateral intercellular spaces of *Necturus* gallbladder to determine space fluid K^+ concentration

EPITHELIA such as that of *Necturus* gallbladder consist of a pavement-like sheet of uniform cuboidal cells of $20 \times 20 \times 20 \mu\text{m}$. As seen in the electron microscope, the clefts between the cells (lateral intercellular spaces, LIS) are ~ 0.1 – $1 \mu\text{m}$ wide. They are closed against the gallbladder lumen by so-called 'tight' junctions—which, however, allow the passage of small ions¹—and at the other end communicate more freely with the interstitial fluid compartment of the body through slits of > 30 – 50 nm width. The lateral membranes of the epithelial cells which face the LIS are highly folded (the surface amplification factor in *Necturus* gallbladder is around 20 (ref. 2) but the basal membranes which face the interstitial fluid compartment directly are not, and so it is evident that virtually all matter which is reabsorbed from the lumen after passing the cell membranes (or the tight junctions) must enter the LIS before it reaches the interstitial fluid compartment proper. It is generally assumed that the LIS have a central role in isotonically transporting epithelia by acting as the main coupling site between salt and water transport. Although elaborate models have been used in attempts to describe the salt and water coupling in detail mathematically^{3–9}, the results have been inconclusive, mainly because there was no definitive experimental proof. A central question, whether or not the LIS fluid is hypertonic as postulated by the standing gradient hypothesis of isotonic fluid absorption³, remained open, until recently, when the first direct measurements of the ion concentrations in the lateral spaces were published¹⁰. These data were obtained by means of electron-probe analysis of frozen tissue sections of rabbit ileum and revealed a substantial hypertonicity. We have tried the alternative approach of measuring the ion concentrations in the LIS directly using ion-selective microelectrodes. Our observations, which are reported here, indicate that as far as the K^+ concentration is concerned the ion accumulation in the LIS of *Necturus* gallbladder is much lower than suggested by the electron-probe data from rabbit ileum.

We chose an *in vitro* preparation of *Necturus* gallbladder¹¹ in which we can recognise the LIS under $\times 640$ magnification with

Table 1 Results from measurements with double-barrelled K^+ -selective electrodes

Potential (mV)	f (%)	c_K (mmol l^{-1})	Localisation	Depth of tip penetration
+0.4	71	3.4	Colour	
–1.9	89	4.6	Colour	
–0.9	97	3.2	Colour	
+1	100	3.0	Colour	
+1.5	100	2.8	Profile	
–0.5	96	4.9	Mark	50–75%
+0.2	95	4.4	Mark	50–75%
–2	77	4.6	Mark	~50%
-0.28 ± 1.28		3.9 ± 0.8		

'Potential' is the LIS potential referred to subepithelial space. f is the fraction of the overall transepithelial resistance which was observed in the LIS; low values of f indicate the presence of some leak between gallbladder lumen and LIS along the electrode path. c_K is the K^+ concentration of LIS fluid. It was measured by filling one electrode barrel with Corning no. 477317 potassium exchanger as described previously¹² and the other barrel with Ringer solution, recording the potential difference between both barrels and reading c_K from a calibration curve¹². 'Localisation' gives the means of localisation. Because bevelling of double-barrelled electrodes resulted in tip diameters of 1.2 – $2 \mu\text{m}$, the last experiments were carried out with unbevelled tips of $< 0.5 \mu\text{m}$ diameter, which did not allow colour ejection. Hence, localisation was achieved by recording the potential profile and the advance of a dye mark on the electrode.

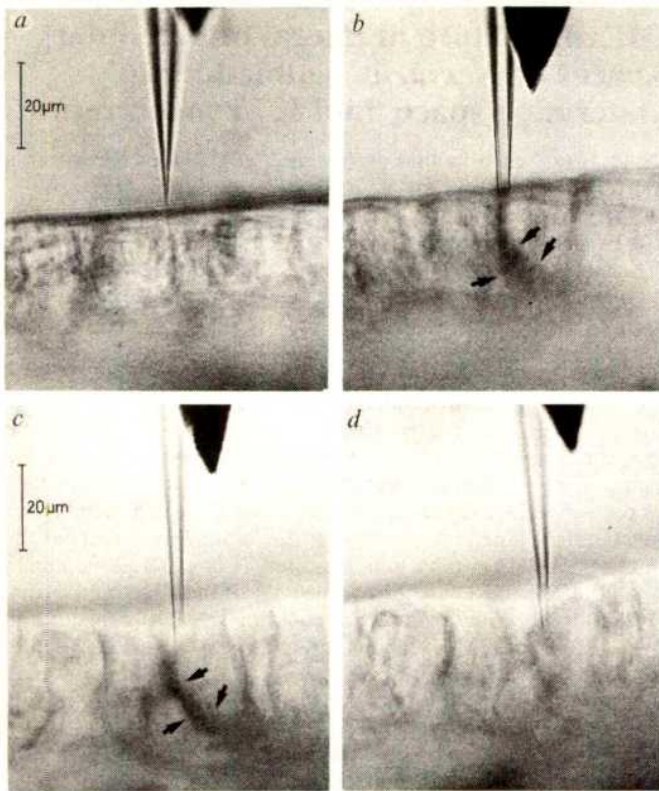


Fig. 1 Photomicrographs of fluid ejection into lateral intercellular spaces. The electrodes were filled with Ringer's solution containing in mmol l^{-1} : NaCl, 110; KCl, 2.5; CaCl_2 , 1; NaHCO_3 , 10, to which 1% of Kiton blue was added. After bevelling of the tip to 0.8–1.2 μm o.d. or, respectively, 70–95 $\text{M}\Omega$ resistance, enough colour could be ejected to give a clear indication of the position of the electrode tip. *a*, Electrode and epithelium before puncture; *b*, after puncture and during fluid ejection at slightly shifted focus. Between the arrows, the colour can be seen to fill a LIS, although the contrast in the black and white reproduction is poor. *c*, A similar picture of another LIS; *d*, the same place after cessation of fluid ejection and after the electrode has been moved slightly laterally.

a water immersion lens. The bladder was mounted between two half-chambers and bathed on either side with Ringer's solution. We first tried to define criteria for successful impalements of the LIS and measured the electrical parameters of the LIS. Glass micropipette electrodes were mounted on a Leitz micro-manipulator and manually advanced from the lumen compartment into a cell and then further in the direction of a LIS. Because the electrode tip is not visible inside the tissue and because the tissue is usually slightly squeezed by the advancing electrode, we verified the location of the electrode tip by ejecting coloured electrode fluid. Essentially four different images could be distinguished microscopically. (1) Injection into a cell. This led to sharply outlined, homogeneous and persistent staining of a single cell. (2) Injection into subcellular cystic spaces. This yielded a sharply defined, small, persistent coloured dot, which tended to grow slightly with time of ejection but never reached the size of a cell. Communication with the gallbladder lumen along the electrode shaft was frequently observed. (3) Injection into a LIS. Depending on the shape of the LIS this resulted in a more or less sharp outline of the space between two cells (Fig. 1), with occasional additional coloration of neighbouring spaces and faint coloration of the immediately underlying subepithelial space. In contrast to cell staining, the coloration of the LIS never persisted, disappearing within approximately 10 s of cessation of ejection from the electrode tip. (4) Injection into the subepithelial space. This resulted in diffuse coloration of a larger subepithelial area with a quite sharply defined front at the basal end of the epithelium but otherwise uncertain delineation. No backflux into the spaces

from underneath was observed. Depending on the amount of colour ejected, the subepithelial space cleared again in less than 1 min.

Although image (1) was correlated with the recording of a cell potential, the potential always dropped towards zero when the tip had entered a LIS (image (3)). In 10 measurements on 8 bladders a stable (> 1 min) potential difference of -1.61 (s.d. ± 0.96) mV (range -2.5 to $+0.8$) was recorded, LIS negative with respect to mucosal compartment. Referred to the serosal potential, which was measured immediately after each successful puncture by advancing the electrode further into the subepithelial space (image (4)), the potential of the LIS was 0.03 ± 0.83 mV negative which is not different from zero. (The transepithelial potential difference averaged 1.58 mV, lumen positive in these experiments.) We also continuously recorded the voltage divider ratio by passing square-wave constant current pulses of up to 100 μA , 1 s width and 0.1 Hz repetition rate across the bladder, and recording the potential deflections in the cell, the LIS and the subepithelial compartment. Expressing the data as fractional resistances (f) it was found that, on its way from the mucosal compartment into the LIS, the electrode tip had passed on average $97.8 \pm 4.7\%$ (range 90–104%) of the resistance between mucosal compartment and subepithelial space, indicating that the electrical resistance from LIS into the serosal fluid compartment was negligible. Image (2) was usually connected with a near-zero potential reading, but the simultaneously recorded fractional resistance was much lower than 100%.

In further experiments, we used double-barrelled micro-electrodes, with one barrel acting as K^+ -sensitive electrode and the other as reference. Because the localisation by fluid ejection was not always possible we determined the position of the electrode tip in some experiments from the potential and resistance profile and from the position of a lacquer mark which had been placed at a known distance of $\sim 40 \mu\text{m}$ from the tip. Thus, we assumed, in agreement with the above observations, that the tip had entered a LIS when, on advancing the electrode, the cell potential collapsed but f remained high, and when subsequent further advancement yielded a new cell potential (when the slightly diagonally moving electrode had crossed the LIS and penetrated the adjacent cell). The latter criterion as well as the depth of insertion, as read from the dye mark, distinguished LIS from subepithelial measurements.

Table 1 gives the results from eight stable measurements in six bladders. They yield a mean K^+ concentration for the LIS fluid of $3.9 \pm 0.8 \text{ mmol l}^{-1}$, which is significantly higher than the K^+ concentration of the mucosal and serosal bathing solution. To test the validity of our measuring technique we also determined the K^+ concentration in the subepithelial space by advancing the electrode from a LIS across the neighbouring cell into the underlying connective tissue. These measurements yield a mean K^+ concentration of $2.4 \pm 0.6 \text{ mmol l}^{-1}$ ($n = 9$) which is similar to that of the Ringer's solution. Although our measurements are not sufficiently advanced to yield complete concentration profiles along the LIS for all ions of interest (attempts to measure the Na^+ concentrations have been unsuccessful because of technical problems with the electrodes) we think that they open a new experimental approach towards a better understanding of the function of the LIS. Our observation that the K^+ concentration of the LIS is only slightly higher than that of the external bathing solutions calls for some caution with respect to the interpretation of concentration profiles from electron-probe data. Even though the latter data¹⁰, which show K^+ concentrations of up to 40 mmol l^{-1} , were obtained on a different tissue of a different species and at higher ambient K^+ concentrations (15 compared with 2.5 mmol l^{-1}), they are difficult to reconcile with the present findings, because there is no reason to believe that part of the space fluid K^+ should be bound (assuming that the sampling area of the electron beam does not include lateral folds² of the cell membranes).

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Deficient production of tyramine and octopamine in cases of depression

THE cause or causes of depressive illness are unknown, although biochemical evidence points to a physical basis for the psychiatric and somatic features of the disease¹. For nearly 20 years, the amine hypothesis² has been the most favoured explanation and putative derangements of noradrenaline and/or 5-hydroxytryptamine economy have been invoked as the systems of primary disturbance. Careful scrutiny, however, has revealed major inconsistencies in this hypothesis³. Some of the strongest evidence in its favour has been the ability of tricyclic antidepressant drugs to act as potent monoamine uptake inhibitors, thus potentially facilitating neurotransmission at the synaptic cleft; but recently, two drugs in this group, iprindole and mianserin, have been shown to be devoid of both noradrenaline and 5-hydroxytryptamine re-uptake-inhibiting ability, although each is a clinically effective antidepressant (for review see ref. 4). Two things should be pointed out in this connection. First, most pharmacological experiments performed to test the hypothesis have focused on noradrenaline and 5-hydroxytryptamine, whilst a possible role for the so-called trace amines⁵ has been almost completely neglected. And second, the direct clinical approach based on the detection of possible metabolic abnormalities has resulted in conflicting and controversial data³. We now report preliminary evidence pointing to a deficient production of two trace amines, tyramine and octopamine, in patients with depressive illness.

Thirteen females (mean age, 56.9 ± 3.2 yr) and 10 males (mean age, 57.6 ± 2.8 yr) suffering from primary depressive illness⁶ were studied. They had no history of mania or of significant physical illness. On admission, all were withdrawn from antidepressant or neuroleptic medication and placed on

placebo tablets for 7–10 days. In certain cases, night-time sedation (nitrazepam, 10 mg) was allowed for a few days. At the end of this 'drug-free' washout period, the patients were assessed for the severity of their disease by the Hamilton rating scale (HRS)⁷ for depression. The assessment on HRS was made by two independent raters who showed a satisfactory concordance ($r = 0.98$; $P < 0.001$). Only patients scoring 16 or more on the first 16 items of the HRS were included in the study. All were assessed on the Newcastle diagnosis scale⁸ and were assigned a diagnostic score placing them along an endogenous–non-endogenous dimension.

The control subjects were drawn from laboratory and hospital staff and were not on any form of medication. They included 17 females (mean age, 48.5 ± 2.2 yr) and 10 males (mean age, 52.9 ± 2.7 yr). Whilst no attempt was made to give patients or controls a standardised diet, patients received a full hospital diet during their drug-free period and controls living in hospital received a diet almost identical to that of the patients.

Mean 24-h urinary excretion values for free phenolic acids, total phenylacetic acid and free phenylethylamine in patients and controls are shown in Tables 1 and 2. Controls living in hospital had a similar excretion pattern of amine metabolites to those living outside hospital. The mean output of both *p*-hydroxyphenylacetic acid and *p*-hydroxymandelic acid (the major metabolites of *p*-tyramine and *p*-octopamine⁹) in depressed patients was significantly lower than that of controls (Table 1). When males and females were compared separately, the difference was also evident but only reached significance in females. There was no correlation with age. These results were examined in relationship to the patients' Newcastle scale scores. The three with high scores (9–11) on this scale, characterised by severe endogenous features and responding poorly to tricyclic drugs¹⁰, had very low excretion values of *p*-hydroxymandelic acid (4.64 ± 0.29 μmol per 24-h) and *p*-hydroxyphenylacetic acid (53.8 ± 13.0 μmol per 24-h) although the other metabolites were normal. The only other significant finding was that male depressives excreted more 4-hydroxy-3-methoxymandelic acid than females. These findings suggest that the deficit was a selective one and did not derive from some technical source of error such as incomplete sample collection.

The trace amines⁵ are represented in the brain in nanogram concentrations only. However, high tissue concentrations of catecholamines and 5-hydroxytryptamine stem not so much from increased production but from the existence of large storage depots. In addition, their assay is relatively simple whereas suitable quantitative procedures for the trace amines and their metabolites have become available only relatively recently⁵. It is important to note that the absence of a specific storage mechanism does not necessarily imply that turnover is any less rapid than that of compounds possessing one. Daily whole-body octopamine production, for example, falls little short of that of the individual catecholamines¹¹.

Although the urinary output values of trace amine breakdown products which we have measured in depressive illness for the

Table 1 Urinary excretion of total phenylacetic acid (PAA) and free hydroxyphenylacetic (HPAA), homovanillic (HVA), *p*-hydroxymandelic (*p*-HMA) and 4-hydroxy-3-methoxymandelic (VMA) acids in control subjects and depressive patients

Group	n	PAA	o-HPAA	Urinary excretion (μmol per 24 h)		HVA	<i>p</i> -HMA	VMA
				m-HPAA	<i>p</i> -HPAA			
Depressive patients								
Males	10	911 ± 66	5.85 ± 0.85	27.2 ± 6.1	80.1 ± 14.7	21.6 ± 2.6	8.74 ± 1.84	27.1 ± 2.9†
Females	13	808 ± 140	4.93 ± 0.92	30.9 ± 5.8	90.9 ± 9.7†	18.0 ± 1.0	5.71 ± 0.89‡	18.6 ± 1.8‡
All patients	23	852 ± 95	5.32 ± 0.66	29.3 ± 4.1	86.2 ± 8.3*	19.5 ± 1.3	7.02 ± 1.01§	22.2 ± 1.8
Controls								
Males	10	896 ± 132	8.41 ± 1.38	34.6 ± 8.4	128.9 ± 23.6	25.2 ± 2.2	12.07 ± 1.90	23.0 ± 2.0
Females	17	881 ± 154	5.45 ± 0.46	40.5 ± 6.2	146.7 ± 15.8†	20.6 ± 1.4	9.46 ± 1.31‡	23.5 ± 1.6
All controls	27	881 ± 110	6.57 ± 0.66	38.3 ± 4.9	140.1 ± 13.1*	22.3 ± 1.0	10.41 ± 1.07§	23.3 ± 1.2

Urine samples (24-h) from each group were collected into 25 ml 6 M HCl and stored at –15°C. Free phenolic acids (*o*-, *m*-, and *p*-hydroxyphenylacetic acids, homovanillic acid, *p*-hydroxymandelic acid and 4-hydroxy-3-methoxymandelic acid¹⁶ and total phenylacetic acid¹⁷ were measured by capillary column flame-ionisation gas chromatography. Phenylacetic acid, *p*-hydroxyphenylacetic acid and *p*-hydroxymandelic acid are the major metabolites, respectively, of phenylethylamine, *p*-tyramine and *p*-octopamine which are, themselves, synthesised sequentially from L-phenylalanine¹⁸. Values are given as means ± s.e.m.

* All controls versus all depressives $P < 0.005$. † Female controls versus female depressives $P < 0.01$. ‡ Male depressives versus female depressives $P < 0.02$. § All controls versus all depressives $P < 0.05$. ¶ Female controls versus female depressives $P < 0.05$.

Table 2 Urinary excretion of free phenylethylamine in control subjects and depressive patients

	n	Urinary excretion of free phenylethylamine ($\mu\text{mol per 24 h}$)
Control subjects		
Males	10	38.7 ± 5.78
Females	16	40.9 ± 6.77
Total	26	40.0 ± 4.70
Depressed patients		
Males	10	51.6 ± 13.1
Females	12	39.2 ± 7.76
Total	22	44.8 ± 8.25

Free phenylethylamine was determined by an electron-capture gas chromatographic method¹⁸. Values are means $\pm$ s.e.m.

first time appear to support this view, it must be remembered that an unknown proportion of these acidic metabolites may derive from diet or gut flora (for extended discussion, see ref. 9). *p*-Hydroxyphenylacetic acid and *p*-hydroxymandelic acid, which presumably reflect quantitatively the turnover of their precursor amines, are metabolically interconnected (Table 1). Even though changes in phenylethylamine production are likely to have been masked by a massive gut flora contribution⁹, our recent findings of significantly low concentrations of phenylacetic acid, the major metabolite of this amine, in cerebrospinal fluid from patients with depressive illness¹² suggests that phenylethylamine too may be deficient in the central nervous system of these patients.

Note also that an as yet unknown proportion of the total production of both tyramine and octopamine derives from the periphery, particularly the sympathetic nervous system and the tissues it innervates¹³. However, even if the changes we have described were to prove to be wholly peripheral, and such a finding need not necessarily detract from their relevance to depression, this disease may well turn out to stem from a generalised upset, connected perhaps with a membrane transport deficit, rather than a disturbance localised solely to the brain¹⁴.

What lessons are we to draw from the present findings? We have no wish to substitute a tyramine-octopamine hypothesis for its competitors. Recent data implicating histamine H₂ receptors in the therapeutic response to tricyclic antidepressant drugs⁴ are particularly compelling but by no means exclude other factors. It seems that an extremely complex sequence of events is responsible for the maintenance of normal affect, in which histamine, catecholamines, 5-hydroxytryptamine, and the trace amines also, may all play their part. Recent trials have indicated that when administered orally, phenylalanine, the precursor of phenylethylamine, tyramine and octopamine, may be beneficial in certain patients with depressive illness¹⁵.

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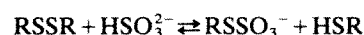
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Sulphonation of cholinergic receptor disulphide bond increases response to ACh

THE presence on the cholinergic receptor of a critical disulphide bond has been demonstrated and localised to within 10 Å of the cholinergic receptor anionic binding subsite¹⁻⁴. Reduction of this bond by dithiothreitol results in a reduced response of the nicotinic cholinergic receptor to acetylcholine (ACh) in many systems^{1,5-8}. I report here that heterolytic cleavage of this disulphide bond is produced using sodium bisulphite, a reagent which shows a high degree of specificity, reactivity and reversibility of interaction with the disulphide bond⁹. The active agent is the bisulphite ion, the reaction being



and resulting in the addition of the strongly nucleophilic sulphonate group to the receptor. The addition of this group seems to modify the ACh receptor-ionophore complex in a unique manner which results in an increase in its response to ACh.

The preparation used was the cutaneous pectoris muscle of the frog *Rana pipiens*. Reagents were added to continuously perfusing frog Ringer (NaCl 115 mM, KCl 2.0 mM, CaCl₂ 0.2 mM, MgCl₂ 5.0 mM, buffered with HEPES, pH 7.2, at room temperature). Sodium bisulphite was effective in concentrations of 10⁻⁵-10⁻³ M. All compounds were bath applied with the exception of the ACh iontophoretic experiments. The amplitude of either the miniature end plate potential (m.e.p.p.) or iontophoretically-induced ACh responses were used as a measure of the postsynaptic response to ACh.

The application of 1 mM NaHSO₃ to the preparation results in an increase in m.e.p.p. amplitude within 5 min, most typically with a 1-min latency. This increase may be up to 300%, although increases of 50-100% are more common. The amplitude increase is not the result of a single class of large m.e.p.ps but rather a shifting of the entire amplitude spectrum to the right (Fig. 1a, b). Iontophoretic application of ACh shows this amplitude increase to be of postsynaptic origin (Fig. 1c). The rise or delay time of the response to ACh (m.e.p.p. or iontophoresis) and the input impedance of the postsynaptic membrane (measured with two intracellular electrodes) are unchanged by bisulphite. The resting potential of the postsynaptic membrane remains constant even after a 2-h application of bisulphite in concentrations up to 5 × 10⁻³ M. (Presynaptic effects of bisulphite, including m.e.p.p. frequency increases shown in Fig. 1a, will be reported elsewhere.)

From the above data, it can be concluded that the increased response to ACh following bisulphite application cannot be attributed to changes in the postsynaptic membrane input resistance (no change in input impedance), to an action on acetylcholinesterase (no change in response decay time) or to changes in presynaptic release (response to iontophoretic ACh increases). An action on the postsynaptic cholinergic receptor molecule thus seems a possibility. As the bisulphite ion has a high degree of specificity of reaction with disulphide bonds⁹ and as a disulphide bond on the cholinergic receptor has been shown to be critical in postsynaptic ACh action¹⁻⁴, the following experiments were carried out using compounds known to

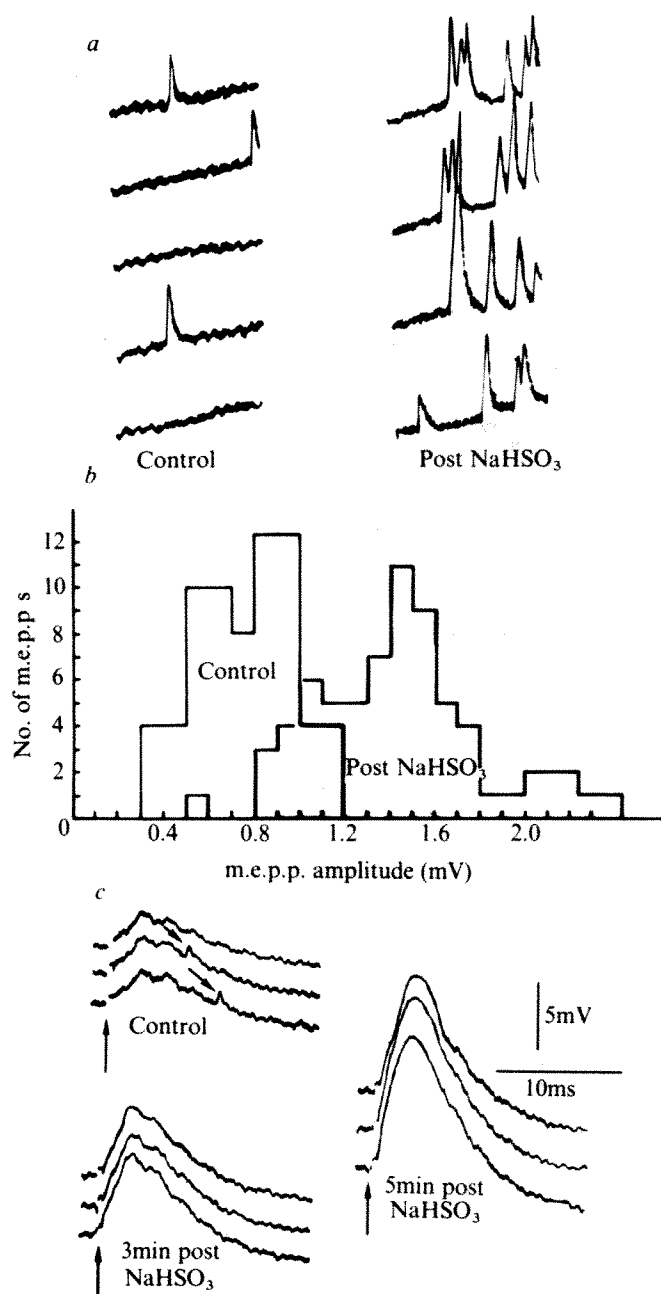


Fig. 1 Increases in response to ACh following sodium bisulphite. *a*, Intracellularly recorded m.e.p.ps before and 15 min after NaHSO₃ application (10^{-3} M); *b*, m.e.p.p. amplitude spectrum of the same muscle fibre during control recording and 15 min after NaHSO₃ treatment (10^{-3} M); *c*, response of postsynaptic membrane to iontophoresed ACh (large arrows) before and after NaHSO₃ application (10^{-3} M). Small arrows indicate m.e.p.ps.

localise the site of action of a reagent to this disulphide bond of the cholinergic receptor^{1-4,8}.

DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] forms an addition product with sulphhydryl groups which then revert to the oxidised state of the disulphide bond⁹. It will reverse the reduction of the disulphide bond on the cholinergic receptor produced by dithiothreitol¹. The effect of DTNB on the bisulphite-induced amplitude increase was thus tested. Application of DTNB (0.2×10^{-3} M) after bisulphite treatment (10^{-3} M) produces a rapid return of the m.e.p.p. amplitude to control values (Fig. 2). DTNB, without prior bisulphite treatment, has no effect on the end plate at this concentration. The accelerated reversal of the bisulphite response by DTNB most probably occurs by combination of DTNB with free -SH groups and -SH groups produced by hydrolysis of the easily reversible sulphonate⁹.

MBTA [4(*N*-maleimido)- α -benzyltrimethylammonium-iodide] was synthesised as an affinity label for sulphhydryl groups located near the cholinergic binding site². MBTA alkylates covalently at its maleimide group and binds reversibly at the quaternary end of the molecule to the cholinergic ligand binding site. Through the irreversible alkylation, MBTA prevents restoration of the disulphide bond by DTNB and at the same time keeps its quaternary group in the vicinity of the cholinergic binding site where it competes with ACh for the receptor site². Application of MBTA (5×10^{-7} M) following application of bisulphite, reduces the postsynaptic response to ACh, presumably by competitive inhibition of the quaternary group of MBTA with the spontaneously released ACh. It also prevents the DTNB reversal of the bisulphite response to control values (Fig. 3). MBTA without prior bisulphite treatment has no effect either pre- or postsynaptically at this concentration.

Occupation of the receptor binding site by ACh has been shown to prevent the reaction of dithiothreitol with the receptor disulphide bond⁸. The experiment was repeated using sodium bisulphite and ACh to ascertain whether the binding of ACh to the receptor would also prevent the action of the bisulphite ion on the receptor. Following a control m.e.p.p. recording, the muscle was exposed for 2 min to ACh (5×10^{-6} M) and then for 20–40 min to ACh (5×10^{-6} M) plus sodium bisulphite (3×10^{-3} M). This concentration of bisulphite would invariably have produced a high m.e.p.p. frequency and large amplitude increase. However, during application and the subsequent 40 min Ringer wash, no change in m.e.p.p. amplitude and only a small change in m.e.p.p. frequency occurred. The contralateral muscle used as a control showed the usual bisulphite effect. These experiments were also repeated with fibre sampling: 20 fibres were sampled, at each experimental point above, with the same results. Occupation of the receptor by ACh seems to inhibit disulphide bond modification either by preventing access to the bond by steric hindrance or by induction of a conformational change after which the disulphide bond is either inaccessible or no longer essential for further receptor-ionophore interaction. Evidence that steric hindrance is not the mechanism of ACh protection is given by the fact that curare, a bulky molecule which binds to the cholinergic receptor, is

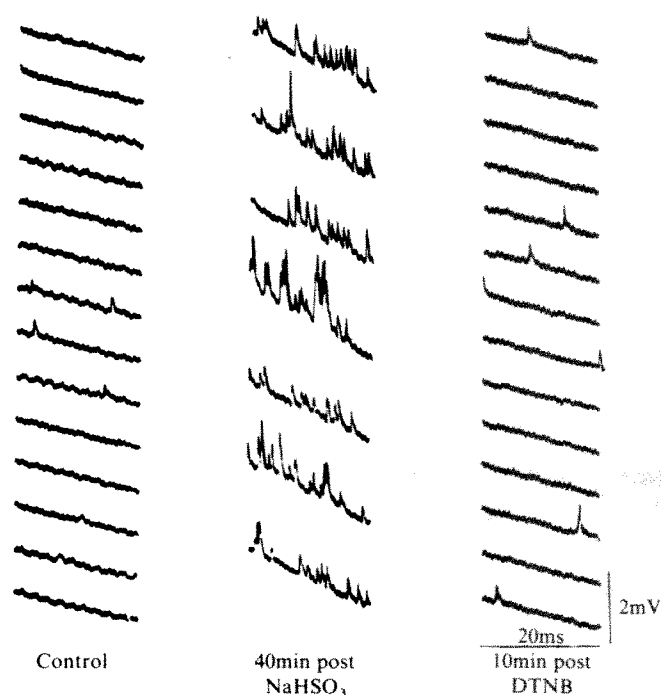


Fig. 2 DTNB reversal of m.e.p.p. amplitude and frequency increase following NaHSO₃ application (10^{-3} M). DTNB (0.2×10^{-3} M) was applied 40 min after NaHSO₃ application, when m.e.p.p. frequency and amplitude were maximal. Reversal begins approximately 2 min post-DTNB and is complete at 20–40 min.

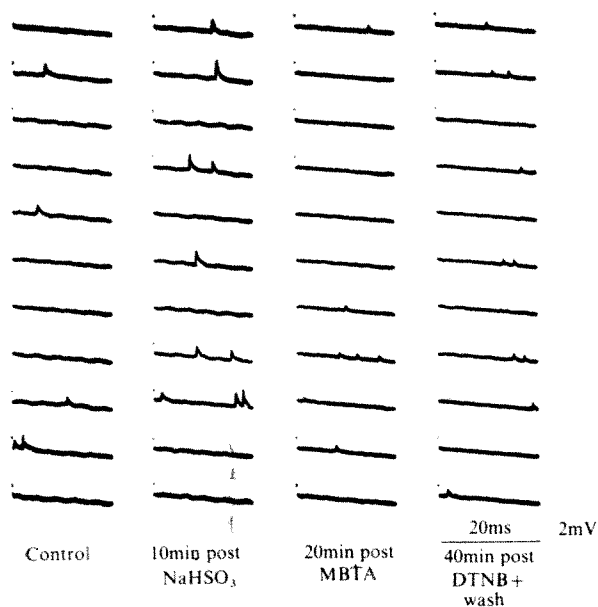


Fig. 3 Use of alkylating affinity label MBTA to demonstrate the presence of the sulphhydryl group produced by NaHSO_3 application. MBTA (5×10^{-7} M) is applied after bisulphite has produced a m.e.p.p. amplitude increase. The production of a sulphhydryl group by bisulphite is shown by the presence of affinity alkylation as demonstrated by (1) failure of DTNB to produce reversal of the MBTA response and (2) reduced m.e.p.p. amplitude produced by competition of spontaneously released ACh with the quaternary group of the affinity label.

unable to block the action of dithiothreitol⁸. Desensitisation of the ACh receptor does not seem to be involved in the process of ACh protection, as it has been shown that receptors in the desensitised state are still accessible for reduction by dithiothreitol⁸.

The above data demonstrate an action of bisulphite on the postsynaptic cholinergic receptor which increases the receptor response to ACh. Bisulphite is known to produce a heterolytic cleavage of disulphide bonds with resultant addition of a sulphonate group⁹. This action has been localised to a disulphide bond in the vicinity of the receptor negative subsite by the use of MBTA and is likely to be the same disulphide bond which has been reduced by dithiothreitol in previous experiments¹⁻⁸. The mechanism of action of this increased response to ACh following sulphonation remains unclear, but must be linked to the addition to the receptor molecule of the negative charge of the sulphonate group.

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A semi-synthetic penicillinase inactivator

MANY pathogenic bacteria secrete penicillinases (β -lactamases, EC 3.5.2.6) as a defence mechanism against β -lactam antibiotics¹. Inhibitors of penicillinase are therefore of potential clinical importance, and interest in this area has been stimulated by the discovery of effective naturally occurring enzyme inactivators²⁻⁴. We have studied the interaction of one of these, clavulanic acid (I) with the exocellular enzyme from *Staphylococcus aureus*, and we report here the synthesis of a novel penicillinase inhibitor whose design was based on the mechanism postulated for the inactivation by clavulanate.

Sodium clavulanate rapidly inactivates the penicillinase from *S. aureus* with close to 1:1 stoichiometry. Partially inactivated enzyme regains 10-15% activity over 10 min at pH 7 and 25 °C; fully inactive enzyme can be prepared by treatment with a small excess of inactivator. The apparent K_m for inactivation is 12 μM , and the enzyme is protected from inactivation by good substrates such as the chromogenic cephalosporin, 87/312 (ref. 5), or by competitive inhibitors such as phenylacetylcephalosporin C. Clavulanate is therefore a highly specific active site-directed inhibitor of *S. aureus* penicillinase.

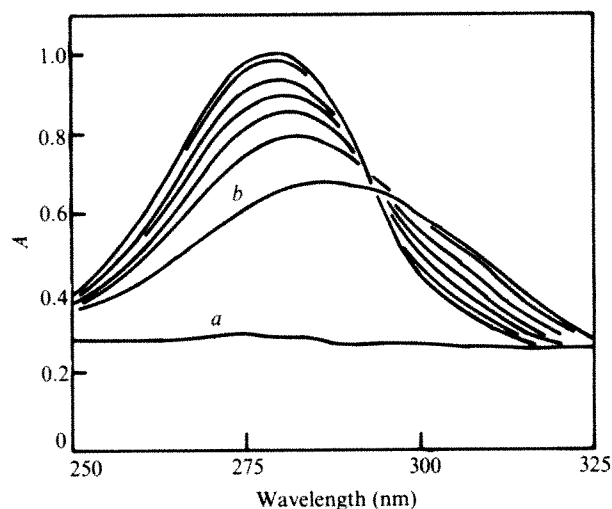
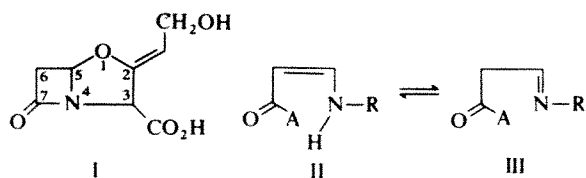


Fig. 1 Difference spectra, (enzyme + clavulanate) - enzyme, for an equimolar mixture of enzyme and inhibitor, 3.5×10^{-5} M, at pH 8 and 25 °C. *a*, Is the baseline, *b*, the spectrum immediately after mixing. The successive spectra with isosbestic point 292 nm were taken 1, 3, 6, 10, 20 and 35 min after mixing.

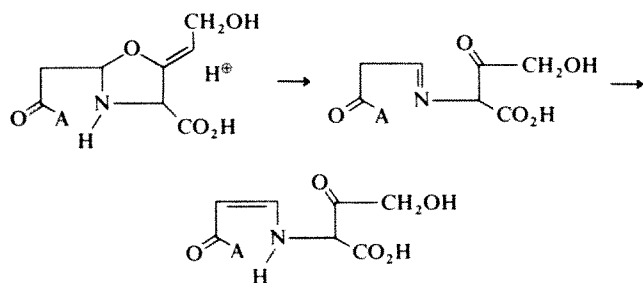
Incubation of the protein with clavulanate leads to the appearance of a new component in the UV spectrum, whose evolution is shown in Fig. 1. Loss of activity is associated with the rapid appearance of a species with maximum absorbance at wavelength (λ) 290 nm. In a slower process, not accompanied by any change in the extent of inactivation, this species is converted into another, with $\lambda_{\text{max}} = 278$ nm and extinction coefficient $21,500 \text{ cm}^{-1} \text{ M}^{-1}$. Inhibited enzyme can be fully reactivated by incubation in acid solution. Figure 2 shows that this restoration of activity is paralleled by loss of absorbance due to the UV chromophore. The kinetics of the reactivation were measured between pH 5 and 10.5. In this range the rapid phase of the reaction is first order, with the rate constant decreasing from 0.12 min^{-1} at pH 5 to $<0.001 \text{ min}^{-1}$ at pH 10.5. The slow phase leading to complete reactivation shows a similar pH dependence. Hydroxylamine has no effect on the reaction.

The simplest hypothesis to explain these results is that inhibition is due to formation of a compound of partial structure II from the β -lactam ring of clavulanate. 'A' represents an enzyme



active site residue. We suggest that this structure is an analogue of an acyl enzyme in the normal reaction pathway, stabilised to hydrolysis by the unsaturation at C5-C6 (ref. 6). A compound containing this structure (ethyl β -benzylaminocrotonate) has $\lambda_{\max}$ 286.5 and 275.5 nm, and extinction coefficients 23,400 and 28,800 $\text{M}^{-1} \text{cm}^{-1}$ in the *cis* and *trans* forms, respectively (in 95% ethanol)⁷, and these values are typical of β -amino- α , β -unsaturated carbonyl compounds⁸. We suggest therefore that the slow change in spectrum (Fig. 2) is due to *cis-trans* isomerisation. Structures of type II are expected to be unstable in acid because of tautomeric equilibria with imines (III), whose hydrolysis is acid catalysed. A model compound, ethyl 3-carboxymethylamino-2-butenate, synthesised by condensation of ethylacetoacetate and glycine, showed the expected spectrum and acid instability.

If this proposal for the mechanism of inactivation is correct, the difference that makes clavulanate an inactivator of the enzyme and closely similar compounds, such as the penicillins (IV) substrates, is the possibility of an elimination across the C6-C5 bond. This elimination could occur by a two-step route



or, as proposed by Charnas *et al.*⁹, by single-step abstraction of a proton from C6 by an enzymatic base. It is not possible to decide between these routes on the basis of present evidence. Either way, elimination requires a sufficiently good leaving group at C5, and a proton which can be lost from C6.

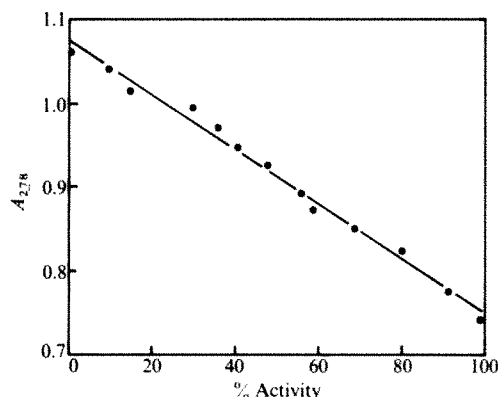
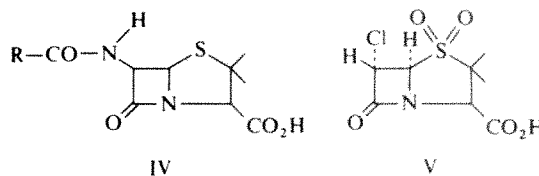


Fig. 2 Correlation of restoration of activity with loss of UV absorbance. Penicillinase (2.7×10^{-5} M) in 0.1 M sodium acetate buffer, pH 5.0, was incubated at room temperature with an equal concentration of clavulanate. The absorbance at 278 nm and the enzymatic activity were measured at intervals over the next 3.5 h.

Oxygenation of sulphur makes it a better leaving group¹⁰, and we therefore synthesised penicillin G sulphone¹¹. This compound was found to be a good substrate, with K_m 20 μM and $V_{\max}$ the same as for penicillin G. The sulphone does not inactivate the enzyme, but a transiently formed (non-enzyme-containing) hydrolysis product has $\lambda_{\max}$ 280 nm. Presumably, in this case the elimination only occurs after the penicilloic acid has left the active site.

Penicillins lack one of the two hydrogen atoms at C6 in clavulanate and elimination by the single-step route may be sterically impossible in penicillins and derivatives. Alternatively, the hydrogen atom in penicillin G sulphone may be too weakly acidic. 6-Chloropenicillanic acid, as usually synthesised¹² (but see also ref. 13), has the opposite configuration at C6 to that in penicillins, and such an electronegative substituent presumably has an acid-strengthening effect on the hydrogen atom. We therefore synthesised 6- α -chloropenicillanic acid, and found it to be a poor substrate for the enzyme ($K_m = 3\text{--}5$ mM, $V_{\max}$ 0.8 relative to penicillin G = 100). The compound is not an inactivator of the enzyme. Oxidation of 6- α -chloropenicillanic acid with KMnO_4 gave the novel 6- α -chloropenicillanic acid sulphone (V).



Incubation of the enzyme with this compound leads to inactivation similar to that produced by clavulanate, except that (1) no reactivation of the enzyme is observed at pH 7, and (2) the enzyme hydrolyses about 100 molecules of the compound for each molecule of the enzyme that is inactivated. This behaviour is thus similar to that found for the reaction of clavulanate with the *Escherichia coli* enzyme^{9,14}. The apparent K_m for the inactivation process is about 100 μM . We anticipate that the stability of the inactivated form of the enzyme will allow identification of the site of attachment of the inhibitor.

English *et al.* have reported¹⁵ that unsubstituted penicillanic acid sulphone inhibits penicillinases, including that from a strain of *S. aureus*. It seems likely that the mechanism of this inhibition is similar to that proposed here.

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In vivo proteolytic cleavage of colicins requires specific receptor binding

COLICINS are plasmid-specified antibiotic proteins produced by some strains of coliform bacteria¹. They are distinguished from other bacterial antibiotics such as aminoglycosides and tetracyclines by their large molecular weights (40–80,000) and their narrow host range. Colicins only kill bacteria having the appropriate cell-surface receptors^{1,2}, and even then the ability of a cell to produce a given colicin usually confers immunity to killing by that colicin. We are interested in the comparative properties of colicins and their similarities to bacterial toxins that are active against mammalian cells, some of which are also encoded by extra-chromosomal elements. A fundamental question concerning the action of both colicins and toxins is how these large, essentially hydrophilic proteins traverse the mainly hydrophobic cell surface to reach the lesion sites. For colicins these include DNA for colicin E2 (ref. 3), RNA (colicin E3 and cloacin DF13)^{4,5}, and presumably the inner cell membrane, where membrane potential and energy metabolism are affected (colicins E1, K and Ia (refs 2, 6, 7)). Here, we show that binding of colicin to its specific receptor can result in a specific proteolytic cleavage to generate two peptide fragments. The smaller of these, derived from the C-terminal region of the colicin, has a greater specific biological killing activity *in vitro* than the parent molecule. We propose that *in vivo* the proteolytically derived

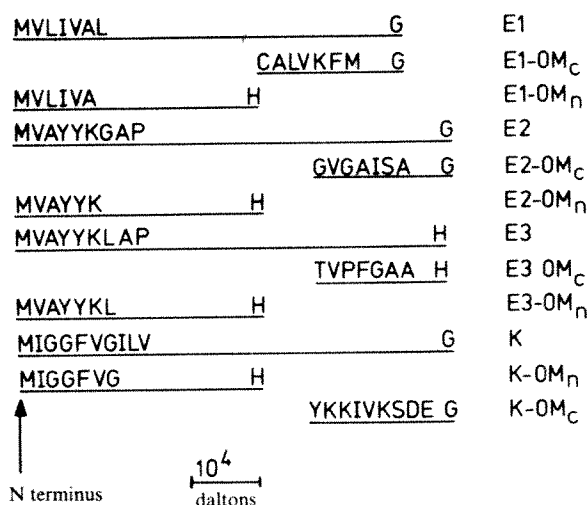


Fig. 1 For analytical purposes, 0.17 nmol purified colicins¹⁰ were incubated with 0.1 A₆₀₀ units of *Escherichia coli* K12 outer membranes¹² in 0.1 M sodium phosphate buffer (pH 7.0) for 15 min at 37 °C. Membranes were removed by centrifugation (78,000g, 90 min) and the peptides resolved by Laemmli SDS (0.1%) polyacrylamide slab gel electrophoresis¹³ (4.5% stacking gel, 12.5% running gel). Co-run MW markers were used in the determination of the MWs of the colicin polypeptides. For preparative gels, 13.3 nmol of digested colicin was run in wide tracks on each gel. Each colicin sample was flanked by a track containing MW markers. The latter tracks only were stained and by reference to the analytical results, each band containing a presumptive colicin-derived polypeptide was cut out, disrupted in distilled water (18 h, 4 °C) and finally dialysed extensively against distilled water. 90% of the product (about 50 nmol) was lyophilised, and then used in determination of N- and C-terminal residues and N-terminal sequences^{17–19}. The remaining sample was precipitated by (NH₄)₂SO₄ (70% w/v) and dialysed against the appropriate reaction buffer before assaying for *in vitro* activities. Intact colicins were treated in the same way before structural determination and *in vitro* assay. C-terminal peptides = OM_C; N-terminal peptides = OM_N. For example, E1-OM_C = C-terminal colicin E1 polypeptide derived from incubation of colicin E1 with outer membranes.

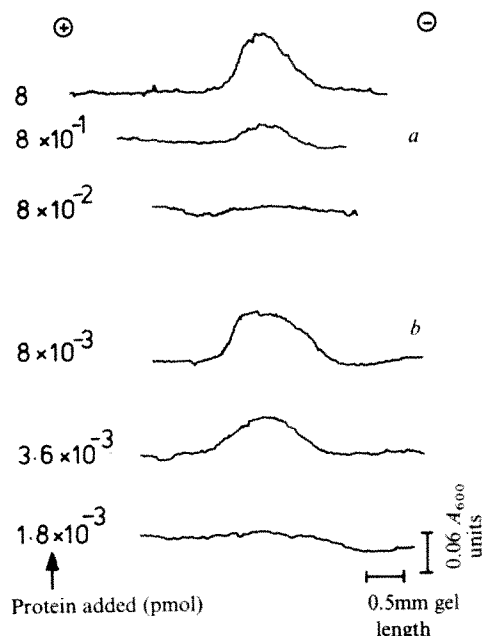


Fig. 2 Colicin E3(a) or E3-OM_C(b) were incubated with ribosomes from sensitive K12 cells²⁰ using the polyacrylamide gel assay method of Lau and Richards⁴. The amount of the nucleotide fragment generated from 16S rRNA was quantified using a Joyce-Loebel densitometer. 80–500 pmol E1-OM_C, K-OM_C and E2-OM_C, colicins E1, E2 and K and all OM_N fragments (Fig. 1) gave no measurable generation of nucleotide fragment (similar traces to the bottom one in a).

C-terminal fragment is transported across the cell surface to the lesion site. This process seems analogous to the apparent cleavage of diphtheria toxin during its entry into mammalian cells⁶.

Because colicins E2 and E3, and cloacin DF13 can be cleaved by pure proteases to yield specific polypeptide fragments, some of which have the nuclease activity of their parent colicin *in vitro* but not *in vivo*^{4,9–11} it seemed possible that colicins, after binding to their specific receptors on whole cells, might be proteolytically cleaved to generate a biologically active fragment which is then transported to the lesion site. To test this for colicins E2, E3, E1 and K, we incubated purified colicins separately with outer membrane vesicles¹², derived either from colicin-sensitive cells (receptor intact) or from resistant cells (receptor non-functional). Incubation of each colicin with colicin-sensitive outer membrane vesicles resulted in a complete conversion of each colicin to two peptides, derived from each end of the colicin molecule (Fig. 1). In contrast, vesicles from an E-colicin-resistant, K-colicin-sensitive (E^r K^s) strain cleaved colicin K but not E, whereas vesicles from an E^s K^r strain cleaved E but not K colicins. None of the colicins were cleaved by outer or inner membranes from E^r K^r strains or by inner membrane vesicles from sensitive cells. Lysozyme, ovalbumin, cytochrome *c* and bovine serum albumin were not cleaved during incubation with any of the vesicles.

Although none of the fragments isolated from preparative SDS-polyacrylamide gels¹³ killed whole cells, even after extensive dialysis, the C-terminal peptides derived from colicins E2 and E3 showed activity in *in vitro* assays. Moreover, their specific activities were higher than those of their parent colicins isolated in the same way. The specific activity of the colicin E3 C-terminal fragment was 300–1,000 times greater than that of the intact colicin (Fig. 2). Colicin E2 DNase specific activity was about 1% of that of its C-terminal peptide (Fig. 3).

In an attempt to assay the *in vitro* activities of colicins E1 and K and their polypeptide fragments, we used the enhanced fluorescence assay of Phillips and Cramer¹⁴. Incubation of colicin E1 (Fig. 4a) or colicin K (Fig. 4b) with intact sensitive cells in

the presence of 8-anilino-1-naphthalene sulphonic acid (ANS) resulted in a large rapid increase in fluorescence, whereas incubation with colicin-resistant cells showed no fluorescence increase. The much smaller increase in fluorescence observed when intact colicins E1 and K were incubated with outer membranes of $E^s K^s$ cells could reflect the binding of colicins to their receptors on the outer membrane. Incubation of C-terminal colicin E1 (E1-OM_c) and C-terminal colicin K (K-OM_c) peptides with inner membranes resulted in a slow but reproducible increase in fluorescence. This may reflect a significant interaction of these polypeptides with their presumptive lesion site in the inner membrane, as N-terminal colicin E1 and K peptides (E1-OM_n, K-OM_n), and colicins E2 and E3 and their peptides had no such effect. If these interactions with inner membranes are of significance, then E1-OM_c and K-OM_c are more active than their respective whole colicins.

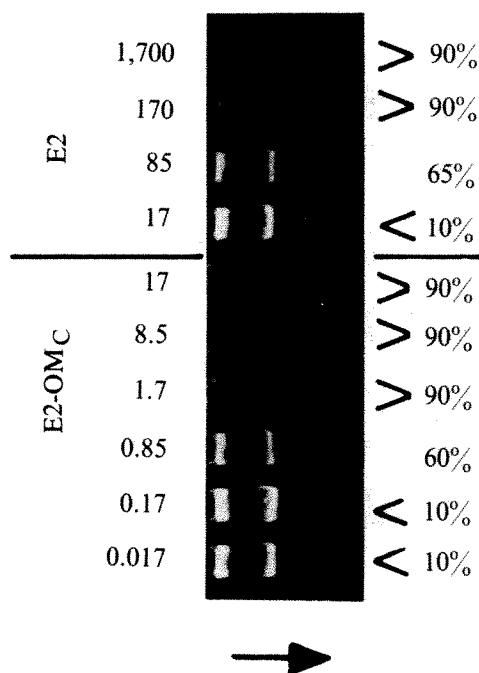


Fig. 3 DNase activity of colicin E2 and its polypeptide fragments. Colicins or their peptides (Fig. 1) were incubated with 0.6 μ g bacteriophage λ DNA for 1 h at 37°C in 16 μ l of the reaction mixture of Schaller and Nomura³. Samples were then assayed on a 0.9% agarose gel⁵. The direction of DNA migration is indicated. Much of the undigested λ has remained at the origin of the gel. We have noted this with many DNA preparations, and it could result from the binding of DNA to non-DNA macromolecules present as contaminants in the DNA preparation. The amount of protein ($\text{mol} \times 10^{-15}$) added for each assay is indicated above each track, and the degree of digestion is indicated below. Colicins E1, K, E3, their peptides and E2-OM_n (not shown), and bacteriophage λ incubated in the absence of added protein, showed no detectable digestion.

Although *in vitro* target activity is associated with the C-terminal fragments, all N-terminal peptides bound cell-surface receptors (Fig. 5). Furthermore, additions of N-terminal fragment in a 10^4 – 10^5 -fold molar excess to homologous colicin inhibited killing activity. Predictably, K-OM_n did not interfere with E-colicin killing and N-terminal colicin E fragments did not affect K-colicin killing. None of the C-terminal fragments effected *in vivo* killing by any colicin.

In conclusion, these data demonstrate that after cell-receptor binding, colicins can be proteolytically cleaved into two peptides with mutually exclusive receptor binding and killing activities. This seems analogous to the proposed action of diphtheria toxin on mammalian cells⁸. Common factors include protein fragmentation into large (molecular weights 38,000 and 35–36,000)

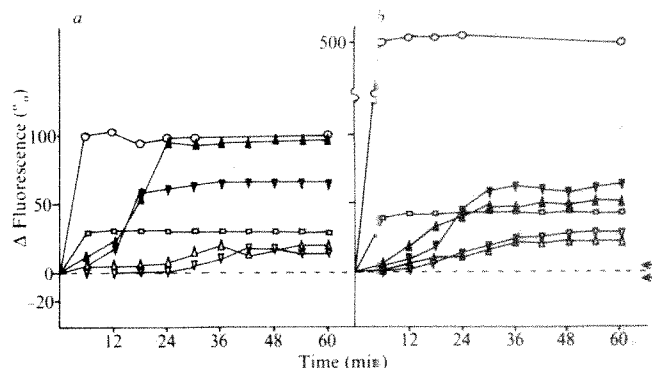


Fig. 4 Fluorescence changes (502 nm) after colicins and their polypeptide fragments (71 pmol per assay; Fig. 1) were added (0 min) to 8 A_{600} units of the indicated cell preparation in 1 ml of M9 salts, equilibrated with ANS (60 μ M). *a*, Colicin E1-derived polypeptides; *b*, colicin K-derived polypeptides. $\circ$, Intact colicin + $E^s K^s$ cells; Δ , intact colicin + inverted spheroplasts²¹ of $E^s K^s$ cells; ∇ , intact colicin + inner membranes¹² of $E^s K^s$ cells; $\square$, intact colicin + outer membranes¹² of $E^s K^s$ cells; $\blacktriangle$, -OM_c peptides + inverted spheroplasts of $E^s K^s$ cells. $\blacktriangledown$, -OM_c peptides + inner membranes of $E^s K^s$ cells. The following combinations of colicin-derived peptides (71 pmol) and cell preparations gave <2% fluorescence change at any time during the 60-min incubation (values within the parallel arrows) (1) Any cell preparation incubated with E1-OM_n; K-OM_n; colicin E2, E3 or any of their fragments. (2) Whole $E^s K^s$ cells or their outer membranes incubated with E1-OM_c and K-OM_c. (3) $E^s K^s$ cells and their outer membranes incubated with intact colicins.

cell binding and small (24,000 and 19–21,000) *in vitro* killing fragments, neither fragment having *in vivo* activity. However, although the action of diphtheria toxin requires cleavage of disulphide bonds, none are present in E and K colicins (D.H.W., unpublished). Another common feature between colicins, diphtheria toxin and some other bacterial toxins is that they are encoded by extra-chromosomal elements: this may have facilitated their spread among bacteria and their evolution¹⁵. Specific cell-surface-associated proteolysis is clearly a key step in the action of some bacterial toxins or bacterial and mammalian cells: whether this same protease is responsible for the cleavage and release of secreted proteins¹⁶ remains to be seen.

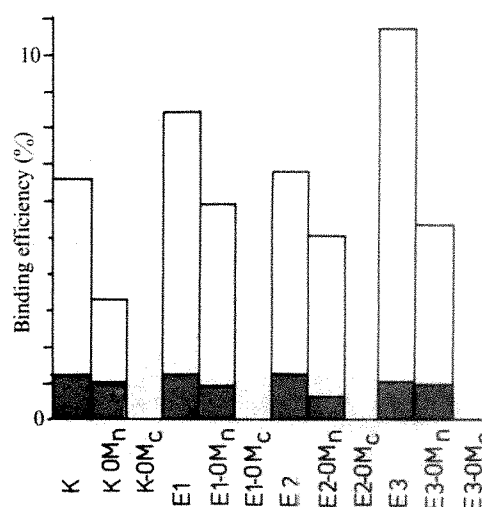


Fig. 5 Five nmol ^{14} C-labelled colicin or proteolytic fragments^{22,23} (see Fig. 1: 65.3–161.2 $\times 10^3$ c.p.m. per 5 nmol) were incubated with sensitive (upper bar) or $E^s K^s$ cells (lower bar, shaded) (10^8 cells ml^{-1}) for 30 min in 1 ml L-broth. After centrifugation and resuspension (boiling 1% SDS), the cell pellet was counted on 1 MM Whatman paper using 0.15% diphenyloxazole in toluene.

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Induction of erythroid differentiation in the human leukaemia cell line K562

MURINE erythroleukaemic cell lines can be induced by various low molecular weight compounds to differentiate *in vitro*¹. Corresponding human cell lines have not previously been described but we now report that human leukaemic cells of the K562 cell line can be induced to red cell differentiation *in vitro*.

Table 1 Accumulation of erythrocyte-like, benzidine-positive particles in sodium butyrate treated cultures

Days in culture	Per cent of benzidine positive particles*				
	0	1	2	3	4
1 mM Na-butyrate	1.4	3.9	12	35	48
Control	1.4	0.9	0.9	1.1	1.4

*Cyto-centrifuged smears were stained with benzidine-Giemsa. Percentages were calculated by counting more than 300 cells.

The K562 line was originally established by Lozzio and Lozzio from the pleural effusion of a patient with chronic myelogenous leukaemia in terminal blast crisis². This cell line, which has been considered to represent an early differentiation stage of the granulocyte lineage³, is commonly used as a sensitive target cell in the human natural killer cell assay⁴.

We have recently analysed the surface glycoprotein patterns of various established human haematopoietic cell lines^{5,6} and those of isolated populations of normal and leukaemic leukocytes⁷⁻⁹. The surface glycoprotein pattern of K562 cells was found to be clearly different from those of benign and malignant cells representing various stages of the myeloblast to granulocyte differentiation sequence. In contrast, we observed several common features of normal erythrocytes and K562 cells. These observations led us to investigate more closely the K562 cells.

The cells were cultivated in RPMI 1640 culture medium supplemented with 10% newborn-calf serum. K562 cells carry on their surface¹⁰ and synthesise¹¹ glycophorin A, which is the major sialoglycoprotein on human red cells¹². This provides additional evidence for the erythroid nature of the K562 cells, as glycophorin A is known to be expressed exclusively on basophilic normoblasts and on later stages of the red cell differentiation in human bone marrow¹³. The K562 cells evidently also contain spectrin as seen by indirect immunofluorescence using rabbit anti-spectrin antibodies on acetone-fixed smears (data not shown).

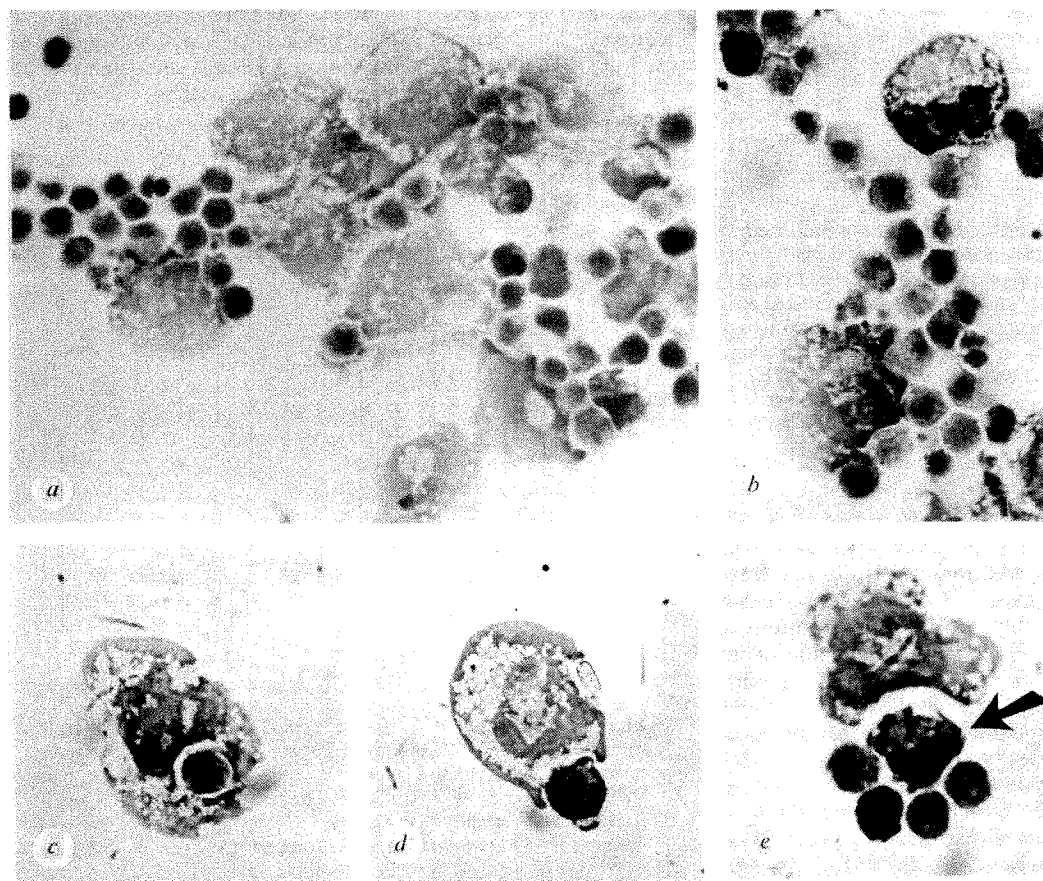


Fig. 1 Microphotographs of cyto-centrifuged smears of K562 cells cultivated for 4 days in the presence of 1 mM sodium butyrate. *a*, Stained with Giemsa-eosin and photographed using a green filter. *b*, Stained with Lephene's benzidine method-Giemsa and photographed using a red filter. *c-e*, Details of the apparent sequence of generation of erythrocyte-like particles in butyrate induced K562 cultures. The arrow indicates a normoblast-like cell. These were stained with Giemsa-eosin and photographed with a green filter.

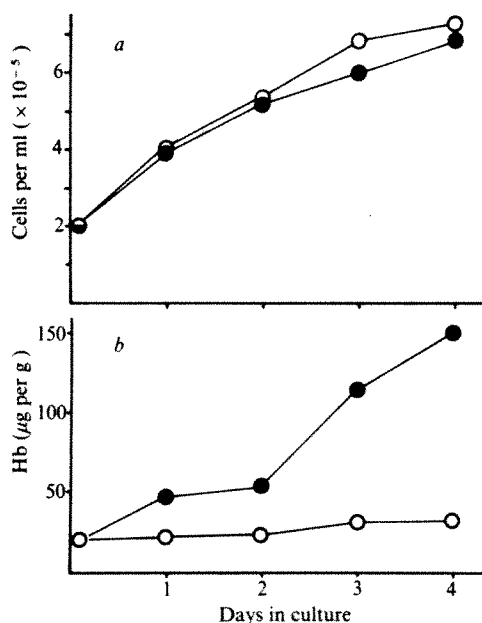


Fig. 2 *a*, Cell counts from K562 cultures treated with 1 mM sodium butyrate (●) and from control cultures without inducer (○). Each point represents the average from duplicate determinations. *b*, Synthesis of haemoglobin in sodium butyrate induced K562 cells as determined by radioimmunoassay. K562 cells were cultivated at an initial density of 0.2×10^6 cells per ml in the presence of 1 mM sodium butyrate (●) or without inducer (○) (the same cultures as in *a*) and aliquots taken on indicated days. After washing the cells three times in phosphate-buffered saline, aliquots were removed for protein determination¹⁵. The rest of the samples were solubilised in Triton X-100 containing buffer and used for radioimmunoassay. Anti-haemoglobin antiserum was prepared in rabbits and anti-rabbit IgG in sheep. Haemoglobin was radioiodinated with ¹²⁵I by the chloramine T method¹⁶. The antibody concentration was calibrated to bind approximately 30% of the radioactivity and more than 95% of the binding was inhibited by nonradioactive haemoglobin. The preparation of antisera and the radioimmunoassay will be described in detail elsewhere. The results are given as μg haemoglobin per g of protein and each point represents the average of duplicate analyses.

As the cultures of K562 cells were found to contain occasional benzidine-positive particles (Table 1) the differentiation capacity of this cell line was further investigated. Because the commonly used inducing agent dimethyl sulphoxide¹ did not cause obvious erythroid differentiation, sodium butyrate was used as inducer¹⁴. When K562 cells were cultivated for 4 days in the presence of 1 mM sodium butyrate (optimal concentration 1–3 mM) differentiation was observed. This amount of sodium butyrate did not significantly affect the growth rate of the K562 cells (Fig. 2*a*). Eosinophilic particles resembling erythrocytes accumulated in the cultures. These particles were surrounded by a membrane and showed positive staining reaction with benzidine (Table 1) indicating the presence of haemoglobin (Fig. 1). The induction of haemoglobin synthesis was confirmed by radioimmunoassay (Fig. 2*b*).

The mode of generation of the erythrocyte-like particles seems to be unusual. Although occasional normoblast-like cells were seen (Fig. 1*e*), most of the erythrocyte-like particles are apparently generated intracytoplasmically (Fig. 1*c*). As shown in Fig. 1*c–e* this seems to start with an accumulation of cytoplasmic eosinophilic inclusions which condense to one or several erythrocyte-like particles which are subsequently expelled. Scanning electron microscopy shows the erythrocyte-like particles to be spherical with a smooth surface resembling spherocytes (in preparation). The mechanism may be similar to the platelet generation by megakaryocytes. The phenomenon of *in vitro* erythrocyte generation by this cell line is being further studied using time-lapse cinematography.

The findings presented here not only prove the erythroid character of the K562 cell line, but also further emphasise that malignant transformation of human haematopoietic cells does not necessarily mean irreversible loss of differentiation capacity. After 8 years in culture *in vitro*, the K562 cell line has retained its ability to react to appropriate stimuli by undergoing erythroid differentiation. The effect of sodium butyrate on human erythroid leukaemic cells might have interesting clinical implications.

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Cleavage site of bacteriophage fd gene II-protein in the origin of viral strand replication

DOUBLE-STRAND DNA replication of small single-stranded bacteriophages such as ΦX174 and filamentous phage fd is initiated by highly specific endonucleases. This was demonstrated *in vitro* with the isolation of $\Phi\text{X cisA}$ protein¹ and more recently with that of fd gene II-protein². Both endonucleases produce one single-strand break in the viral strand of superhelical RFI providing an origin for double-strand (ds) DNA replication^{3,4}. For gene II-protein the strand specificity of nicking was demonstrated by cleavage of asymmetrically labelled fd RFI (viral strand ³²P-labelled, complementary strand ³H-labelled) and subsequent alkaline sucrose sedimentation. As a result the viral strand was cleaved into a full length linear form, whereas the complementary strand remained circular (T.F.M. and K.G., submitted). Here we report the position of the nick in the nucleotide sequence of the fd genome.

Gene II-protein was purified from *Escherichia coli* cells infected with phage fd mutants in gene V using the stimulation of DNA synthesis on fd RFI as an assay. The purification steps were ammonium sulphate precipitation and various columns. Details of the isolation procedure will be published elsewhere (T.F.M. and K.G., submitted). The pure enzyme has a molecular weight of 46,000 on SDS gels and under non-denaturing conditions which agrees with the size of gene II-protein from membranous cells structures. The protein was not found in cells infected with phage mutants in gene II.

Superhelical fd RFI DNA was incubated with purified gene II-protein as described in the legend to Fig. 1 resulting in the open circular form (RFII₀). (Phage fd RFII₀ is defined as the cleavage product of fd RFI by gene II-protein.) To determine the exact position of the nick RFII₀ was cleaved with restriction

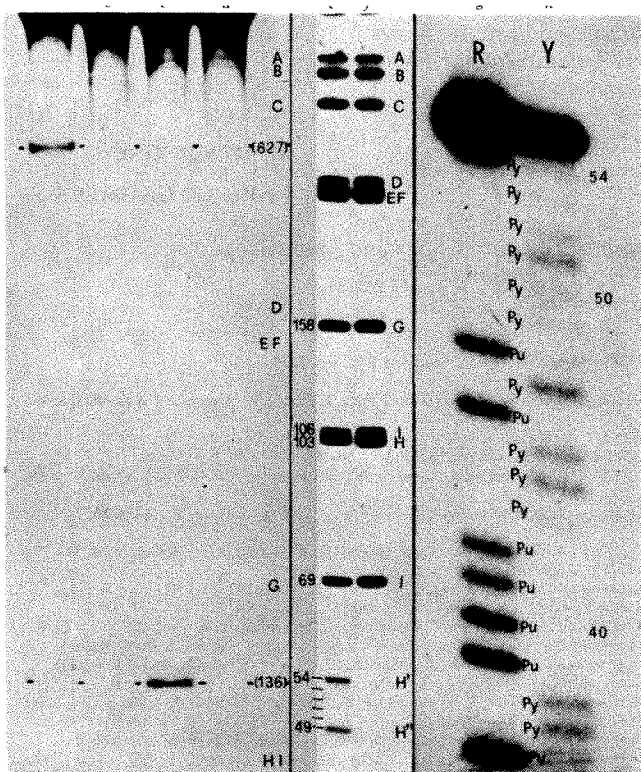


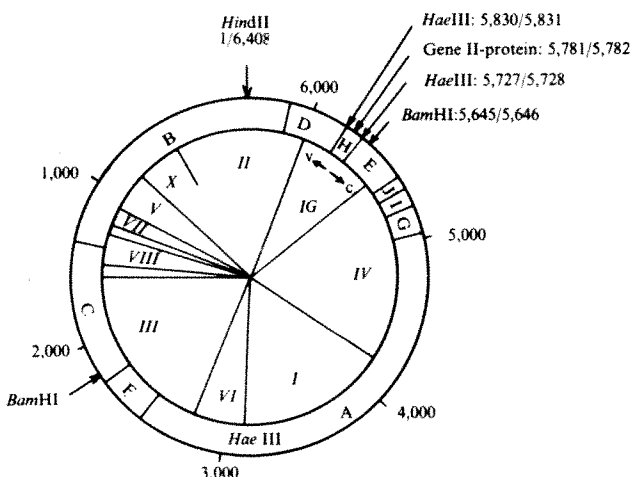
Fig. 1 Localisation of the gene II-protein cleavage site on the fd genome by gel electrophoresis of labelled DNA fragments. The cleavage reaction was carried out with 0.5 pmol (2 μ g) fd RFI and 0.3 units of purified fd gene II-protein (T.F.M. and K.G., submitted) in 150 μ l 10% sorbitol, 20 mM Tris-HCl, pH 8.1, 80 mM NaCl, 5 mM β -mercaptoethanol, 5 mM MgCl₂ and 200 μ g bovine serum albumin per ml for 10 min at 30 °C. The reaction product was then digested with restriction endonucleases *Hind*II (tracks a, b), *Bam*HI (c, d), or *Hae*III (e, f). The DNA was denatured in the presence of 5 μ g fd single-stranded DNA in 0.2 M NaOH for 20 min at 37 °C. The sample was mixed with 200 μ l phenol and 50 μ l 1 M KH₂PO₄, the aqueous phase desalted on a Sephadex G50 column and lyophilised. Tracks b, d and f are controls without gene II-protein. Labelling at the 3'-end (a-d) was done with nucleotidyl transferase (EC 2.7.7.31) (5 units) in 1 mM CoCl₂, 5 mM MgCl₂, 50 mM imidazol-HCl, pH 6.8 and 1.2 μ M [α -³²P]dTTP (2 $\times$ 10⁶ c.p.m. in the 25 μ l assay) for 30 min at 37 °C. The reaction products were analysed on a 5% acrylamide gel. Label at the 5'-ends (e, f) was introduced by T4-polynucleotide kinase (EC 2.7.1.78) after pretreatment with alkaline phosphatase (EC 3.1.3.1) and the fragments separated under denaturing conditions on a 10% polyacrylamide gel¹⁰. The numbers in the figure give the nucleotide length of the fragments generated and the letters the separated *Hae*III fragments, which were also a marker for the tracks a and b. Tracks g and h show a 20% sequencing gel¹⁰ covering the 3' terminus of the 54 nucleotide fragment H'. Strong and weak Py bands correspond to C and T residues, respectively. The very strong bands on top are non-degraded 54 nucleotide fragment.

endonucleases (Fig. 1). The generated fragments were denatured in alkali terminally labelled and analysed by electrophoresis on acrylamide gels. When RFII₀ was cleaved with endonuclease *Hind*II, an additional fragment of about 650 nucleotides was found, when cut by enzyme *Bam*HI, a fragment of about 130 nucleotides appeared in comparison with the control experiment using RFI DNA (Fig. 1). These results indicate that the gene II-protein cleavage site maps around position 5,750 between the *Bam*HI site at nucleotides 5,645/5,646 and the *Hind*II site at nucleotide 6,408/1 within the intergenic region of the phage (Fig. 2). For a more precise analysis restriction enzyme *Hae*III was used. Gel analysis of the denatured reaction products showed two new fragments of 54 and 49 nucleotides derived from the 103 nucleotide *Hae*III fragment (Fig. 1). Nucleotide sequences at the 3'-end of the two fragments (Fig. 1) and comparison with the known fd nucleotide sequence⁵ revealed the position of the gene II-protein cleavage site in the viral strand between nucleotide 5,781 and 5,782 (Fig. 3). As gene II-protein is a component of fd RF replication with purified proteins⁴, it seems likely that the enzyme creates a start for DNA synthesis. This origin of phage fd viral strand synthesis lies within the segment derived *in vivo* for the start of DNA replication in the related phage f1 (ref. 6).

Cleavage of fd RFI by gene II-protein gives a 5'-phosphate end and a 3'-OH end. The viral strand at the nick can be extended in the 3' direction with nucleotidyl transferase (small fragment in Fig. 1, track c). The 5'-end can be labelled with polynucleotide kinase only after phosphatase treatment (fragment of 49 nucleotides in Fig. 1, track e). Labelling was as efficient as at the *Hae*III created 5'-end of the adjacent fragment H'. Thus, gene II-protein is not covalently bound to the DNA ends at the nick after alkaline treatment, but it may well be attached to the 5'-end in native conditions. A covalent linkage was postulated for Φ X174 *cisA* protein at the 5'-end of the cleaved viral strand, as label could not be introduced at this end⁷.

The position of the nick between nucleotides 5,781 and 5,782 has several consequences for phage DNA replication and recombination (see Fig. 3). (1) The cleavage site is separated from the origin of complementary strand synthesis⁸ by 24 nucleotides. The complementary strand can therefore only be initiated on the displaced viral strand after a full round of viral strand synthesis. (2) The nick lies in a nucleotide sequence with

Fig. 2 Physical and genetic map of the bacteriophage fd genome. The inner segments represent the genes and the intergenic region (IG) of the phage. The outer segments give the fragments produced by restriction endonuclease *Hae*III. The arrows at the outside indicate the cleavage sites of restriction endonucleases *Hind*II and *Bam*HI, and of gene II-protein, respectively. The arrows in the IG region indicate start and direction of viral strand (v) and complementary strand (c) synthesis. Counterclockwise movement on the map corresponds to the 5' $\rightarrow$ 3' direction of the viral strand DNA. The numbers at the outside indicate the nucleotide position on the genome.



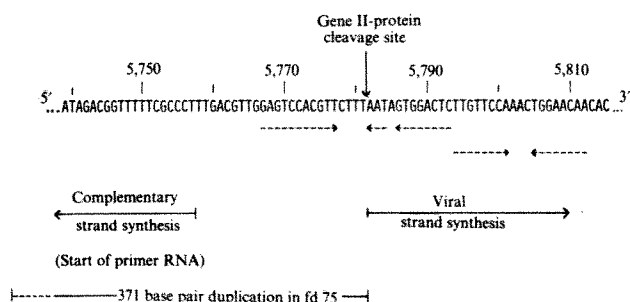


Fig. 3 Nucleotide sequence of the origins of phage fd replication. The interrupted arrows indicate sequences of twofold symmetry around the cleavage site of gene II-protein, which may be in equilibrium with a hairpin in single-stranded viral DNA and in superhelical RF DNA. Viral and complementary strand synthesis start closely neighboured but without overlap. In the larger phage fd 75 (H. F. Lauppe and U. Hess, unpublished) a tandem duplication of base pairs reaches from the gene II-protein cleavage site into gene IV (ref. 9).

twofold symmetries (Fig. 3). As gene II-protein neither reacts with fd single strands nor with relaxed doubly closed fd RF (T.F.M. and K.G., submitted), we assume that transient hairpin structures in the locally disturbed DNA double helix are recognised by the enzyme. The self-annealing structure may also be required for circularisation of linear single strands in RF and viral strand synthesis. (3) The nick in the RF also represents a hot spot for recombination with homologous sequences in other parts of the genome which result in duplications of DNA segments found in variants of filamentous bacteriophages⁹.

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Premature termination during adenovirus transcription

INITIATION of transcription, the first event in gene expression, is probably the most critical possible site for gene regulation. However, it has become clear from recent studies with bacteria and bacteriophages that a transcriptional start does not ensure the expression of a gene. For example, the phenomenon of 'attenuation', that is, premature termination of an mRNA chain before transcription of the structural gene region, is a key regulatory event for the synthesis of enzymes that make amino acids in bacteria¹⁻⁴. We report here evidence for premature chain termination during transcription of an animal virus genome.

Late in adenovirus type 2 (Ad2) infection of HeLa cells a large group of virus-specific mRNAs⁵⁻⁷ are produced by processing

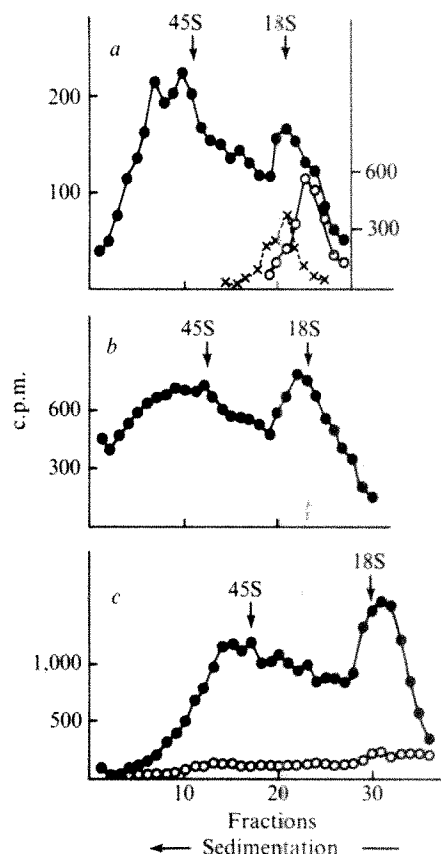


Fig. 1 Sedimentation analysis of 'nascent' Ad2-specific nuclear RNA. *a, b*, Labelled nuclear RNA was extracted from Ad2-infected HeLa cells after a 45-s label with ³H-uridine. $1-2 \times 10^8$ cells were collected 16-18 h after infection, resuspended at 4×10^6 cells ml⁻¹ with 1 mCi ³H-uridine ml⁻¹, final concentration 0.05 μ mol uridine ml⁻¹; after 45 s the cells were poured over frozen crushed medium. The extracted RNA was treated with dimethyl sulphoxide at 65 °C as described previously^{8,17} and sedimented at 25 °C in sucrose SDS gradients. Markers were either A₂₆₀ furnished by ribosomal RNA or ³²P-labelled ribosomal RNAs. The positions of 45S and 18S are indicated. Fractions of the gradients equivalent to 10^7 cells were hybridised²⁷ to 2 γ of Ad2 DNA adsorbed to nitrocellulose filters (●). RNA:DNA hybridisation was carried out as described at 65 °C in 2 $\times$ TES buffer containing 0.3 M NaCl, 0.01 M TES, 0.01 M EDTA and 0.2% SDS. Hybridised RNA resistant to ribonucleases A and T was scored. In panel A samples equivalent to 3×10^7 cells were hybridised to 2 μ g equivalent of Sma1 B, 18.2-36.7, (x) and Sma1 F, 11.1-18.2 (○) per filter. *c*, Nuclei from cells infected with Ad2 for 19 h were isolated^{8,9} and labelled for 2 min with ³H-UTP either with or without 0.5 μ g α -amanitin. Nuclear RNA was extracted, sedimented and hybridised to total Ad2.

the primary transcripts from a single large rightward reading transcription unit⁸⁻¹⁴. The location of the promoter site for this large transcript at 16.45 on the Ad2 genome^{9,15} makes an assay for the frequency of transcription of the beginning of this transcription unit compared to the transcription of the remainder of the region possible. (The linear Ad2 genome is divided into 100 units of 355 bases each with 0 at the left end of the DNA strand that is transcribed to the right.) This assay utilises labelled 'nascent' RNA (unfinished chains labelled only at their growing ends^{8,9,16,17}) hybridised to appropriate Ad2 fragments produced by restriction endonuclease digestion. From the present experiments it appears that as many as 70-80% of the RNA polymerase molecules that initiate Ad2 RNA chains at 16.45 fail to continue for more than ~2,000 nucleotides. The polymerases that are responsible for producing the primary transcripts from which the majority of the late Ad2 mRNA is derived continue to ~98.5, a distance of ~29 kilobases^{8,9,14-16,18,19}.

If cells or isolated nuclei are exposed very briefly to labelled RNA precursors, the labelled RNA chains from a single transcription unit will form an equimolar series of nascent chains labelled at their growing 3' termini^{8,9,16,17}. All chain lengths between very short polynucleotides and the finished length should be equally labelled if no premature termination and no processing occurred during transcription. Figure 1c shows that Ad2-specific RNA labelled *in vitro* in nuclei isolated from Ad2 infected cells presented a bimodal distribution of Ad2-specific chains. A broad continuous distribution of Ad2-specific RNA from about 2–20 kilobases was observed with a peak sedimenting faster than 45S pre-ribosomal RNA (14 kilobases). Also, a distinct peak of shorter Ad2-specific RNA that sedimented about equally to 18S (~2 kilobase) rRNA was observed. This result repeats that of an earlier experiment⁸, where it was further shown that the 10–18S *in vitro* labelled Ad2-specific RNA was complementary mainly to DNA fragments 11.1–18.2 and 18.2–36.7, that is, to regions near the start site (16.45) for late Ad2 RNA synthesis. To test whether the same pattern of transcription also occurred *in vivo*, 'late' Ad2-infected HeLa cells were labelled with ³H-uridine for 45 s. The nuclear RNA was isolated and separated by sedimentation and the various sized RNA chains were hybridised to Ad2 DNA. Two experiments are shown in Fig. 1a, b. In both a bimodal distribution of large (~45S pre-rRNA) and small (~18S and less) Ad2-specific RNA sequences was observed. In approximately five *in vivo* experiments the sedimentation pattern of Ad2-specific RNA was similar. The amount of 'short' Ad2-specific RNA varied from a peak about one-half as great as the large RNA to approximately twice as great as the large Ad2 RNA.

To determine the origin of the short *in vivo* labelled Ad2-specific RNA the 6–18S region from one *in vivo* labelled preparation was pooled and hybridised to a series of fragments of the Ad2 genome prepared by restriction endonuclease digestion (Table 1). Over 75% of the smaller Ad2-specific chains hybridised to DNA fragments 11.1–18.2 and 18.2–36.7 that contain the first part of the transcription unit, in accord with the *in vitro* result^{8,9,15}. Most of the remainder of the small RNA hybridised to the 3–11 region where two or three independent transcription units are known to exist^{9,20,21}. If every polymerase which started at ~16 continued through the entire major late transcription unit (to a terminus near ~99 (ref. 19)) the distribution of labelled chain lengths would be unimodal in a sucrose gradient. The bimodal distribution with an enrichment

Table 1 Hybridisation to Ad2 DNA restriction fragments of small (10–18S) *in vitro* labelled RNA

Coordinate of DNA fragment	c.p.m. hybridised	% Total
3. –10.7	185	5%
11.1–18.2	897	25%
18.2–36.7	1,963	53%
36.7–40.5	66	
40.5–52.6	85	
58.5–70.7	72	
70.7–75.9	90	
75.9–83.4	33	
83.4–89.7	70	

³H-uridine labelled RNA of ~6–18S (equivalent to fractions 21–27 in Fig. 1a, b) was collected from cells that were pulse-labelled (45 s) at 18 h post-infection (see Fig. 1). The input was equal to the 6–18S RNA from 2×10^7 cells. Three successive rounds of hybridisation were carried out to DNA filters containing 10 µg equivalent of each fragment (that amount of a given fragment that would be contained in 10 µg of total Ad2 DNA). The hybridisation was for 48 h at 65 °C in $2 \times$ TES (0.01 M N-tris(hydroxymethyl)methyl-2-aminoethane sulphonic acid, 0.01 M EDTA, 0.2% SDS)²⁷. After the first round of hybridisation the supernatant solution was withdrawn and the filters were washed twice at 65 °C with $2 \times$ SSC (standard saline citrate; 0.15 M NaCl, 0.01 M Na citrate). All the unbound RNA was collected by precipitation with ethanol and the second round of hybridisation was carried out. The third round was then performed. Approximately 60% of the hybrids formed in the first round, 30% in the second round, and 10% in the third round.

of sequences complementary to the promoter proximal regions of the transcription unit is evidence that premature RNA chain termination occurs after about 1,000–2,000 nucleotides both *in vitro* and *in vivo*.

To determine the frequency of premature termination the amount of RNA synthesised in 45 s that was complementary to a variety of DNA fragments was measured. The amount of labelled RNA complementary to any DNA fragment divided by the length of the fragment that is transcribed is a measure of the 'molar' amount of RNA synthesised from that DNA region^{8,9,14}. The length of the 11.1–18.2 region that is transcribed was taken to be 600 nucleotides, from 16.45 to 18.2, the distance from the presumed promoter to the end of this fragment¹⁵. All of the other fragments except the terminal fragments 89.7–100 were

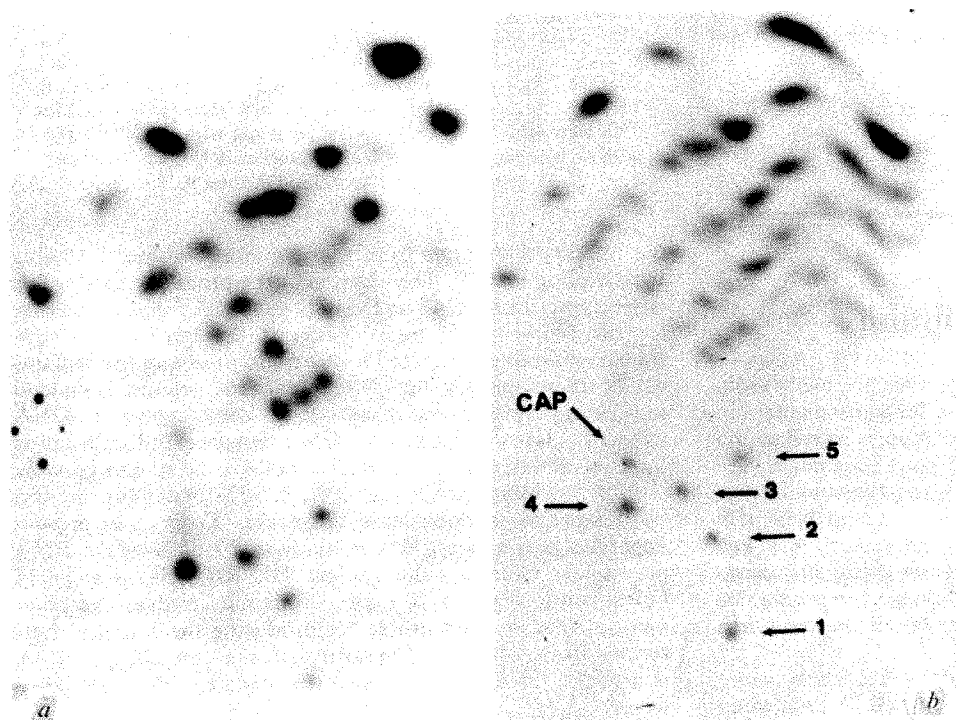


Fig. 2 Two-dimensional oligonucleotide analysis of RNA hybridised to the 11.1–18.2 region of Ad2 DNA. Ad2-infected HeLa cells were labelled with ³²P (2×10^8 cells were exposed to 50 mCi carrier-free ³²P-orthophosphate 14–18 h after infection) or nuclei from infected cells were prepared 18 h post-infection and labelled by *in vitro* incorporation of [α -³²P]UTP for 2 min⁸. Nuclear RNA was extracted from both samples and the *in vivo*-labelled RNA > 28S was selected by sedimentation. The RNA was hybridised to the Sma1 F DNA fragment 11.1–18.2 using 100-µg equivalent filters per 10^8 cell equivalents, eluted after T1 digestion and extensive washing and fingerprinted as described previously¹⁵. Oligonucleotides labelled 1–5 were eluted and secondary analyses (pancreatic digestion followed by DEAE electrophoresis¹⁵ confirmed that the same sequences were present in the *in vivo* and *in vitro* labelled oligonucleotides. For the *in vitro* labelled sample the extramolarity for Sma1 F, 11.1–18.2, was demonstrated as in Table 2 and 95% of the Sma1 F-specific incorporation was inhibited by α -amanitin.

Table 2 Molarity of transcription of regions of Ad2 genome

DNA fragments	Length transcribed* (kilobases)	Expt 1		Expt 2		Expt 3		Expt 4	
		c.p.m. in hybrid	Molarity†	c.p.m. in hybrid	Molarity	c.p.m. in hybrid	Molarity	c.p.m. in hybrid	Molarity
11.3–18.2	0.6	1,319	6.7	1,385	6.8	521	6.4	2,678	3.3
29.1–36.7	7.5	–	–	–	–	990	0.98	–	–
29.1–52.6	3.4	–	–	–	–	–	–	3,227	0.71
36.7–40.5	1.1	381	1.05	395	1.1	–	–	–	–
40.5–52.6	4.4	940	0.65	1,239	0.84	–	–	–	–
58.5–70.7	4.3	1,240	0.86	1,104	0.75	493	0.85	3,256	0.6
75.9–83.4	2.8	460	0.51	500	0.55	–	–	–	–
83.4–89.7	2.0	655	1.0	672	1.0	270	1.0	2,685	1.0
89.7–100	3.2	–	–	–	–	290	0.65	3,121	0.72

³H-uridine labelled nuclear RNA was prepared after a 45-s exposure of Ad2 infected HeLa cells (16–18 h post infection) and the total RNA sample was hybridised as indicated to various Ad2 DNA restriction fragments (see Fig. 1 for details). Two or three rounds of hybridisation were carried out in each case.

* The length transcribed, in kilobases, was taken to be the whole fragment except for the 11.1–18.2, *Sma*I F fragment where only 0.6 kilobases from 16.45–18.1 is transcribed^{8,14} and for the 89.7–100 *Eco*C fragment. It is probable that the termination site for transcription is at ~98.5 (ref. 19) and the DNA transcribed in this fragment would be 3.2 kilobases.

† Molarity was calculated by dividing the c.p.m. in hybrid by the length transcribed for each fragment and normalising to 1.0 for 83.4–89.7 fragment which was used in every experiment.

assumed to be completely transcribed. Transcription of the terminal fragment was taken to be 89.7–98.5 (ref. 19). When the molar amount of synthesis from each DNA region was calculated, there was three- to sixfold more labelled RNA complementary to 16.45–18.2 than to any other DNA fragment (Table 2). The remainder of the fragments were essentially equally transcribed, a result which has been obtained in other experiments^{8,14,19}.

In calculating the molarity of transcription from the 11.1–18.2 region an assumption was made that all transcription reads rightward beginning at 16.45. This assumption is justified from previous experiments²¹ that showed 98.5% of total late transcription to be rightward reading. In addition, other recent experiments by Sehgal *et al.*²² showed that 90% of the nuclear RNA labelled in a 2-min pulse that was complementary to the 11.1–18.2 region was complementary to the *r* strand.

Evidence that the short and presumably prematurely terminated RNA contains the same sequences and thus begins at the same site as those molecules that eventually give rise to mRNA is given in Fig. 2. Infected cells were labelled with ³²PO₄³⁻ and nuclear RNA from molecules larger than 5,000 nucleotides was hybridised to the 11.1–18.2 fragment and unpaired RNA digested with T1 RNase. The filters were then washed extensively and the RNA eluted, digested with RNase T1 and fingerprinted. The hybridised RNA (Fig. 2a) revealed the characteristic large oligonucleotides that were previously shown to be near the 5' end of long nuclear RNA transcripts¹⁵. These T1 oligonucleotides were all predicted on the basis of direct sequence analysis to be part of rightward reading RNA molecules beginning at 16.45. As previously reported no oligonucleotides characteristic of sequences to the left of 16.45 were observed¹⁵. For comparison, isolated nuclei which continue synthesis of chains already initiated⁸ and which exhibit premature termination (Fig. 1c) were labelled with [α -³²P]triphosphates, and RNA complementary to 11.1–18.2 isolated. A fivefold molar excess of the *in vitro* labelled ³²P-labelled RNA to the 11.2–18.1 region was detected by hybridisation of a small portion of the preparation to a series of DNA fragments as had been done in the experiments of Table 2 (data not shown). When this ³²P-labelled RNA was bound and eluted from the 11.1–18.2 DNA, subjected to T1 digestion and analysed by fingerprinting, the same characteristic large oligonucleotides as those observed in the *in vivo* ³²P-labelled RNA were most prominent. In addition, many smaller oligonucleotides coincided (Fig. 2). Thus the prematurely terminated chains appeared to start at the same site as the chains that eventually are processed into mRNA. It is not possible, of course, from this experiment to be certain that the same exact 5' end is present on the prematurely terminated

RNA. However, preliminary evidence from *in vivo* labelled RNA (Fraser, Sehgal and J.E.D., unpublished) indicates that the short Ad2 chains that accumulate in a long ³²P label in infected cells have a similar fingerprint as that of Fig. 2 and contain the same capped oligonucleotide characteristic of the large late transcription unit.

Thus, the major result of the present experiments is that three to six times as much transcription occurs, presumably beginning at 16.45, as in other regions of the large late transcription unit. The sucrose sedimentation of pulse-labelled RNA (Fig. 1a, b) suggested that the extra transcription could continue as far as 2,000 nucleotides and this conclusion was also supported by the hybridisation of the slower sedimenting second peak of Ad2-specific RNA mainly to the 11.1–18.2 and 18.2–36.7 DNA fragments (Table 1 and Fig. 1a). From the present data we cannot conclude whether there are specific termination sites. The extra-molar synthesis does not extend past 25.5–27.9 because pulse-labelled RNA complementary to this region was earlier shown to be equimolar with all other regions to the right of 25 (refs. 8, 14). Thus the third of the leader sequences at 26.7 that is spliced to late Ad2 mRNAs^{11–13} does not appear to be represented in the extra synthesis, although each late mRNA is believed to contain all three leader sequences^{6,11–13}. Furthermore, it seems very unlikely that the prematurely terminated chains lead to production of mRNA molecules containing sequences that lie exclusively within the first 2,000 nucleotides of the transcription unit. Extensive searches for mRNA:DNA duplexes by electron microscopy^{6,11} have not revealed mRNA that lies within the 16–25 region and fingerprint analysis of cytoplasmic RNA does not show the distinctive oligonucleotides of Fig. 2 which lie within the first 500 nucleotides of the transcription unit¹⁵. Thus very few if any of the extra-molar premature transcripts appear as stable mRNA in the cytoplasm.

One final point concerns the nature of the enzyme responsible for the short prematurely terminated RNA chains. In the experiments of Figs 1 and 2, a portion of the isolated nuclei during incorporation of labelled precursor was treated with 0.5–1.0 μ g ml⁻¹ of α -amanitin, a dose of this inhibitor that almost completely inhibits synthesis by RNA polymerase II but not RNA polymerases I or III (ref. 23). The synthesis of >90% of the Ad2-specific RNA, both short chains and long chains, was sensitive to α -amanitin (Fig. 1c, legend to Fig. 2). Therefore, it seems that it is RNA polymerase II that prematurely terminates RNA chains within the first 2,000 nucleotides of the large Ad2 transcription unit. Other recent studies have shown that polymerase II transcription termination also frequently occurs in cell heterogeneous nuclear RNA formation (ref. 26, M. Salditt-Georgieff, M. Harpold and J. E. D., in preparation).

Transcription termination as a regulatory mechanism has been extensively documented in the temperate bacteriophages^{24,25} in addition to the attenuation phenomenon in the amino acid operons in *Escherichia coli*¹⁻⁴. In these instances the frequency of termination is very high, occurs on a region immediately preceding structural gene clusters and probably involves interactions between ribosomes translating the new 5' terminus of the RNA chain and the polymerase molecule that is synthesising the RNA chain. If there is any regulatory role at all for premature termination on mammalian cell or Ad2 DNA it would be unlikely to involve ribosomal interaction with growing chains of RNA in the nucleus of a mammalian cell and so no mechanism of premature termination parallel to bacterial attenuation seems likely. A cause for premature RNA chain termination other than a direct regulatory role might be found in reports about the complexity and steps of Ad2 mRNA formation¹⁴. Based on the close linkage in time between transcription, selection of a site and addition of poly(A), polymerase II may be accompanied by processing enzymes or may possess processing subunits¹⁴; possibly if polymerase-processing complexes that initiate transcription are incomplete they may be rejected after a certain distance from the origin of transcription.

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Sequence of three introns in the chick ovalbumin gene

WE now know that many genes are split, that is, that the DNA is not co-linear with its RNA, but is interrupted by regions of DNA about 10-1,000 residues long which have been called intervening sequences or introns¹. This organisation poses special problems for the biosynthesis and processing of mRNA², but is widespread in occurrence³⁻¹⁷, although some genes are known not to be split^{18,19}. Little is known of the detailed sequence of introns. In the case of globin and ovalbumin genes, hybridisation experiments^{20,21} indicate an absence of extensive sequence homology with other unrelated genes. Detailed sequence analysis of the β -globin genes in mice and rabbits²² suggest that during evolution introns diverged in sequence and in length

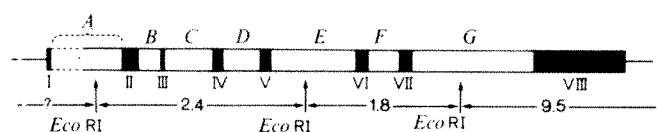


Fig. 1 Arrangement of introns (light, A-G) and exons (dark, I-VIII) in the chick ovalbumin gene. The positions of the cloned 1.8, 2.4 and 9.5 kilobase fragments separated by *EcoRI* restriction sites are shown. The 5' *EcoRI* fragment containing exon I has not yet been characterised so that the length of A is unknown.

more rapidly than the expressed sequences or exons, although the position of the exon-intron junction is precisely maintained²². The ovalbumin gene is a particularly extreme example of an interrupted gene. It has seven introns, and is spread over a distance of at least 6 kilobases (Fig. 1), but this distance is compressed at least 7 times into less than 800 residues of mRNA sequence; all seven known splice points occur between residues 47 and 830 in the 5' half of the ovalbumin mRNA^{23,24}. This gene is thus a favourable model gene for studying both the general properties of introns and the mechanism of splicing. Previously we and others sequenced selected short regions in two cloned fragments (the 1.8 and 2.4 kilobase fragments) of the gene to define the possible recognition site of the presumed splicing enzyme^{24,25}. We now report the sequence analysis of three introns of the 2.4-kilobase fragment of the chick ovalbumin gene.

A clone of the 2.4-kilobase ovalbumin fragment (pOv2.4) contained within the plasmid pBr322 and the vector *Escherichia coli* had been previously isolated and the major restriction sites mapped^{23,26}. After transfer of the pOv2.4 to *E. coli* RRI suitable restriction fragments were prepared which were labelled either at their 5' or 3' ends with ³²P for sequencing by the chemical degradation method of Maxam and Gilbert²⁷. Figure 2 summarises the sequence evidence and the legend gives details of some technical improvements in the methods. It can be seen that most of the sequence was read off both strands of DNA to ensure 100% sequence accuracy. Where data from one strand were

Table 1 Known restriction sites in the 2.4-kilobase fragment

Enzyme	Residue nos	Sequence†
<i>PstI</i>	1,966	CTGCA/G
<i>HaeIII</i>	623	CG/CC
<i>HinfI</i>	766, 968, 1,063	G/ANTC
<i>HphI</i>	317, 489, 806, 1,425, 1,656, 2,058	TCACC and GGTGA
<i>AluI</i>	8, 374, 432, 579, 661, 717, 785, 860, 895, 1,055, 1,119, 1,544, 1,727, 1,792, 1,879, 1,964, 1,984, 2,085, 2,111, 2,121	AG/CT
<i>MboII</i>	404, 983, 1,179, 1,397, 1,478, 1,678	TCTTC and GAAGA
<i>EcoRII</i> *	450, 470, 762, 1,102, 1,996	CC(A/T)GG
<i>Sau3A</i>		
(<i>MboI</i> *)	1,109, 1,521, 1,971	/GATC
<i>XbaI</i>	300	T/CTAGA
<i>SstI</i>	373, 1,983	GAGCT/C
<i>PvuII</i>	1,963	CAG/CTC
<i>AsuI</i>	1,215	G/GNCC
<i>MnlI</i>	33, 129, 585, 620, 809, 830, 881, 1,018, 1,167, 1,414, 1,634, 1,933, 1,936, 2,158, 2,238, 2,270, 2,285, 2,306, 2,314, 2,332	CCTC and GAGG
<i>HpaI</i> }	181	GTT/AAC
<i>HindII</i> }		
<i>KpnI</i>	2,240	GGTAC/C
<i>HindIII</i>	2,110	A/AGCTT

There are no sites for *HaeII*, *HhaI*, *HpaII*, *HgaI*, *AvaI*, *BamHI*, *BglII*, *SmaI*, *Sall*, *TaqI* and *XhoI*. *EcoRI* sites define the two ends of the fragment. The residue number given is the first (5') nucleotide of the recognition sequence and not the point of cutting. Where this is within the recognition sequence it is indicated by a vertical bar.

* These enzymes fail to cut pOV2.4 grown in *E. coli* strain RRI because of methylation of C(*EcoRII*) or A(*MboI*) residues (see text).

† See Roberts²².

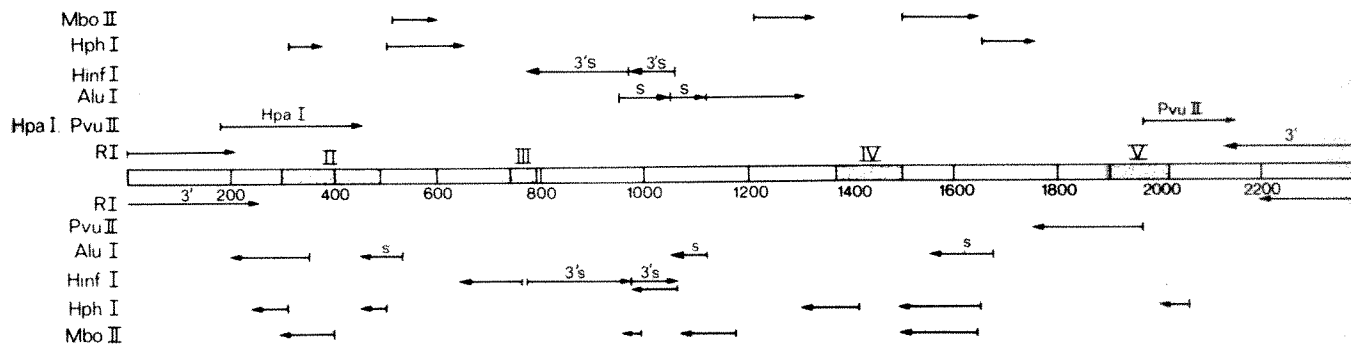


Fig. 2 Diagram of gel evidence showing the overlapping data used to derive the sequence of the 2.4-kilobase fragment (represented by the coding strand 5'-3', left to right). Lines indicate the length of sequence read and the restriction enzyme used to prepare the fragment. Above, the sequence was of the coding DNA strand; below, the sequence of the complementary non-coding strand. Arrows point away from the labelled position. 5'-labelling with ^{32}P was carried out with $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$ and T4 polynucleotide kinase²⁸ using restriction fragments derived by *Eco*RI, *Hpa*II, *Pvu*II, *Mbo*II, *Hinf*I, *Hph*I and *Alu*I digestion of either the purified 2.4-kilobase ovalbumin gene fragment (purified by 3.5% acrylamide gel electrophoresis²⁸) or the intact pOv2.4 plasmid directly, so as to obtain higher yields of labelled fragments. 3'- ^{32}P -labelled, *Eco*RI and *Hinf*I labelled fragments were also prepared by 'filling in' with a single $[\alpha\text{-}^{32}\text{P}]\text{dATP}$ using the Klenow fragment of DNA polymerase I (*E. coli*) (F. Sanger and J. Hindley, personal communications). Complementary strands in the size range of 20–200 residues were separated by heat denaturation in formamide and electrophoresis on 20% acrylamide 7 M urea gels (G.G.B., unpublished). Separated strands or labelled fragments, recut by another restriction enzyme, were subjected to chemical modification according to Maxam and Gilbert²⁷ using the alternate G, alternate A, C and C+T degradation and fractionation on an 8% or 20% acrylamide, 7 M urea thin²⁹ gel (0.3 × 200 × 400 mm). Longer (800 mm) thin gels (8%) were used to 'read' sequences 200–300 residues from the site of labelling. Sensitive autoradiography was according to Laskey and Mills³⁰. All the exon sequence was established independently of the mRNA except: (1) 10 residues across the *Hinf*I overlap (residue 766) in exon III; (2) 60 residues between the *Hph*I site (1,425) and *Mbo*II site (1,478) in exon IV; (3) 10 residues across the *Pvu*II site in exon V. A computer file was kept on the sequence as different restriction fragments were sequenced and this was edited using the SEQEDT program of Staden³¹, as new data became available. The file was also routinely searched for possible restriction sites³¹.

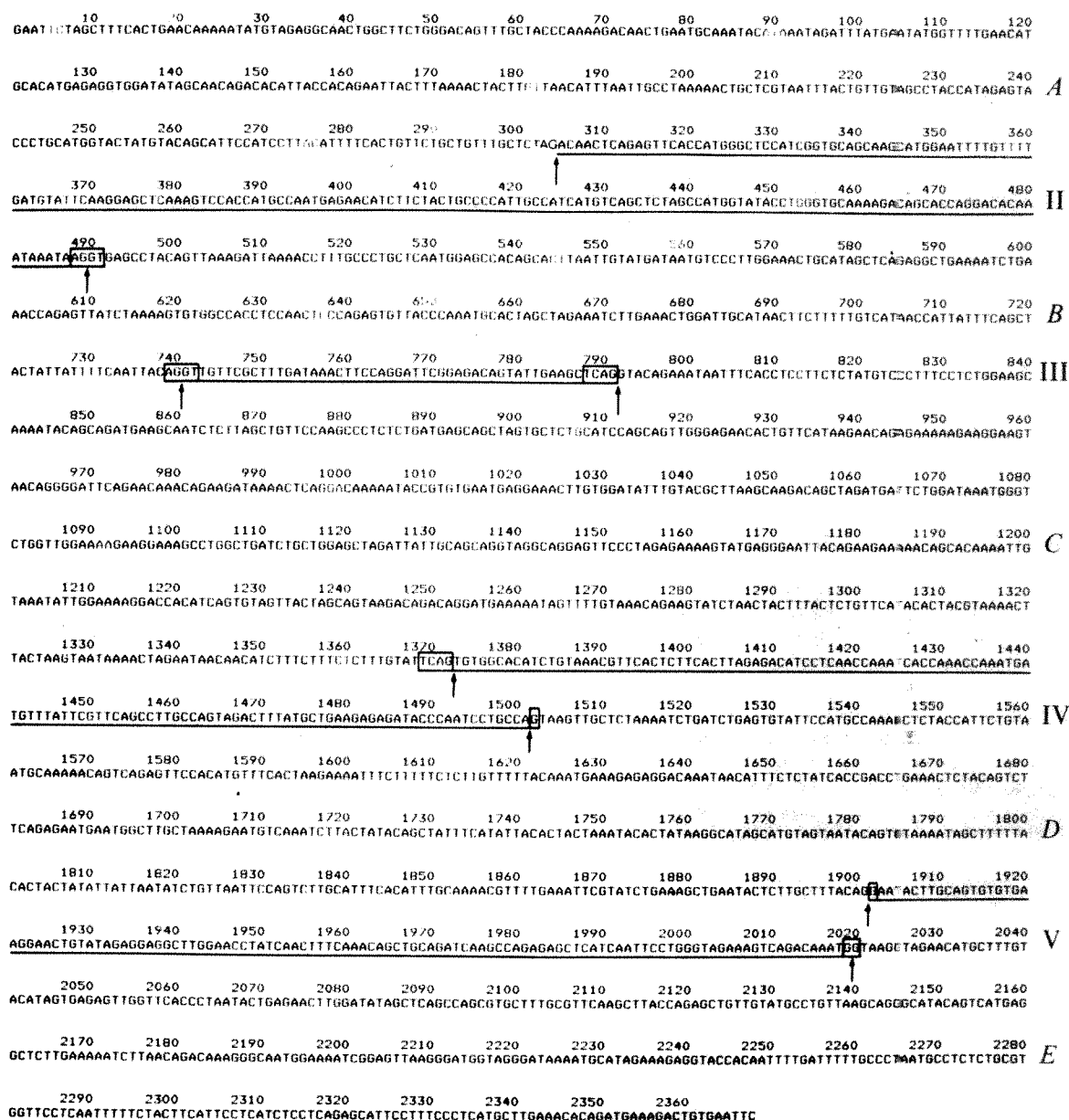


Fig. 3 Sequence of 2.4-kilobase fragment. The exons II–V and introns A–E are identified, as are the boxed regions showing terminal redundancy at each end of the introns. Arrows indicate the predicted splice points (see text).

Table 2 Simple parameters of the 2.4-kilobase sequence

Region (Fig. 3)	Length (no. of residues)	A + T%	CG (frequency)	AA (frequency)
A*	304	63	1	30
B	251	64	0	28
C	581	62	3	81
D	400	68	3	49
E*	349	59	1	31
II	185	54	1	16
III	51	58	2	3
IV	129	57	2	11
V	118	56	0	13

*Introns A and E are incomplete, so that the length and other parameters quoted here reflect only the proportion of these introns present in the 2.4-kilobase fragment.

used, only completely clear gel evidence was used to construct the sequence. A single arrow was invariably constructed from many independent gels.

The restriction enzymes *Mbo*I and *Eco*RII failed to cut the recombinant plasmid grown in *E. coli* RRI because of a methylation of the A residue (in the sequence GATC) or the second C residue (in the sequence CC(A/T)GG (ref. 28)). An anomalous degradation was also present in the sequencing gels, in that 6-methyl A gave a weaker band than an unmodified A in the alkaline degradation (alternate A) procedure²⁷. Similarly, 5-methyl C was resistant to hydrazinolysis, resulting in possible misreading of the gel¹⁹. Therefore, in all such cases of possible ambiguity the sequence was carefully checked on the opposite strand of the DNA.

Figure 3 shows the sequence of the 2,368 residues present in the 2.4-kilobase fragment, and Table 1 gives the position of most of the known restriction sites within this sequence. The exon sequences are underlined in Fig. 3 and were identified by their presence in the mRNA. It codes for 26% of the mRNA, from residue number 48 to 530, out of a total of 1,859 residues²⁸. No differences were found in these regions between the gene and the previously determined mRNA sequence²⁸, although about 80 residues of exon were not established independently (see legend to Fig. 2). Figure 3 indicates the previously noted terminal redundancy at each end of the introns B, C and D and the predicted splice points^{24,25}, either before or after a GT or AG doublet, depending on which end of the intron it is. A general summary of properties such as the length, base composition, expressed as % A+T, and frequency of CG doublets relative to AA doublets are given in Table 2. This shows that the three complete introns (B, C and D) vary by 251–581 residues in length. Exons are remarkably short; the smallest, exon III, is only 51 residues. The entire sequence is A + T rich, but is slightly higher for introns (varying by 62–68%) than for exons (54–58%). CG doublets occur very infrequently (compared with AA doublets) in the exons, as noted before²⁸, and also in all introns. No other doublet behaves like CG.

The main purpose of the sequence analysis of this part of the ovalbumin gene was to focus on introns. We have sequenced three introns completely and in all, nearly 1,900 residues of intron sequence are presented here. We had hoped to find specific primary sequence or secondary structures which might give an insight into the mechanism of RNA splicing. However, despite extensive computer analysis, we have been unable to find any convincing features. We will therefore defer presentation of this analysis until the full structure of the gene is known.

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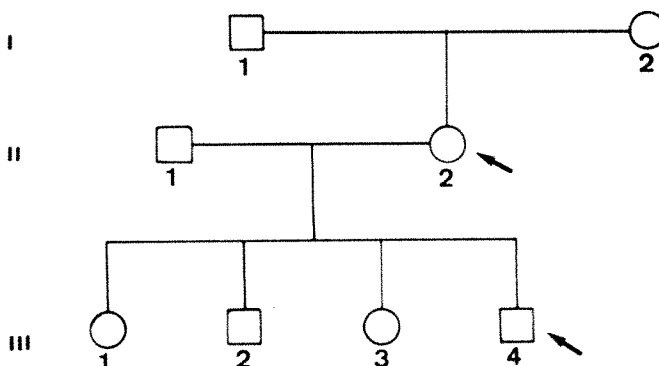
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Corrigendum

In the letter 'High frequency recombination in centromeric and histone regions of *Drosophila* genomes' by R. F. Grell, *Nature* **278**, 78–80 (1978), an additional reference should be included following the citation of refs 15 and 16: Goldring, Brutlag & Peacock in *Arrangement of the Highly Repeated DNA of Drosophila melanogaster* (eds Peacock & Brock) 47–59 (Australian National University Press, Canberra, 1975).

Errata

In the article 'Isotopes of tellurium, xenon and krypton in Allende meteorite retain record of nucleosynthesis' by R. V. Ballad *et al.*, *Nature* **277**, 615–620, paragraph 6 line 22 reads: 'Allende's spinels is...'. In paragraph 11, line 56 reads: '... Interference from the (n, 2n) reaction...'.
 In the letter 'Human chimaera detectable only by investigation of her progeny' by W. R. Mayr, V. Pausch and W. Schnedl, *Nature* **277**, 210–211, Fig. 1 was omitted, and is reproduced here.



matters arising

Solar modulation of atmospheric electrification and the Sun-weather relationship

MARKSON¹ has provided a useful summary of reasons for looking to atmospheric electrical structure for an explanation of 'Sun-weather' correlations, but the specific global and local mechanisms which he proposes do not, in the light of available evidence, apparently produce such effects frequently enough to explain the observations. Effects more directly related to magnetospheric and auroral phenomena would seem to show more promise in explaining the observed coupling of solar activity to lower atmosphere electrical phenomena.

The global effect proposed by Markson is that the 'Forbush decrease'² in cosmic ray intensity associated with solar flares results in a decrease of atmospheric ionisation with consequent effects on the 'global circuit'. Measurements made on a high mountain by Reiter³ have shown that the fair-weather electric field and current show 10–30% effects related to solar magnetic sector boundary crossings. Forbush measured decreases of the order of 1% at low latitudes and even at higher altitudes and latitudes, the effects rarely exceed a few per cent.⁴ As Markson points out atmospheric conductivity is expected to vary as the square root of the Forbush decrease, (ionisation rate) it is difficult to see how this mechanism could produce the observed effects, as decreases of the order of 50% in cosmic ray flux at low latitudes would be required.

The local effect proposed by Markson involves the direct penetration of ionising radiation effects to the tops of thunderclouds, (~20 km at mid and high latitudes). While this is perhaps possible in some rare solar proton (PCA) events⁵, such as the large flare of August, 1972, there is little evidence that substantial conductivity variations occur as low as this after more modest events. Yet balloon measurements at high latitudes^{6,7} typically show a 30% increase in the vertical field following geomagnetically disturbed periods. Thus it would seem that we must look for effects more directly related to magnetospheric and auroral processes to explain the reported data. One problem

would seem to be Markson's acceptance of the 'classical picture' of atmospheric electricity, where an 'equipotential surface is considered to exist at about 60 km', which does not allow the penetration of higher altitude electrical effects. The earliest rocket-borne conductivity measurements in 1950 (ref. 8) showed that the classical exponential increase of conductivity did not persist above 40 km, a result attributed to aerosol particle layers which has since been frequently confirmed⁹. Mühleisen⁷ has observed horizontal electric field gradients totalling ~100 kV between high and mid latitudes following magnetic disturbances, and Tyutin¹⁰ in the USSR has reported a 'permanent' mesospheric vertical field of similar magnitude. (We have tentatively confirmed the USSR measurements and suggested that the 'shorting out' of the vertical field by ionisation produced by aurorally related radiation might produce local changes in the lower atmosphere vertical field and also the observed horizontal potential differences, by adding the permanent mesospheric potential to the lower atmosphere circuit at high latitudes¹¹.) These measurements clearly demonstrate the invalidity of the classical picture for studying many possible coupling mechanisms.

Other promising avenues of investigation would seem to be the direct 'mapping' of ionospheric fields to low altitudes¹² and of aurorally produced charge separation¹³ inducing displacement currents and electric fields in the lower atmosphere. Magnetospheric and auroral phenomena would seem to have a chance of explaining the 22-yr 'magnetic cycle' Sun-weather effects cited by Markson, because of the likelihood that they could be influenced by the details of the solar magnetic field structure. An intermediate mesospheric coupling circuit modulating the lower atmosphere circuit would have the attractiveness of providing a mechanism responsive to auroral particles or bremsstrahlung rather than requiring rare high intensity events, and could thereby provide a more prevalent coupling effect due to the much greater frequency of such events.

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MARKSON REPLIES—Hale's objection may be summarised as: (1) he does not believe that the requisite changes in ionisation occur; nor (2) that the classical picture of atmospheric electricity is valid. He is inaccurate in his quotation and citation, and there is some question regarding his interpretation of data and their significance.

I wish to point out the following: conductivity variations occur sufficiently low in the atmosphere and frequently enough to be a significant factor in solar modulation of current flow in the global circuit. Hale refers to relatively small changes in the galactic component of cosmic radiation measured on the ground which are not representative of the much larger variations of ionising radiation in the lower stratosphere. Winckler¹, in summarising his balloon program in which cosmic rays were measured at mid-latitude (Minneapolis) states "Probably the single most significant finding of the entire series (of flights) was the frequent occurrence of intense low energy cosmic rays. They originated from large solar flares but had energy spectrum so steep that the particles were not detected by the extensive network of sea-level cosmic ray monitors established during IGY. The particles were measured directly, however, by means of balloons at altitudes greater than 20 or 25 km". Not only are solar controlled changes in ion production larger and more frequent in the 20–30 km height range compared to near the Earth, they generally are of the opposite sense, increases instead of decreases^{1–3}. Akasofu and Chapman⁴ (Hale's reference) discuss three Forbush decreases in a 16-day period which resulted in a cumulative 20% reduction in cosmic ray rate at the

Earth's surface. During this interval, Winkler's balloons³ recorded 2–10 fold increases in ion production rate at 24 km, half an electrical scale height above 20 km. Such events, if they were over 20 km high thunderstorms, would at least double the current flow from them. There is also a long period variation in cosmic radiation which is inversely correlated with the sunspot cycle⁵. My Fig. 1, averaged data from Neher and Anderson⁶, illustrated that through a solar cycle at 15 km (100 g cm^{-2}) ionisation increases 51% (conductivity 23%) while at 20 km (50 g cm^{-2}) ionisation increases 76% (conductivity 33%). While these data are for high latitude, Fig. 4 in my article⁷ depicts the variation with latitude of ion production at 20 km. At mid-latitude the conductivity variation through a solar cycle is $\sim 18\%$, near the Equator it is $\sim 10\%$. As these are average values, larger variations must occur at times.

Changes in fair-weather electric field intensity (a measure of ionospheric potential) related to solar sector crossings⁸ does not imply that the electric field is controlled through geomagnetic processes. However, thunderstorms (which maintain the ionospheric potential) have been found to vary with solar sector crossings^{9–11}.

Almost all students of atmospheric electricity accept the general premises of the classical global circuit concept because there is strong evidence supporting this view^{12–15}. As fair-weather electric fields vary simultaneously all over the world and correlate with the universal time variation of global thunderstorm activity^{16,17}, even in the polar cap region¹⁸, upper atmosphere generators (which follow local time) can not be the cause of the ionospheric potential. In addition, the latter would not vary simultaneously over large distances¹⁷ unless the ionosphere had the characteristics of an equipotential surface. It is well known that potential differences can exist across the auroral oval; this does not invalidate the classical picture which is necessary to explain the maintenance and characteristics of the Earth's electric field at sub-auroral latitudes, that is over 85% of the Earth's surface (including most of the portion with thunderstorms). Satellites that record 70–200 kV across the auroral oval do not measure significant potential differences between the auroral zone and mid- to low-latitudes (R. G. Roble, personal communication). Magnetospheric electric fields impressed in the polar ionosphere are attenuated to 10% of their high-latitude value by mid-latitude¹⁹.

The proposed mechanism⁷ does not depend on 'Forbush decreases', this is a generic term used to describe decreases in galactic cosmic radiation following some solar flares. Conductivity, of course, does not vary as the square root of Forbush decreases.

Hale erroneously believes the proposed

mechanism requires a 50% decrease in cosmic ray flux at low latitudes although my article discusses effects on thunderstorm currents at all latitudes and does not mention a 50% decrease in cosmic rays. Perhaps he is referring to the increase in ionising radiations causing a 50% decrease in columnar resistance over the 'global generator' that I used for arithmetic simplicity to illustrate the sensitivity of the entire global circuit to conductivity changes within the small volume over thunderstorms.

The average ground level Forbush decrease is 5% (with a maximum of 20%), not the 1% implied by Hale²⁰.

While the August 1972 flare was unusual in having a very high number density of low to moderate energy particles, numerous other flares have had a larger density of particles in the high energy end of the spectrum²¹. The latter particles penetrate deepest into the atmosphere and cause the effects under discussion.

Ionising radiation does not have to reach the tops of thunderclouds to increase the current flow from them to the ionosphere; it only has to lower the total columnar resistance over the clouds. If, however, ionising radiation did reach the cloudtops, cloud electrification might be enhanced²². Increased ionisation following solar flares has been reported in the 15–20 km height range^{2,3,23}.

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

Tyutin's rocket measurements²⁴ (evidently similar to ones made by Hale) indicate that the fair-weather field reverses above 10 km. It is surprising that Hale accepts such data which are contrary to fundamental physical principles. As the vertical air–Earth current is unidirectional from the ionosphere to Earth, electric fields at all heights must be of the same sign.

Concerning aerosol layers above 40 km, such conditions would have little effect on atmospheric electricity in the

lower atmosphere unless the columnar resistance through this upper atmospheric region were a significant fraction of the columnar resistance through the lower atmosphere. There are no measurements indicating that such conditions exist.

Hale cites Akasofu and Chapman⁴ as saying that even at high altitudes and latitudes Forbush decreases rarely exceed a few per cent. This statement is not only erroneous^{1–3}, but it does not appear in the book. Hale's ref. 13 is a paper by Cole and Pierce, not by Freier.

An attempt to explain solar–weather relationships as due to ionospheric and magnetospheric electrical processes is understandable, however, these do not seem applicable to the problem. Upper atmospheric currents and particles would introduce energy into the atmosphere orders of magnitude too small to affect weather processes^{25,26}. They would be important to the extent they influenced thunderstorm currents and cloud electrification in the lower atmosphere⁷.

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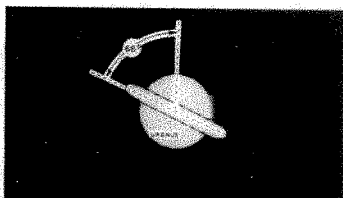
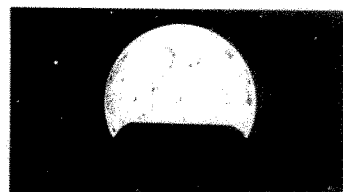
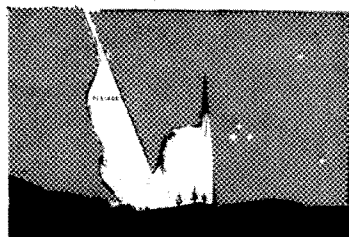
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reviews

Exploration of the Solar System

J. Veverka

The Realm of the Terrestrial Planets.
By Z. Kopal. Pp. 223. (The Institute
of Physics: Bristol, UK, 1979.) £7.50.

THIS book is described as a completely up-to-date account of recent advances in the exploration of the Solar System. Written by a well known astronomer who has made important contributions to our understanding of the Moon and the Solar System, the book is well illustrated and pleasant to read. Unfortunately, it abounds in factual errors. From the nature of most of these, it is clear that the author is writing about the Solar System based on information available, for the most part, a decade ago. It is certainly not an up-to-date account of our current understanding of the terrestrial planets, and is especially dangerous to the initiated reader who has no way of distinguishing fact from error.

The factual errors are too numerous to list in detail. For example, in one single chapter, which consists of 39 pages, there are more than a dozen serious factual errors, not to mention an equally large number of potentially misleading and inaccurate statements. For instance, there is much confusion about the polar caps of Mars. Even though recent Viking results are alluded to, no distinction is made between the annual CO₂ frost caps which form every year and disappear during spring and summer, and the permanent (or residual) polar caps, which survive through the Martian summer. In fact, on page 114, we find a restatement of the old misconception that the southern polar cap of Mars vanishes completely on occasion during summer. It is now known that, although the annual cap is composed of carbon dioxide, the residual caps consist of vast deposits of frozen water, a fact apparently unknown to the author. He is unaware of the fact that even the annual caps can have substantial thickness in places because we read on page 119 that the snowfields in the Martian polar regions must be "less than a few centimetres deep", although numerous studies in recent years have shown that several metres of frost can be deposited in the polar regions during Martian winter.

On page 124, we find further evidence of a thorough misunderstanding of Viking data. It is stated the

Martian sky appears pink in daytime, but that it is blue near sunset and sunrise. There is no evidence to support such an assertion, or the claim that the atmosphere is dust free after sunrise but becomes dusty as the sun rises higher and convection begins to lift up surface dust. Published analyses of the Viking Lander data by Pollack and others clearly demonstrate that the dust does not settle out of the atmosphere daily, but remains suspended for periods on the order of the Martian year. There is no evidence in the Viking data to substantiate the statement made on page 125 that a significant number of dust particles are stirred and lifted into the atmosphere by diurnal convection.

On the same page, there is further evidence that the author has not kept abreast of Mars studies in the last seven years. He states that:

"While the bright markings were identified correctly with arid desert plains and their colour ascribed to that of ferric oxides long before the Viking Landers provided the final proof, the dark markings were thought to owe their lower reflectivity to moisture and even to vegetation flourishing in the spring and watered by systems of canals, natural or artificial from the melting polar caps. Their seasonal variations were regarded as indications that at least plant life existed on this planetary neighbour of ours. These expectations were destined to total disappointment by the outcome of the Viking missions."

This is an astounding statement! Soon after the first Mariner 9 pictures were analysed in 1971-72, it became clear that there is no vegetation in the dark areas of Mars, and that seasonal variations are due to the redistribution of dust, by Martian winds. Similarly, at about the same time, Earth-based telescopic measurements by McCord and others clearly demonstrated that the dark areas of Mars are dark because they are less oxidised than the bright areas. No one in the last seven years has proposed seriously that the dark areas are dark either because of moisture, or because of vegetation.

There are several indications that Kopal does not believe or is not convinced that the large structures such as Olympus Mons are really volcanoes. Although one could sympathise with such a cautious attitude, it is much more difficult to take kindly unsupported statements such as that the

whole of Olympus Mons is not more than 10 Myr old.

Perhaps the most colossal blunder in the entire chapter involves sinuous channels. Kopal shows a Mariner 9 picture of some faults on Mars which have never been called 'sinuous channels' by anyone, except Kopal, and have never been attributed to erosions by liquid water by anyone. On the following page, he shows an example of similar features on the Moon and writes, "Note the striking similarity between these lunar features and those on Mars as shown in the previous plate. The origin of these features on the Moon has nothing whatever to do with water". Kopal cautions the reader "against jumping to conclusions about erosion caused by flowing water. For whatever may be a merit of such a suggestion as regards to Mars, it certainly would not apply to the Moon". The whole argument is embarrassing. Nowhere does Kopal show a real Martian sinuous channel. Of course, linear faults were not produced by flowing water.

One should not get the impression that the Mars discussion is the only one marred by serious errors. For example, the discussion of the Mars satellites, Phobos and Deimos, covers only five pages, but contains at least four serious errors. The largest crater on Phobos is not 3 km across; Phobos is not the darkest body in the Solar System: grooves on Phobos most likely do not represent the effects of "cosmic abrasion"; and it is certainly not true that such grooves are "also characteristic of certain parts of the Moon".

There is little point in picking apart other chapters in similar detail—most contain similar serious factual errors and misconceptions.

The Realm of the Terrestrial Planets, although a superficially attractive, well illustrated book, is too full of errors to be recommended to any reader, except as a record of what a noted astronomer thinks the inner Solar System might be like, based mostly on data generally available about ten years ago. It could have been a much more important book if it were really based on current information. □

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Koobi Fora hominids

The Fossil Hominids and an Introduction to Their Context, 1968-1974. (Koobi Fora Research Project, Vol. 1). Edited by M. G. Leakey and R. E. Leakey. Pp. 191. (Clarendon/Oxford University Press: Oxford, 1978.) £15.

THIS is the initial volume of a projected series of monographs on interdisciplinary earth sciences and palaeoanthropological researches in the Koobi Fora area, northeastern Turkana basin, Kenya. That combined research effort unquestionably represents one of the most lastingly important and fruitful investigations into the earlier evolutionary history of Hominidae and the natural setting and biotic associations of Plio-Pleistocene times. This volume provides an historical and methodological introduction (R. E. Leakey); followed by chapters on geology, palaeontology, archaeology and fossil hominids.

A summary of the lithostratigraphy, sedimentary environments and palaeogeography of the Kubi Algi and Koobi Fora formations is provided by I. C. Findlater. This chapter sets out the stratigraphical nomenclature now used for these formations, the members of which are defined on the basis of several spatially extensive tephra. Five principal sedimentary environments are recognised, two lacustrine and three alluvial; and their areal relationships are shown diagrammatically. Eight generalised maps depict palaeogeographical settings of the area of successive temporal intervals during the accumulation of the Koobi Fora Formation. Maps show the locations of the principal hominid finds in the major fossiliferous areas; their temporal relationships and environments of deposition are indicated in stratigraphical section and usefully summarised in a table.

An annotated inventory (and listing) of 87 mammal taxa (exclusive of micromammals) in those formations, as well as mollusc assemblages and their concurrent range zones, and notes on other vertebrate groups is given by J. M. Harris. The fauna of the Kubi Algi Formation is not specified, but is stated to overlap in part that of the (lower) portion of the Koobi Fora Formation. The Ileret, Koobi Fora and Kubi Algi sectors have been each subdivided into a series of numbered areas (19 in the former two, 5 in the latter) for purposes of geological mapping and fossil collection. Tephra deposits are widespread, some demonstrably

spatially extensive and useful in correlation. Certain (named) tuffs serve as marker horizons to differentiate and to subdivide the several formations; however, the relationships, spatially and temporally, of many other (numbered) tuffs are uncertain. Three fossil 'collection units' are now recognised for the Kubi Algi Formation, and five such 'units' for the Koobi Fora Formation, the base of each being defined in a particular area by a named or numbered tuff. However, in the Koobi Fora Formation just over half of all fossiliferous areas in the Ileret sector and nearly half of all such areas in the Koobi Fora sector have lithostratigraphically undefined lower boundaries. Thus, the 'collection unit' concept seems to have limited utility at best, as it is an unfortunate mix of both lithostratigraphy and biostratigraphy.

An overview of the stratigraphical and areal situation, depositional environments and associations, and nature of all major archaeological occurrences, and their content and industrial characteristics is provided by G. L. Isaac and J. W. K. Harris. The

remainder of the volume comprises a well-illustrated inventory, with data on finder, provenience and depositional environment (including many microstratigraphic sections) of all fossil hominids (numbering 129) recovered in the seven years of the project, until 1974 (R. E. and M. G. Leakey and A. K. Behrensmeyer), complemented by an appendix of 30 tables of assorted measurements of those specimens. The scale on some hominid illustrations is unfortunately incorrect. Hominid taxonomy is only briefly mentioned (p89); but not many workers personally familiar with this vast corpus of fossil material will agree with all the attributions suggested. Finally, there is a full bibliography (through 1976) of the project.

This is an immensely useful, well executed and well produced volume of major import to all students of human evolution.

F. Clark Howell

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Coastal sedimentation

Coastal Sedimentary Environments. Edited by Richard A. Davis, Jr. (Springer: New York and Berlin, 1978.) \$22; DM44.

THIS book is intended as a student text and to provide a background on coastal environments for geologists, engineers, oceanographers and coastal managers. Being a multi-author text means that there are certain differences of approach between the chapters; however, this is offset by the obvious expertise that has been brought to bear. The emphasis is on geological description of the morphological features and of the processes that have been interpreted largely from changes in the morphology and from the analysis of internal structures. The chapters have a review style and frequently, to provide an explanation satisfactory for students, would necessitate lectures tailored to the book, or the student to follow up extensively the quoted papers. An example of this is the very brief coverage of longshore transport, which in itself would warrant a chapter in a more specialist book. The bibliographies are reasonably comprehensive and there are abundant and very good photographs, especially of tidal deltas.

There are chapters on river deltas by L. D. Wright, which particularly concentrates on the processes in the Mississippi delta, and on coastal bays by R. B. Biggs, which actually emphasises suspended sediment in estuaries. There is a particularly comprehensive description of American East Coast salt marshes by R. W. Frey and P. B. Basan, and coastal dunes are described by V. Goldsmith. R. A. Davis describes the beach and nearshore zone, and mesotidal inlets and estuaries are covered by J. C. Boothroyd. The latter concentrates largely on tidal deltas. The final two chapters are by J. C. Kraft on the coastal stratigraphic sequences produced mainly by transgression, and by W. T. Fox on modelling coastal environments.

Virtually all of the examples quoted are drawn from the American East or Gulf Coasts. Consequently the extensive body of European literature is largely neglected, together with such features as high range tidal estuaries and coasts. Nevertheless, this will be a useful addition to texts on coastal sedimentation and should be recommended reading on courses covering coastal geomorphology and sedimentology, as well as providing useful background in a wider range of disciplines.

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Cloud physics

Microphysics of Clouds and Precipitation. By H. R. Pruppacher and J. D. Klett. Pp.706. (D. Reidel: Dordrecht, The Netherlands, 1978.) Dfl. 85; \$39.

DURING the past decade the major emphasis in cloud physics has been directed away from fundamental microphysical processes—which had hitherto largely dominated the evolution of the subject, and in which progress had been based primarily upon laboratory investigation—to field investigations of larger-scale effects, in which the cloud dynamics are recognised to play a central role. This change of approach has been dictated in part by the development of superior experimental techniques—radar, optical particle samplers, high-speed data processing systems, and so on—and in part by the recognition that the major obstacle to progress has been the absence of comprehensive and reliable observational data. Accordingly, the publication of a textbook devoted exclusively to microphysical processes might appear at first sight to be somewhat anachronistic. In fact, however, I feel that this comprehensive and rigorous critical analysis of current understanding of the physics of the multitude of processes which form the basis of the subject, will be of invaluable assistance to research workers. The classic text in this field for two decades has been Mason's massively authoritative *The Physics of Clouds* (Clarendon Press, Oxford, 1957) but an enormous amount of material has been published since the second edition of this work appeared in 1971, and the small number of generally excellent monographs that have been produced in the intervening years have been insufficiently detailed to take full account of this new work.

The range, tenor and emphasis of the book can readily be appreciated by studying the titles of the chapters which follow the short historical review: microstructure of atmospheric clouds and precipitation (the only chapter concerned predominantly with field observations); the structure of water substance; equilibrium between water vapour, water, aqueous solutions and ice in bulk; surface properties of water substance; equilibrium behaviour of cloud drops and ice particles; homogeneous nucleation; the atmospheric aerosol; heterogeneous nucleation; hydrodynamics of single cloud and precipitation particles; cooling of moist air; mechanics of the atmospheric aerosol; diffusion growth and evaporation of water drops and ice crystals; cloud particle interactions—collision coalescence and breakup; growth of cloud drops by collision and coalescence;

microphysics of ice particle-drop interactions; the electrical state of the atmosphere and its effect on cloud microphysics. In addition, there is a series of useful, mathematically rigorous and sometimes novel appendixes, on more specialised topics.

A feature of this book is the consistent attempt to provide a critical appraisal of published work. In general, these analyses are penetrating and balanced. Another impressive aspect is the bibliography which contains about 2,000 papers, concerning research carried out up to 1978.

A disadvantage of the analytical, microphysical approach adopted by the authors is that although individual topics are critically examined a broader overview of the status of areas of cloud physics is not consistently presented.

The emphasis on the physics rather than the dynamics and raw data is a deliberate and defensible policy which, however, is not uniformly successful. For example, the important research leading us towards a solution of the problem of thunderstorm electrification has been in the field—the laboratory and theoretical studies on which the authors have concentrated attention are of secondary consequence. These limitations—if valid—are minor, however, and there is little doubt that this impressive book will be of central importance to cloud physicists and scientists working in related fields.

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Inorganic analysis

Vogel's Textbook of Quantitative Inorganic Analysis. Fourth edition. Revised by J. Bassett, R. C. Denney, G. H. Jeffery and J. Mendham. Pp.925. (Longman: London and New York, 1978.) £14.

This fourth edition of 'Vogel' by four members of the late Dr Vogel's staff is considerably different from the previous versions that served as the standard textbook of quantitative inorganic analysis for several generations of British chemists. It uses SI units throughout, but in line with the recommendations of the Analytical Division of IUPAC it retains the concept of 'the equivalent' and the 'normal' solution, where it is more convenient and less ambiguous so to do. This is sound common sense in an era that spans the old and the new systems of nomenclature, and it serves the needs of a wide range of chemists and of industrial and academic requirements. The authors are to be congratulated on their pragmatic approach which may, of course, be denigrated by the pundits on either side, but which is eminently sensible at the present time.

Inevitably the new edition shows signs of fairly drastic pruning in the area of classical gravimetric and titrimetric analysis in the sense that the number of alternative classical procedures has been considerably reduced. The methods that have been retained are generally, in the reviewer's opinion, those that are most useful both on a practical analytical basis and for the purpose of training the next generation of students how to undertake

the elements of classical analysis that are still much used in industry and are likely to be required for many years to come. The decision to expand the section on sampling procedures is doubly welcome as all too many textbooks ignore this vital function without which most analyses are virtually pointless.

A new chapter has been devoted to thermoanalytical procedures and the one on infrared analysis which featured in the third edition has been deleted. This has allowed considerable expansion in other areas of spectrochemistry, for example, atomic absorption and emission spectrography, and a new chapter has been added on gas chromatography. The new arrangement of the material in the section on electroanalytical chemistry is also a considerable improvement on the previous edition. There have also been some changes in the extremely useful appendixes that have always characterised Vogel, for example, the omission of 'chemical factors' and the little used five-figure logarithms, though the four-figure tables are still there to help the chemist with his pH calculations.

Taken all round Vogel is still an excellent book and, in this reviewer's opinion at least, the authors have got the balance right. It contains a very useful blend of the best of classical and neo-classical techniques with the basis of many modern instrumental techniques set out in a way that the student can assimilate readily and follow subsequently with the selected list of references and recommendations for more advanced or detailed reading. The balance between theoretical background and laboratory practice is probably much better than it was even in the previous editions. The book is neatly printed and set out, there are

many useful illustrations and not too many 'commercial' pictures and line diagrams. A mine of information? Yes, but also a very interesting and stimulating one for all who have the opportunity to quarry therein. Unquestionably this is one of the best books available in this area. I only wish the

publishers had had more faith in its success to reduce the price and thus allow a greater audience of students to expose themselves to having their own copy.

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Condensed state helium

The Physics of Liquid and Solid Helium. Part II. Edited by K. H. Bennemann and J. B. Ketterson. Pp.750. (Wiley: New York and Chichester, UK, 1978.) £45.85.

THE helium atom probably comes closer than any other to the miniature "billiard ball" of classical kinetic theory. One might expect, therefore, that the physics of liquid and solid helium would be exceptionally simple and even—perhaps, rather dull. The reality is far otherwise, thanks mainly to the profound influence of quantum statistics: a collection of these small, rigid spheres displays a quite unparalleled richness and variety of physical phenomena some of which, such as bulk superfluidity, are unique on earth (although they may, perhaps, also manifest themselves within the nucleonic fluids in the interiors of dense stars).

In the 1960s it was still possible (just) for a single author to prepare a research monograph providing a reasonable coverage of virtually everything that was known about condensed state helium; but, such has been the expansion of knowledge over the intervening years, this is hardly the case any more in the late 1970s. The admirable texts by Wilks (*The Properties of Liquid and Solid Helium*; Clarendon: Oxford, 1967) and by Keller (*Helium-3 and Helium-4*; Plenum: New York, 1969) are thus almost certainly the last of their kind. In presenting the fruits of more recent researches, Bennemann and Ketterson have very sensibly chosen to solicit individual chapters from a selection of experts. Although inevitably lacking the coherence, internal self-consistency and the helpful cross-referencing associated with a single-author monograph, the result is a collection of weighty, authoritative and, on the whole, very interesting review articles.

This second volume is strongly biased towards helium-3 which is, of course, entirely appropriate given the enormous progress made towards elucidating the properties of its super-

fluid phases following their discovery in 1972. The first three chapters are tough going, relatively speaking, being mostly devoted to a formal development of the theory: Baym and Pethick deal with the theory of normal (non-superfluid) liquid ^3He and with ^3He - ^4He solutions; Anderson and Brinkman cover anisotropic superfluidity in liquid ^3He . Most graduate students would be well advised to start instead on chapter 4, by Lee and Richardson. Running to more than 200 pages their contribution is virtually a book in its own right. They explain and discuss in considerable detail almost all the experimental work carried out on superfluid ^3He up to a cut-off date in mid 1976, conveying in an admirable way the relationship between the experiments

and the growing theoretical structure.

The remaining chapters are devoted to articles on three very disparate topics: by Dash and Schick on two-dimensional helium, concentrating, in particular, on the recent fascinating investigations of helium adsorbed on grafoil substrates; by Brewer on multilayer helium films in restricted geometries; and by Price on neutron scattering from helium. Each of these is excellent in its own way and, like the articles on ^3He , they should be a real help both to people entering, as well as to those already working in, the field in question.

The quality of production is high, as indeed it should be, considering the price. Very few private individuals will be able to afford personal copies; but the book provides a substantial and probably lasting contribution to the literature on condensed state helium and, as such, it will obviously be an essential purchase for libraries.

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Bile salts

The Biological Importance of Bile Salts. By G. A. D. Haslewood. Pp.206. (North-Holland: Amsterdam, New York and Oxford, 1978.) \$55.75; Dfl.125.

THE interest in bile salts of the author of this useful publication is of very long standing. However, the author points out in the preface that this volume does not represent a second edition of his earlier publication entitled *Bile Salts* (1967). As remarked later in the present book, its purpose is to put the reader into the biological picture (physiological, biochemical, taxonomic and medical) regarding bile salts.

How can such a slim volume, with only some 200 pages, expect to achieve this aim? Professor Haslewood has succeeded by being selective. The book does not, for example, deal in any detail with the chemistry of bile salts, and the references (up to 1978) are described as a personal choice selected partly for their general interest. The author considers that, in these times of computer storage and retrieval, completeness seems an unnecessary aim and may make reading difficult and dull. Several chapters therefore commence by providing appropriate documentation to comprehensive earlier sources for background information.

The monograph has been produced to a high standard, with few printer's errors. It has five main chapters, of which the longest reflects the author's interest in the distribution of bile salts in the animal kingdom—for example, "Thus, crocodilian bile is quite primitive and generalised: a halt on the main route of bile salt evolution". The other chapters deal with the nature and function of bile, chemistry and methods of separation and analysis of bile salts, their biosynthesis and artefacts of the enterohepatic circulation, and their medical importance.

An Appendix contains 16 pages of tabulated data consistent with the intention that, throughout their studies, the author and his colleagues have tried to publish records of all bile salts discovered. The possible medical relevance of such compilations is shown by the observation (p189) that the tree "shrew" has advantages as a laboratory animal since this primate has bile much more like man than other small laboratory species, and can easily be induced to form cholesterol gallstones.

This monograph will thus be useful to many workers concerned with a number of different aspects of bile salts in biology and medicine.

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Recent scientific and technical books

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- RACHMAN, S. J. Fear and Courage. (A Series of Books in Psychology.) Pp.ix + 319. ISBN-0-7167-0087-5. (San Francisco and Reading: W. H. Freeman and Company, 1978.) Hardcover £8.90; Softcover £3.50.
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- SINGER, Jerome L., and POPE, Kenneth S. (edited by). The Power of Human Imagination: New Methods in Psychotherapy. (Emotions, Personality, and Psychotherapy.) Pp.xx + 405. ISBN-0-306-31140-2. (New York and London: Plenum Press, 1978.) \$15.75.
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- WENDLAND, W. L. Elliptic Systems in the Plane. (Monographs and Studies in Mathematics, 3.) Pp.xi+404. ISBN-0-273-01013-1. (London, San Francisco and Melbourne: Pitman, 1978.) £28.
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- COLLINS, H. George W. The Virial Theorem in Stellar Astrophysics. (Astronomy and Astrophysics Series.) Pp.viii+135. ISBN-0-912918-13-6. (Tucson, Arizona: Pachart Publishing House, 1978.) np.
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- HODGE, Paul W., and WRIGHT, Frances W. The Small Magellanic Cloud. Pp.ix+69+atlases. ISBN-0-295-95387-X. (Seattle and London: University of Washington Press, 1977.) £35.
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- ZHARKOV, V. N., and TRUBITSYN, V. P. Physics of Planetary Interiors. Translated, edited, and with additional material by W. B. Hubbard. (Astronomy and Astrophysics Series, Vol. 6.) Pp.xi+388. ISBN-0-912918-15-2. (Tucson, Arizona: Pachart Publishing House, 1978.) np.

Physics

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- BALTES, H. P. (edited by). With contributions by H. P. Baltes *et al.* Inverse Source Problems in Optics. (Topics in Current Physics, Vol. 9.) Pp.xi+204. ISBN-3-540-09021-5. (Berlin and New York: Springer-Verlag, 1978.) DM 45; \$24.80.
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- BISHOP, A. R., and SCHNEIDER, T. (edited by). Solitons and Condensed Matter Physics. (Proceedings of the Symposium on Nonlinear (Soliton) Structure and Dynamics in Condensed Matter, Oxford, England, June 27-29, 1978.) (Springer Series in Solid-State Sciences, Vol. 8.) Pp.xi+341. ISBN-3-540-09138-6. (Berlin and New York: Springer-Verlag, 1978.) DM 59; \$32.50.
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- DUPUY, J., and DIANOUX, A. J. (edited by). Microscopic Structure and Dynamics of Liquids. (NATO Advanced Study Institutes Series. Series B: Physics, Vol. 33.) Pp.ix+524. ISBN-0-306-35733-X. (New York and London: Plenum Press, 1978.) £31.18.
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- HALLEY, J. Woods (edited by). Correlation Functions and Quasiparticle Interactions in Condensed Matter. (NATO Advanced Study Institutes Series. Series B: Physics, Vol. 35.) Pp.xi+665. ISBN-0-306-40018-9. (New York and London: Plenum Press, 1978. Published in co-operation with NATO Scientific Affairs Division.) £37.48.
- HAYES, William, and LOUDON, Rodney. Scattering of Light by Crystals. Pp.x+360. ISBN-0-471-03191-7. (New York and Chichester: Wiley-Interscience, John Wiley and Sons, 1978.) £19.50; \$38.
- KALMAN, Gabor, (edited by). CARINI, Paul (assistant editor). Strongly Coupled Plasmas. (NATO Advanced Study Institutes Series. Series B: Physics, Vol. 36.) Pp.x+656. ISBN-0-306-40039-1. (New York and London: Plenum Press, 1978. Published in co-operation with NATO Scientific Affairs Division.) £37.48.
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- ROSENBERG, H. M. The Solid State: An Introduction to the Physics of Crystals for Students of Physics, Materials Science, and Engineering. (Oxford Physics Series.) Pp.274. ISBN-0-19-851844-7. (Oxford: Clarendon Press; London: Oxford University Press, 1978.) Boards £5.50 net; Paper £2.95 net.
- SMITH, R. A. Semiconductors. Second edition. Pp.xiii+523. (Cambridge, London and New York: Cambridge University Press, 1978.) Hardcover £27.50; Paperback £8.95.
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Chemistry

- ABRAHAM, R. J. (senior reporter). Nuclear Magnetic Resonance, Vol. 7. (A Review of the Literature published between June 1976 and May 1977. A Specialist Periodical Report.) Pp.xxvi+361. ISBN-0-85186-312-4. (London: The Chemical Society, 1978. Obtainable from The Chemical Society, Blackhorse Road, Letchworth, Herts; American Chemical Society, 1155 Sixteenth Street, N.W., Washington, DC.) £25; \$55.
- AERSTIN, Frank and STREET, Gary. Applied Chemical Process Design. Pp.xvii+294. ISBN-0-306-31088-0. (New York and London: Plenum Press, 1978.) £15.75.
- BARD, Allen K. (executive editor). In association with LUND, Henning. Encyclopedia of Electrochemistry of the Elements: Organic Section, Vol. 12: Carbonyl Compounds, Carboxylic Acids, Esters, and Anhydrides, Organic Sulfur Compounds. Pp.xii+512. ISBN-0-8247-2512-3. (New York and Basel: Marcel Dekker, Inc., 1978.) \$88.
- BUCKINGHAM, A. D., LIPPERT, E., and BRATOS, S. (edited by). Organic Liquids: Structure, Dynamics, and Chemical Properties. Pp.x+352. ISBN-0-471-99673-4. (Chichester and New York: Wiley-Interscience, John Wiley and Sons, 1978.) £18.
- CARNDUFF, John. An Introduction to Organic Chemistry. Pp.xiii+194. ISBN-0-471-99647-5. (Chichester and New York: John Wiley and Sons, 1978.) £8.50.
- CHAPMAN, N. B., and SHORTER, J. (edited by). Correlation Analysis in Chemistry: Recent Advances. Pp.xiii+546. (New York and London: Plenum Press, 1978.) £31.18.
- CLEANING OUR ENVIRONMENT: a Chemical Perspective. (A Report by the Committee on Environmental Improvement.) Pp.ix+457. (Washington, DC: American Chemical Society, 1978.) \$9.50.

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- GINELL, Robert. Association Theory: The Phases of Matter and Their Transformations. (Studies in Physical and Theoretical Chemistry, 1.) Pp.xiii+224. ISBN-0-444-41753-2. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1979.) Dfl. 97; \$43.

Earth sciences

- BACH, Wilfrid, PANKRATH, Jürgen, and KELLOGG, William (edited by). Man's Impact on Climate. (Proceedings of an International Conference held in Berlin, June 14-16, 1978.) (Developments in Atmospheric Science, 10.) Pp.xxiii+327. ISBN-0-444-41766-4. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1978.) Dfl. 120; \$53.25.
- BOGLI, Alfred. Karsthydrographie und Physische Speläologie. Pp.xii+292. ISBN-3-540-09015-0. (Berlin and New York: Springer-Verlag, 1978.) DM 58; \$31.90.
- HERSCHY, R. W. (edited by). Hydrometry: Principles and Practices. Pp.xi+511. ISBN-0-471-99649-1. (Chichester and New York: Wiley-Interscience, John Wiley and Sons, 1978.) £24.
- HUSAR, R. B., LODGE, Jr., J. P., and MOORE, D. J. (edited by). Sulfur in the Sulfur in the Atmosphere. (Proceedings of the International Symposium held in Durbrovnik, Yugoslavia, 7-14 September 1977.) Pp.816. ISBN-0-08-022932-8. (Oxford and New York: Pergamon Press, 1978.) £15.
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- ROBERTS, P. H., and SOWARD, A. M. (edited by). Rotating Fluids in Geophysics. Pp.xvii+551. ISBN-0-12-589650-6. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) £17.50; \$36.25.
- SELLEY, Richard C. Ancient Sedimentary Environments and Their Sub-surface Diagnosis. Second edition. Pp.287. ISBN-0-412-14750-5. (Science Paperbacks.) (London: Chapman and Hall, Ltd., 1978.) £4.95 net.

Biology

- AKOYUNOGLU, G., and ARGYROUDI-AKOYUNOGLU, J. H. (edited by). Chloroplast Development. (Proceedings of the International Symposium on Chloroplast Development held on the Island of Spetsai, Greece, July 9-15, 1978.) Pp.xvii+888. ISBN-0-444-80084-0. (Developments in Plant Biology, Vol. 2.) (Amsterdam, New York and Oxford: Elsevier/North-Holland Biomedical Press, 1978.) Dfl. 220; \$98.
- ANDERSON, John. Sulphur in Biology. (The Institute of Biology's Studies in Biology, No. 101.) Pp.59. ISBN-0-7131-2711-2. (London: Edward Arnold (Publishers), Ltd., 1978.) £1.60 net.
- ANGELL, Tony. Ravens, Crows, Magpies and Jays. Pp.112. ISBN-0-295-95589-9. (Seattle and London: University of Washington Press, 1978.) £10.50.
- ANNETT, Marian. A Single Gene Explanation of Right and Left Handedness and Brainedness: The Human Handedness Right Shift Factor Behaves Like a Single Gene. Pp.20. ISBN-0-9506464-0-7. (Coventry: Marian Annett, Department of Applied Social Studies, Lanchester Polytechnic, 1978.) £1; \$3.
- BARASH, David P. Sociobiology and Behavior. Pp.xv+378. ISBN-0-435-62046-0. (London: Heinemann Educational Books, Ltd., 1978.) £3.95 net.
- BERLINER, Lawrence J., and REUBEN, Jacques (edited by). Biological Magnetic Resonance, Vol. 1. Pp.xiv+345. ISBN-0-306-38981-9. (New York and London: Plenum Press, 1978.) £22.05.
- BLACKBURN, S. (edited by). Amino Acid Determination: Methods and Techniques. Second edition, revised and expanded. Pp.x+365. ISBN-0-8247-6349-1. (New York and Basel: Marcel Dekker, Inc., 1978.) Sw.fr.82.
- BOURNE, G. H., and DANIELLI, J. F. (edited by). Assisted by JEON, K. W. International Review of Cytology, Vol. 55. Pp.ix+384. ISBN-0-12-364355-4. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) \$29.50; £19.15.
- CLOUDSLEY-THOMPSON, J. L. Dietary Adaptations in Animals. (Patterns of Progress: Zoology Series.) Pp.69. ISBN-0-900541-83-0. (Shildon, Co. Durham: Meadowfield Press, Ltd., 1976.) UK £2.80; Overseas \$8.40.
- CLOUDSLEY-THOMPSON, J. L. Evolutionary Trends in the Mating of Arthropoda. (Patterns of Progress: Zoology Series.) Pp.86. ISBN-0-90054182-2. (Shildon, Co. Durham: Meadowfield Press, Ltd., 1976.) UK £2.80; Overseas \$8.40.
- CLOUDSLEY-THOMPSON, J. L. The Size of Animals. (Patterns of Progress: Zoology Series.) Pp.58. ISBN-0-900541-80-6. (Shildon, Co. Durham: Meadowfield Press, Ltd., 1977.) UK £2.80; Overseas \$8.40.
- CLOUDSLEY-THOMPSON, J. L. The Water Temperature Relations of Woodlice. (Patterns of Progress: Zoology Series.) Pp.84. ISBN-0-90054185-7. (Shildon, Co. Durham: Meadowfield Press, Ltd., 1977.) UK £2.80; Overseas \$8.40.
- CLOUDSLEY-THOMPSON, J. L. Why the Dinosaurs Became Extinct. (Patterns of Progress: Zoology Series.) Pp.85. ISBN-0-9004095-29-0. (Shildon, Co. Durham: Meadowfield Press, Ltd., 1978.) UK £2.80; Overseas \$8.40.
- CROWE, John H., and CLEGG, James S. Dry Biological Systems. (Proceedings of the 1977 Symposium of the American Institute of Biological Sciences held in East Lansing, Michigan.) Pp.x+357. ISBN-0-12-198080-4. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) \$18.50; £12.
- DAVIES, Donald G., and BARNES, Charles D. (edited by). Regulation of Ventilation and Gas Exchange. (Research Topics in Physiology.) Pp.xii+308. ISBN-0-12-204650-1. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) £22; £14.30.
- ELLENBERG, Heinz. Vegetation Mitteleuropas mit den Alpen in Ökologischer Sicht. Zweite, völlig neu bearbeitete Auflage, 499 Abbildungen und 130 Tabellen. Pp.982. ISBN-3-8001-3418-7. (Stuttgart: Verlag Eugen Ulmer, 1978.) DM 120.
- FABER, Donald S., and KORN, Henri (edited by). Neurobiology of the Mauthner Cell. Pp.xii+290. ISBN-0-89004-233-0. (New York: Raven Press, 1978.) £32.50.
- FALKNER, Frank, and TANNER, J. M. (edited by). Human Growth. Vol.2: Postnatal Growth. Pp.xviii+634. ISBN-0-306-34462-9 (v.2). (New York and London: Plenum Press, 1978.) £22.05.
- FREIFELDER, David. Molecular Biology and Biochemistry: Problems and Applications. (A Series of Books in Biology.) Pp.xv+308. ISBN-0-7167-0068-9. (San Francisco and Reading: W. H. Freeman and Company, 1978.) £4.10.
- GARLAND, P. B., and HALES, C. N. (organized and edited by). Substrate Mobilization and Energy Provision in Man. (Biochemical Society Symposium No. 43, held at University College, Cardiff, July 1977.) Pp.ix+228. ISBN-0-904498-07-7. (London: The Biochemical Society, 1978.) £15; \$32.
- GILLHAM, Nicholas W. Organelle Heredity. Pp.xv+602. ISBN-0-89004-102-4. (New York: Raven Press, 1978.) \$64.35.
- GUINCHET, Marcel, et de VILMORIN, Roger. Flore de France, Fascicule 3. Pp.819-1199. ISBN-2-222-02171-5. (Paris: Editions du Centre National de la Recherche Scientifique, 1978.) F.80.
- HALL, Brian K. Developmental and Cellular Skeletal Biology. Pp.x+304. ISBN-0-12-318950-0. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) £21; £13.65.
- HARRISON, R. J., and RIDGWAY, Sam H. Deep Diving in Mammals. (Patterns of Progress: Zoology Series.) Pp.51. ISBN-0-900-54189-X. (Shildon, Co. Durham: Meadowfield Press, Ltd., 1976.) UK £2.80; Overseas \$8.40.
- HAZELBAUER, G. L. (edited by). Taxis and Behavior: Elementary Sensory Systems in Biology. (Receptors and Recognition, Series B, Volume 5.) Pp.x+341. ISBN-0-470-26483-7. (London: Chapman and Hall, 1978. Distributed in the USA by Halsted Press, a Division of John Wiley and Sons, Inc., New York.) £18.

announcements

Meetings

9–10 April, **Enzyme Inhibitors as Drugs**, London (Mrs J. Kruger, Dept of Pharmacology, School of Pharmacy, Brunswick Square, London WC1).

11 April, **Computers in Drug Research and Development**, London (Society for Drug Research, c/o Institute of Biology, 41 Queen's Gate, London SW7, UK).

17–18 April, **1st European Conference on the Protection of Farm Animals**, Amsterdam (Barban Public Relations Ltd, 43 Great Marlborough Street, London, W1, UK).

18–19 April, **The Terrestrial Environment and the Origin of Land Vertebrates**, Newcastle upon Tyne (Dr A. L. Panchen, Dept of Zoology, The University, Newcastle upon Tyne, UK).

23–24 April, **Stereodynamics of Molecular Systems**, New York (Dr R. H. Sarma, State University of New York at Albany, 1400 Washington Avenue, Albany, New York 1222).

29 April–2 May, **Ceramic Quality, Durability and Reliability**, Cincinnati (American Ceramic Society, 65 Ceramic Drive, Columbus, Ohio 43214).

10 May, **Safety in Research Laboratories**, London (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

22–25 May, **13th International Leuko-**

cyte Culture Conference, Ottawa (K. Charbonneau, c/o National Research Council of Canada, Ottawa, Canada KIA OR6).

10–30 June, **The Transformed Cell**, New York (Cold Spring Harbor Laboratory, New York 11724).

10–30 June, **Advanced Bacterial Genetics**, New York (Cold Spring Harbor Laboratory, New York 11724).

21–22 June, **Lysosomes in Endocrine Secretion**, Marseille (Prof. C. Simon, Laboratoire d'Endocrinologie Cellulaire, Université de Provence, Centre St-Charles, 3 Place Victor Hugo, 13331 Marseille Cedex 3, France).

25–27 June, **Amino Acid Analysis in Clinical Chemistry and Medical Research**, Edinburgh (Dr J. M. Rattenbury, Dept of Paediatric Biochemistry, Royal Hospital for Sick Children, Edinburgh, UK).

27–29 June, **2nd International Conference on Lubrication Challenges in Metal-Working and Processing**, Illinois (IIT Research Institute, 10 West 35 Street, Chicago, Illinois 60616).

27–29 June, **Implantable Transducers and Systems: Packaging Methods and Testing Criteria**, Stanford (Prof. J. B. Angell, Electrical Engineering Dept, Stanford University, California 94305).

3–23 July, **RNA-TV**, New York (Cold Spring Harbor Laboratory, New York 11724).

3–23 July, **Molecular Biology and Developmental Genetics of Drosophila**, New York (Cold Spring Harbor Laboratory, New York 11724).

25 June–7 July, **Medical Physics**, Varenne (Prof. M. Della Corte, Istituto di Fisica Medica e Medicina Nucleare, Viale Morgagni, 85, 50134 Firenze, Italy).

9–21 July, **Nuclear Structure and Heavy Ion Collisions**, Varenna (Prof. R. A. Ricci, Istituto di Fisica Galileo Galilei, Università di Padova, Via Marzola, 8, 35100 Padova, Italy).

23 July–4 August, **Physics of the Earth's Interior**, Varenna (Prof. E. Boschi, Istituto di Geofisica dell'Università, Via Irnerio, 46, 40126 Bologna, Italy).

25 July–14 August, **Yeast Genetics**, New York (Cold Spring Harbor Laboratory, New York 11724).

30 July–12 August, **Immunogenetics**, New York (Cold Spring Harbor Laboratory, New York 11724).

13–16 August, **Impact of Intensive Harvesting on Forest Nutrient Recycling**, Syracuse (Dr A. L. Leaf, SUNY College of Environmental Science and Forestry, Syracuse, New York 13210).

24–30 August, **The Ammonoidea**, York (Dr J. R. Senior, Dept of Extra-Mural Studies, 32 Old Elvet, Durham, UK).

Gordon Research Conferences; Frontier of Science; 1979 Schedule—New Hampshire

	Colby-Sawyer College New London, NH	New Hampton School New Hampton, NH	Kimball Union Academy Meriden, N.H.	Tilton School Tilton, NH	Proctor Academy Andover, NH	Holderness School Plymouth, NH	Brewster Academy Wolfeboro, NH	Plymouth State College Plymouth, NH
11–15 June	*Elastin	Nucleic Acids	Cyclic Nucleotides	Theoretical Biology and Biomathematics	Free Radical Reactions	Physical Organic Chemistry (Hydrocarbon Chemistry)	Space Plasma Physics	*Red Cells
18–22 June	Nuclear Chemistry	Environmental Sciences: Air Proteins	Lipid Metabolism	Animal Cells and Viruses	Plant Cell and Tissue Culture	Mammary Gland Biology	Magnetic Resonance Atomic Physics	Calcium Phosphates
25–29 June	Catalysis		Atherosclerosis	Carbohydrates, Chemistry of Polymer Colloids	Polyamines	Biological Regulatory Mechanisms		Molecular Pharmacology
2–6 July	Fiber Science	Bacterial Cell Surfaces	Interfaces, Chemistry at Bones and Teeth, Chemistry, Physiology and Structure of Enzymes, Coenzymes and Metabolic Pathways	Nuclear Structure Physics	Cell Adhesion and Movement	*Analytical Pyrolysis	Nonlinear Optics	Fuel Science
9–13 July	Polymers	Heterocyclic Compounds, Chemistry of	Point and Line Defects in Semiconductors		Physical Metallurgy	Solids, Chemistry and Physics of	Molecular Pathology	Quantum Solids and Fluids, Dynamics of
16–20 July	Elastomers	Organic Reactions and Processes	Safety Evaluations	Biomaterials, Science and Technology of	Energy Coupling Mechanisms	Chemotherapy of Experimental and Clinical Cancer	Macromolecules and Behavior	Quantitative Structure Analysis
23–27 July	Corrosion	Natural Products		*Food Microbiology	Organic Photochemistry	Drug Metabolism	Microbiological Degradation	Tumor Immunology
30–3 August	Food and Nutrition	Statistics in Chemistry and Chemical Engineering	Toxicology and Safety Evaluations	Muscle Ionic Channels in, and Other Excitable Membranes	Developmental Biology	Fertilization and the Activation of Development	Ceramics, Solid State Studies in	*Ion Containing Polymers
6–10 August	Medicinal Chemistry	Inorganic Chemistry	Glycoproteins and Glycolipids	*Epithelial Differentiation and Keratinization	Coatings and Films, Chemistry and Physics of Catecholamines	Postharvest Physiology	Micellar and Macromolecular Catalysis	Magnesium in Biochemical Processes
13–17 August	Separation and Purification	Analytical Chemistry	Fluids in Permeable Media	Laser Interaction with Matter		Liquids, Chemistry and Physics of Inorganic Geochemistry	Few Body Problems in Chemistry and Physics	*Dynamics of Gas-Surface Interactions
20–24 August	Cancer	Adhesion, Science of	Ion Exchange	Transport Phenomena in Lipid Bilayer and Biological Membranes	Elementary Particle Interactions		Molten Salts and Metals	*Remote Sensing of the Earth's Surface from Space

Reports and Publications

UK and Ireland—February

- Countryside Commission. New Long Distance Path Leaflets. Offa's Dyke Path. The Cleveland Way. Pembrokeshire Coast Path. The Penine Way. North Downs Way. South Downs Way. South Devon Coast Path. The Ridgeway Path. Cornwall Coast Path. Dorset Coast Path. Somerset and North Devon Coast Path. (Cheltenham, Gloucestershire: The Countryside Commission, John Dower House, Crescent Place, 1979.) **gratis.** [12]
- Nutrition in Eye Health and Disease. By Stanley C. Evans. Pp. 38. (London: Roberts Publications, 225 Putney Bridge Road, SW15, 1978.) £1.50. [12]
- The Association of Commonwealth Universities. Annual Report of the Council 1977-78. Pp. 50. (London: The Association of Commonwealth Universities, 36 Gordon Square, WC1, 1979.) [12]
- United Kingdom of Great Britain and Northern Ireland. Report on the Implementation of the Convention on International Trade in Endangered Species of Wild Fauna and Flora in the United Kingdom, 1 January-31 December 1977. Pp. 74. (London: Department of the Environment, 2 Marsham Street, SW1, 1979. Available from Department of the Environment, Wildlife Conservation Licensing Section, Tollgate House, Houlton Street, Bristol.) [12]
- Ministry of Overseas Development. Report on Research and Development. 1978. Pp. 76. (London: HMSO, 1978.) £2 net. [22]
- Health and Safety Executive. Health and Safety Statistics, 1976. Pp. vi+65. (London: HMSO, 1979.) £1.75 net. [22]
- Tropical Products Institute. Grain Processing Losses Bibliography: Covering Threshing, Shelling, Hulling, Milling, Grinding etc. and Excluding Harvesting and Storage. By Ruth Kasasian and D. A. V. Dendy. Pp. iv+47. (G117). (London: Tropical Products Institute, 56/62 Gray's Inn Road, WC1, 1979.) £1.35. [22]
- The Glasshouse Crops Research Institute. Annual Report, 1977. Pp. 226. (Rustington, Littlehampton, West Sussex: The Glasshouse Crops Research Institute, 1978.) [52]
- The Universities Central Council on Admissions. Sixteenth Report, 1978-77. Pp. 28. (Cheltenham, Glos.: The Universities Central Council on Admissions, 1979.) 65p. [52]
- The Hannah Research Institute. (University of Glasgow). Report 1978. Pp. 126. (Ayr, Scotland: The Hannah Research Institute, 1979.) £2.50. [72]
- Health and Safety Executive. Packaging and Labelling of Dangerous Substances: Regulations and Guidance Notes. Pp. iii+23. (London: HMSO, 1978.) 60p plus postage. [82]
- Health and Safety Executive. Safety in the Use of Timber Pallets. (Guidance Note PM 15.) Pp. 8. (London: HMSO, 1979.) 30p plus postage. [82]
- Department of the Environment: Central Unit on Environmental Pollution. Pollution Control in Great Britain: How It Works. (Pollution Paper No. 91) Pp. vi+107. (London: HMSO, 1978.) £2.25 net. [92]
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- An Introductory Guide to Information Sources in Physics. By L. R. A. Melton. Pp. 44. (Bristol and London: The Institute of Physics, 1978.) £1.25. [122]
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Laboratory timer. Nuclear Enterprises. The laboratory timer 2546, designed by Nuclear Enterprises, measures accurately to 1,000th of a second, and is ideal for radiation dosimetry and for general laboratory timing. The pocket sized, battery operated timer provides accurate measurements of elapsed time and incorporates two ranges of 999.999 s and 9999.99 s. These are displayed digitally on a liquid crystal, 7-segment element display.

Circle No. 51 on Reader Enquiry Card.



Nuclear Enterprises laboratory timer

Ultra-fine temperature probe. Bailey. An implantable temperature-sensing microprobe, believed to be the smallest of its type ever made, is announced by Bailey Instruments. Type IT-21 is a 12-inch Teflon tube with an o.d. of 0.013 ins having a microthermocouple in the sealed tip and terminating in a miniature copper/constantan type T plug, for connection to any Bailey BAT thermometer.

Circle No. 52 on Reader Enquiry Card.

CO₂ Incubators. Burkard. A new CO₂ incubator range, the Lab-Line Imperial 111 Modular CO₂ incubators, with complete system control, is available from Burkard Scientific. Features include the use of digital set points for temperature and CO₂ and a calibrated set point for relative humidity. Humidity, CO₂ level and temperature are readable at all times by direct digital LED readout. A new feedback system, with a two stage output for fast recovery to set chamber conditions, keeps CO₂ consumption to a minimum.

Circle No. 53 on Reader Enquiry Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Enquiry Card bound inside the cover.

Fluorescence detection micro flow cells. Perkin-Elmer. A new rectangular liquid chromatography micro flow cell for fluorescence detection from Perkin-Elmer offers a signal-to-noise value 4.5 times better than that obtained with cylindrical cells. Two models have been introduced: one for use with the Company's line of MPF instruments and the other for use with model 204 fluorescence spectrophotometer. Measured signal-to-noise values of the new 20 μ l cell approach values obtained with standard 3 ml cells.

Circle No. 54 on Reader Enquiry Card.

Gel filtration medium. Pharmacia. Pharmacia Fine Chemicals present a new high performance medium, Sephacryl S-300 Superfine, for gel filtration of proteins, polysaccharides and other biopolymers. This medium provides excellent resolution over an unusually wide fractionation range, exclusion limit for globular proteins approximately 2 million, making it ideal as a general purpose gel filtration medium. As well as good separation characteristics, Sephacryl S-300 Superfine has good flow properties. For separations where all the components of interest are smaller than about 250,000 (globular proteins), Sephacryl S-200 Superfine is recommended. Sephacryl S-300 Superfine and Sephacryl S-200 Superfine are available in packs containing 750 ml pre-swollen gel.

Circle No. 55 on Reader Enquiry Card.

Microchopper. Rofin. The micro-chopper from Rofin is designed to fit into the smallest spaces, into OEM applications and into experiments. It measures only 31 mm diameter and is frequency programmable over the range 50–2,000 Hz, or 10–500 Hz. The unit features a digital LED readout, and has a printed circuit board built in behind the chopping wheel which enables the user to plug in a TO5 or similarly sized detector.

Circle No. 56 on Reader Enquiry Card.

Silicon avalanche photodiode. Coherent. The Coherent picosecond diode, consists of the fastest avalanche photodiodes commercially available together with a precision 50 Ω coaxial assembly. The picosecond diode may be used wherever general high speed research work is undertaken. It requires neither precision mounting nor focusing optics.

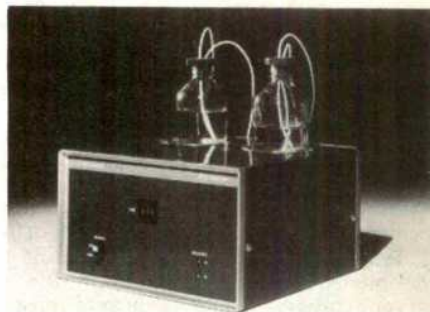
Circle No. 57 on Reader Enquiry Card.

Dispensing pipette. Brand. A selection of micro litre dispensing pipettes by Brand are now available. Use of disposable tips with the pipettes enables volumes in the range 5–1,000 μ l to be dispensed accurately and efficiently. Called the transferpette, it has an overall length of 270 mm, and is operated by pressing a button on the top of the pipettor body. An insulating jacket minimises any effect created by hand heat and a PTFE sleeve and flexible O-ring provide positive internal sealing in the transferpette.

Circle No. 58 on Reader Enquiry Card.

Laboratory computer system. Digital. Designated DEClab-11/MNC, the new Digital laboratory computer system uses twin R101 disk storage units for a standard 10-megabyte capacity and its fundamental programming is done in ANSI-standard FORTRAN IV, supplemented with Digital-supplied subroutine packages. The new system is housed in a 41-inch (104 cm) high cabinet. The DEClab-11/MNC system design permits the use of the system's configuration through use of plug-in 'MNC modules.'

Circle No. 59 on Reader Enquiry Card.



Pye Unicam HPLC aid.

HPLC Aid. Pye Unicam have launched a new addition to their LC-XP range of high performance liquid chromatography instruments. Known as LC-XP Dialamix, this unit delivers any desired isocratic mixture of two solvents to the inlet of a reciprocating pump. With its integral degassing facilities and highly efficient dynamic mixing system, the unit provides a significant time saving compared with traditional hand-mixing techniques. The desired solvent composition is rapidly selected by means of a digital front panel switch which initiates mixing in the three-port PTFE solenoid valve and magnetically stirred chamber.

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APPOINTMENTS VACANT



LECTURESHIP IN GEOGRAPHY

Applications are invited for the post of Lecturer in Geography. Candidates should have a specialist interest in Geomorphology and preference may be given to those with research interests in Pleistocene studies.

Salary Scale: £4317-£8487. Appointment is likely to be made in the range £4317-£5235. There is a non-contributory pension scheme.

Interested persons should, in the first instance, telephone the Staff Office, Trinity College, on Dublin 772941 Ext. 1678/1775.

The closing date for receipt of applications will be 17th April 1979.

W6(A)

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF GEOGRAPHY LECTURESHIPS IN GEOGRAPHY

Applications are invited for two posts. 1) Lecturer in hydrology or meteorology. Preference may be given to candidates who can demonstrate competence in both fields.

2) Lecturer in the geographical aspects of planning. Preference may be given to candidates with special interests in industry or resource management.

Salary will be in the range £3,883 to £7,754 (under review) plus superannuation. Maximum starting salary will be according to age, experience and qualifications, but appointment will be towards the lower end of the scale.

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (3 copies) including full curriculum vitae and naming 3 referees, should be sent by Thursday April 19, 1979.

Please quote ref: NHS. 156(A)

IMPERIAL COLLEGE LONDON TWO BIOCHEMISTS/ NEUROCHEMISTS

Two Scientific Research Assistants with two or three years post-doctoral research experience are required for M.R.C.-funded projects on cellular biochemical aspects of the organisation and function of neurotransmitter systems at synapses in the mammalian C.N.S.

For one post a background in neurochemistry is not a strict requirement, but experience with incubating tissues, subcellular fractionation, affinity chromatography, ligand-binding assays, or protein chemistry would be advantageous.

The second post is for a neurochemist/neuropharmacologist with experience in catecholamine or indoleamine transmitter systems. The successful candidates would join a team of ten working on closely related topics.

One post is available from April 1 and would be renewable on an annual basis for at least five years, the second will be available on October 1.

Salary would be commensurate with experience. Applicants should send curriculum vitae and the names of two referees to Dr. H. F. Bradford, Department of Biochemistry, Imperial College, London SW7 2AZ. 150(A)

EDINBURGH & S.E. SCOTLAND REGIONAL BLOOD TRANSFUSION SERVICE Royal Infirmary, Edinburgh POSTDOCTORAL RESEARCH FELLOW OR GRADUATE RESEARCH ASSISTANT

Required to undertake a project which involves the development of procedures for isolating, purifying and characterising red cell antigens, and the use of purified antigens for the production of antisera. The work will be carried out in a laboratory producing a range of immunological reagents, and will involve collaboration with other laboratory staff in evaluation of antisera as potential red cell typing reagents. Applicants should possess an Honours B.Sc. or a Ph.D. in Biochemistry or a related discipline.

The post is for three years. Salary range £3,486 to £3,798, N.H.S. Whitley Council conditions of service apply. Starting date by arrangement.

Applications to and further details from Dr. D. B. L. McClelland, Regional Transfusion Centre, Royal Infirmary, Edinburgh. Tel: 031-77291, by April 16, 1979. 123(A)

FACULTY POSITION IN INSECT PATHOLOGY DEPARTMENT OF ENTOMOLOGICAL SCIENCES, COLLEGE OF NATURAL RESOURCES UNIVERSITY OF CALIFORNIA Berkeley

Full-time, 11-month research (75%) and teaching (25%) position available in insect pathology at the Assistant Professor level available July 1, 1979. Expertise mandatory in one of the following areas: bacteriology, comparative virology, mycology, or protozoology. Ph.D. and background in pathology required and postdoctoral experience desirable.

Responsibilities will be in basic and applied research and teaching in insect pathology and pest management. Experience in pathogen-insect relationships, pathogenicity, and the manipulation of pathogens for insect control will be weighting factors when judging applicants.

Send full curriculum vitae, official transcripts, copies of significant publications or manuscripts, and names of three references by May 1, 1979 to Evert I. Schlinger, Acting Chairman, Department of Entomological Sciences, 137 Giannini Hall, University of California, Berkeley, California 94720. The University of California is an equal opportunity, affirmative action employer. W21(A)

EDINBURGH & S.E. SCOTLAND REGIONAL BLOOD TRANSFUSION SERVICE Royal Infirmary, Edinburgh RESEARCH ASSISTANT

Research assistant required for a three-year supervised project on the development of sensitive specific methods for detecting and quantitating immunoglobulin and complement bound to the human red cell. The work will involve the isolation of highly purified plasma proteins, production of antisera, and development of assay systems. Applicants should possess an honours degree or an equivalent qualification in Biochemistry or a related subject.

The post is available from April 1, 1979. Salary range £3,486 to £3,798, N.H.S. Whitley Council conditions of service apply.

Applications as soon as possible to Dr. D. B. L. McClelland, Regional Transfusion Centre, Royal Infirmary, Edinburgh, from whom further information may be obtained. 122(A)

WYE COLLEGE (University of London) LECTURER IN BIOCHEMISTRY

Applications are invited for the post of Lecturer in Biochemistry. The post is a permanent one. Candidates are expected to possess an honours degree in Biochemistry with a Ph.D. and post-doctoral research experience in some aspect of animal biochemistry. Experience in lipid biochemistry would be an advantage.

Applications to be forwarded to the Secretary, Wye College, Wye, Ashford, Kent TN25 5AH (from whom further particulars of the post may be obtained) by April 25, 1979 at the latest. 149(A)

DESIGNER (SCIENCE PROJECTS) OPEN UNIVERSITY PRODUCTIONS

Are you interested in the presentation of science through television? There is an opportunity to work on the design and preparation of scientific and technological experiments and demonstrations for Open University television programmes. Applicants must have mechanical workshop skills and should have experience of electronics to HNC level or equivalent. Ability to work in a wide range of scientific areas, e.g. Chemistry, Biology, Geology and Physics is essential.

Based at Alexandra Palace, North London, but it is anticipated that Open University Productions will move to Milton Keynes in 1981.

Salary (under review): £5,890 p.a. (may be higher if qualifications exceptional) by £225 to £7,015 p.a. Relocation expenses considered.

Telephone or write immediately for an application form, enclosing addressed envelope and quoting reference 2183/NA, to **Appointments Department, BBC, London W1A 1AA. Telephone: 01-580 4468, ext. 4619.**

168(A)

CELL BIOLOGIST/BIOCHEMIST. Ph.D. 1970. Teaching and research experience. Established research program and publications in areas of cardiac and skeletal muscle ultrastructure, muscle membrane ion transport. Extensive experience in conventional electron microscopy as well as freeze fracture. Desire position in U.K. Curriculum vitae and references gladly sent to serious inquiries. Reply to Box 5(B), c/o Nature, 3 Dyers Buildings, Holborn, London EC1N 2NR. W5(A)

**Chemical
Engineers
Metallurgical
Engineers
Instrument
Engineers
Analytical Chemists
Industrial
Chemists**

The National Institute for Metallurgy, which is one of the largest mineral-processing laboratories in the World, and which is situated on the northern outskirts of Johannesburg, is at present, as a result of its very considerable list of scientific and technological successes, together with the rapid growth of the mineral industry in South Africa, entering a major expansion phase. The Institute has advanced equipment and very modern laboratories, workshops, and pilot-plant facilities, located in a pleasant rural atmosphere within easy reach of the city. To cope with this expansion, the Institute is looking for research staff to work on projects covering a wide field in minerals and metals.

Applicants should be in possession of a research degree in chemical engineering, extractive metallurgy, instruments or electronic engineering, analytical chemistry, industrial or pure chemistry, material science, minerals processing, or a related field. Post-graduate experience will be a recommendation, but is not essential.

The low cost-of-living structure and attractive salaries, based on education and experience, coupled with challenging opportunities, are some of the attractions of employment at the Institute.

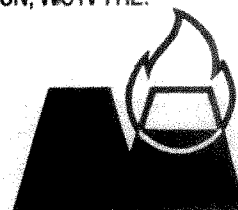
Appointments may also be made on contract for two or three years, after which time the Institute will pay return fares for the applicant and his family.

The field of research covers hydrometallurgy, pyrometallurgy, refractory metals, the use of computers, the design and operation of pilot plants, and the automation of processes and the application thereof to full-scale plants. It also includes mineralogy, the study of surface chemistry, electro-chemistry, the properties of solutions, analytical chemistry, and the development of specialized electronic instruments. The large and well-equipped workshop can handle precision engineering jobs and pilot plant construction in a wide variety of materials. A large proportion of the work of the Institute is done on a joint venture basis in close collaboration with industry.

Benefits include a pension scheme, medical aid, a housing-subsidy scheme, 5-day working week, generous leave privileges (in addition to normal vacation leave, the Institute closes for the period between Christmas and New Year), flexitime, subsidized cafeteria facilities, sports facilities such as squash, tennis, swimming, snooker, etc., and an annual leave bonus. There are possibilities for advanced studies at universities, with substantial bursaries from the Institute.

The Institute will pay the fares to South Africa of the applicant and his family, contribute to removal expenses and grant a settlement allowance under certain conditions.

Please apply giving full details of age, qualifications, experience, etc., to:
The Office of the Scientific Counsellor,
South African Embassy, Chichester House,
278 High Holborn, LONDON, WC1V 7HE.



National Institute for Metallurgy

KEEPER

Conservation & Technical Services
£10,560-£11,820

The Department of Conservation and Technical Services of the British Museum, Bloomsbury, is primarily concerned with the conservation of items from the Museum's collections and elsewhere, and with scientific research into new methods and materials for conservation. It provides advice on conservation matters in liaison with other institutions and organisations in this field both in the United Kingdom and abroad.

The Keeper's duties include the general administration of the Department, the initiation and direction of research into problems of conservation, and the organisation and development of the practical and academic training of conservation staff.

Candidates, normally aged 35-54, must be of high academic standing in a relevant scientific field, preferably chemistry,

physics, conservation or materials science, with considerable experience in an appropriate field of work; particular attention would be paid to conservation experience. They must also have successful experience of managing staff and of initiating and directing research. Relevant contacts with international specialists in conservation are desirable.

Salary (under review) in the range shown above; non-contributory pension scheme.

For further details and an application form (to be returned by 17 April 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours).

Please quote ref: S/5091/79/3.

BRITISH MUSEUM

102(A)

UNIVERSITY OF EAST
ANGLIA
NORWICH

CHAIR IN DEVELOPMENT
STUDIES
(NATURAL RESOURCES)

Applications are invited for the Chair in Development Studies (Natural Resources) in the School of Development Studies. Candidates should have a particular interest in ecological and agricultural aspects of natural resource development, but other specialisations will not be excluded from consideration if there is evidence of concern with the wider implications of resource assessment, use, planning and management.

Candidates should be prepared to play a leading role in evolving programmes of teaching and research within an interdisciplinary framework, while also having a strong command of their own discipline and being concerned with practical solutions to development problems. The person appointed will be a member of the Overseas Development Group organised by the School, and will normally spend up to one-third of his or her time on outside financed assignments, usually overseas. Practical experience of operational or advisory work for government or international organisations will be useful.

Appointment will be at an appropriate point on the Professorial scale £9,443 to £11,532 (under review) plus U.S.S. benefits and will be tenable from October 1, 1979, or as soon as possible thereafter.

Applications (one copy only) giving the names of three persons to whom reference may be made, should be lodged with the Registrar and Secretary, University of East Anglia, Norwich NR4 7TJ (telephone 0603 56161, extension 2215) from whom further particulars may be obtained, not later than April 27, 1979. No forms of application are issued. In naming three referees you are particularly requested to give only the names of those who can immediately be approached. 165(A)



LEICESTER UNIVERSITY

CHAIR OF PHYSICS

Applications are invited for an additional Chair of Physics tenable from 1st October 1979 or an agreed later date. Candidates should be experienced in some branch of experimental physics.

Further particulars from the Registrar to whom applications should be sent by 21st April 1979. Candidates in the U.K. should submit 15 copies of their application (overseas candidates may submit one copy).

157(A)

UNIVERSITY OF
THE WITWATERSRAND,
Johannesburg, South Africa
DEPARTMENT OF
GENERAL PHYSIOLOGY
SENIOR LECTURER/
LECTURER

Applications are invited from persons suitably qualified in the medical, dental, veterinary or basic sciences, for appointment to the above post on the staff of the Department of General Physiology in the Faculty of Dentistry.

Senior Lecturer: Candidates should have a higher scientific, dental, veterinary or medical qualification, or have considerable teaching experience. Salary range attached to the post is R12,474 to R14,700 per annum.

Lecturer: Salary range for this post is R7,278 to R13,515 per annum.

The initial salary and level of appointment will be determined according to the qualifications and experience of the successful applicant. Duties to be assumed as soon as possible.

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion or colour. Further particulars relating to this practice and the the above post are included in an information sheet obtainable from the London Representative, University of the Witwatersrand, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, 2001 South Africa, to whom applications should be lodged by April 12, 1979. 129(A)

UNIVERSITY OF
ADELAIDE
CHAIR OF
CLINICAL PHARMACOLOGY

Applications are invited from qualified persons with teaching and research interests in Clinical Pharmacology who have a medical degree registrable in South Australia.

The new Professor will be invited to accept Chairmanship of a new Department of Clinical and Experimental Pharmacology formed from within the existing Department of Human Physiology and Pharmacology.

Present salary: \$A33,061 a year with provision for superannuation. In addition to the basic salary the Professor will receive, on account of his registrable medical qualification, a clinical loading at the rate of \$A5,000 per annum.

Further information, including the Formal Terms and Conditions of Appointment, the provisions of Departmental Government and details required in an application, is available from the Registrar of the University or from the Secretary General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF; further information about responsibilities, facilities, research interests in the Department, etc., may be sought from the Dean of the Faculty of Medicine, in the University.

Applications, in duplicate, giving the particulars listed in paragraph 10 of the statement should reach the Registrar of the University, G.P.O. Box 498, Adelaide, South Australia, 5001, not later than June 15, 1979. 100(A)

Chemical/ Metallurgical/ Instruments/ Mineral and Processing/ Technologists

The National Institute for Metallurgy, which is one of the largest mineral-processing laboratories in the World, and which is situated on the northern outskirts of Johannesburg, is at present, as a result of its very considerable list of scientific and technological successes, together with the rapid growth of the mineral industry in South Africa, entering a major expansion phase. The Institute has advanced equipment and very modern laboratories, workshops, and pilot-plant facilities, located in a pleasant rural atmosphere within easy reach of the city. To cope with this expansion, the Institute is looking for research staff to work on projects covering a wide field in minerals and metals.

Applicants should preferably hold a diploma in mineral processing, metallurgy, chemical

technology, measurement and control, or instrumentation.

Persons not holding such a diploma, but with considerable experience in any of these fields, are also welcome to apply.

The low cost-of-living structure and attractive salaries, based on education and experience, coupled with challenging opportunities, are some of the attractions of employment at the Institute.

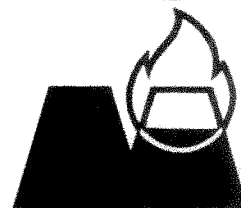
Appointments may also be made on contract, for two or three years, after which the Institute will pay return fares for the employee and his family.

Benefits include a pension scheme, medical aid, a housing-subsidy scheme, 5-day working week, generous leave privileges (in addition to normal vacation leave, the Institute closes for the period between Christmas and New Year), flexitime, subsidized cafeteria facilities, sports facilities such as squash, tennis, swimming, snooker, etc., and an annual leave bonus. There are possibilities for advanced studies at universities, with substantial bursaries from the Institute.

The Institute will pay the fares to South Africa of the applicant and his family, contribute to removal expenses, and grant a settlement allowance under certain conditions.

Please apply giving full details of age, qualifications, experience, etc., to:

The Office of the Scientific Counsellor,
South African Embassy, Chichester House,
278 High Holborn, LONDON WC1V 7HE.



National Institute for Metallurgy

Post-Doctoral Organic Chemist

Miles Laboratories Ltd. are an international pharmaceutical company based in an attractive part of South Buckinghamshire.

We are currently seeking a Ph.D. level Organic Chemist to join a small synthetic team engaged in research into allergic diseases. Candidates should have 2-3 years post-doctoral experience, be capable of supervising graduate chemists, and will be expected to make a significant contribution to the Department's scientific programme. Preferred age 26-30.

In return we offer excellent conditions of employment, four weeks holidays, subsidised staff restaurant, free life and private health insurance, contributory pension scheme, flexible working hours.

For an application form please write or ring my secretary on Farnham Common 2151:

Mrs. Nancy Morgans
Personnel Manager
Miles Laboratories Limited
Stoke Court, Stoke Poges
SLOUGH. 107(A)



National Blood Transfusion Service (Wales)

SCIENTIFIC OFFICER

Salary: £2,991 to £4,275 per annum dependent upon qualifications.

A graduate in biological or natural sciences is required to assist in the investigation of platelet storage, testing and quality control. Work will involve the use of controlled rate cell freezing techniques, evaluation of platelet morphology and function and assessment of statistical data resulting from the use of platelet concentrates in the treatment of patients.

Applicants should preferably have experience in a relevant field and be prepared to take part in the normal research and development activities of the department. This appointment is on the basic grade Scientific Officer scale.

Terms and Conditions of Service.

All terms and conditions of service are in accordance with the Professional and Technical "A" Whitley Council and General Whitley Council for the Health Service.

Applications form and job description from: **National Blood Transfusion Service, Rhydlafar, St. Fagans, Cardiff. Tel: 0222 890302.**

Closing date: April 6, 1979.

169(A)

COMMONWEALTH AGRICULTURAL BUREAU Vacancy for SCIENTIFIC INFORMATION OFFICER

at
NATIONAL INSTITUTE OF
AGRICULTURAL ENGINEERING
Wrest Park, Silsoe
Bedford MK45 4HS, U.K.

Duties of Post: The scrutiny of technical literature, selection and preparation of abstracts on all subjects related to agricultural and horticultural engineering (this may involve visits to outside libraries), indexing, proof-reading, and dealing with technical enquiries.

Qualifications: Applicants should have a knowledge of both engineering and foreign languages. They should have a degree or similar qualification in engineering or a relevant subject and a high competence in at least one foreign language, or a foreign language degree with a sound background knowledge of engineering. The ability to write clear concise English is essential.

Salary: In the scale £2,839 to £5,448, plus a compensatory allowance to offset personal contribution to superannuation. Starting salary according to qualifications and experience. Promotion to higher scales on merit.

Applications forms and full particulars from:

Executive Director,
Commonwealth Agricultural
Bureaux,
Farnham House,
Farnham Royal,
Slough SL2 3BN.

Closing date for applications: April 6, 1979. 138(A)

RESEARCH ASSOCIATE

Applications are invited for a possible appointment as research associate in theoretical astrophysical plasma physics, space plasma physics and/or cosmic-ray physics. The successful applicant will be expected to spend a substantial part of his or her time working on problems in solar or interplanetary physics.

Appointments will begin in Fall or Winter 1979. Applicants should possess a recently acquired Ph.D. in a relevant area of physics, astronomy, or planetary sciences.

Inquiries should be addressed to Prof. J. R. Jokipii or Prof. E. H. Levy, Department of Planetary Sciences, Lunar and Planetary Laboratory, University of Arizona, Tucson 85721.

Applications should be accompanied by resume, complete bibliography, and at least two letters of recommendation from persons who are well acquainted with the applicant's background and potential. All material should be received by June 1, 1979. An Equal Opportunity, Affirmative Action, Title IX, Section 504 Employer. W17(A)

THE UNIVERSITY COLLEGE OF WALES ABERYSTWYTH DEPARTMENT OF ZOOLOGY LECTURESHIP IN IMMUNOLOGY

Applications are invited for the above post which is tenable from October 1, 1979. It will be an advantage to have had a Biological training and to be able to take part in the teaching of vertebrate Biology.

Salary at the appropriate point within the range £3,883 to £7,754 per annum (under review).

Application forms and further particulars can be obtained from the Registrar, The University College of Wales, Old College, King Street, Aberystwyth, Dyfed, SY23 2AX, to whom completed forms should be returned no later than Friday, April 13, 1979. 112(A)

FACULTY POSITION AVAILABLE IN RETINAL HEMODYNAMICS AND BLOOD FLOW STUDIES

University of Illinois
Eye and Ear Infirmary
and
Abraham Lincoln School
of Medicine

The University of Illinois
at the

Medical Center, Chicago

Equal Opportunity/Affirmative
Action Employer

Applications are invited for a faculty position at the assistant or associate professor level. The major responsibility will be supervision of research of vascular flow in the retina, utilising a unique non-invasive computerised ophthalmoscopy system. Active interchange with engineers and clinical ophthalmologists will be fostered. An advanced degree (Ph.D. or M.D.) is a prerequisite.

Those interested should direct inquiries together with a curriculum vitae and bibliography to:

Morton F. Goldberg, M.D.
Professor and Head
Department of Ophthalmology
University of Illinois Eye and
Ear Infirmary
1855 West Taylor Street
Chicago, Illinois 60612

W2(A)

FACULTY POSITION IN NATURAL PRODUCTS CHEMISTRY

DEPARTMENT OF
ENTOMOLOGICAL SCIENCES
COLLEGE OF NATURAL
RESOURCES
UNIVERSITY OF
CALIFORNIA
Berkeley

Full-time, 11-month research (80%) and teaching (20%) position available in Natural Products Chemistry at the Assistant Professor level. Ph.D. in organic chemistry or other field(s) relevant to natural products required with evidence of interest in chemicals active in insects.

Responsibilities will be in basic and applied research involving bioactive chemicals from insects, plants, and other biological materials that influence insect growth, development, reproduction, and behaviour. Research will include identification and synthesis of active components, and studies of their potential use in managing insect populations. Teaching duties may include developing a course in chemical ecology, environmental chemistry, insect biochemistry, or chemical pest control.

Send full curriculum vitae, official transcripts, copies of significant publications or manuscripts, and names of at least three references by May 1, 1979 to Evert I. Schlinger, Acting Chairman, Department of Entomological Sciences, 137 Giannini Hall University of California, Berkeley California 94720. The University of California is an equal opportunity affirmative action employer. W20(A)

UNIVERSITY COLLEGE LONDON DEPARTMENT OF ANATOMY AND EMBRYOLOGY LECTURER/SENIOR LECTURER

An applicant is sought who is committed to any field of biomedical research. Facilities for research are outstanding.

Applications, including curriculum vitae and publications list to Assistant Secretary (Personnel), University College London, Gower Street, WC1E 6BT from whom further particular may be obtained. 179(A)

CSIRO

AUSTRALIA

Research Scientists

Centre for animal research
and development
Bogor, Indonesia

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees — 2,400 of whom are research and professional scientists — located in Divisions and Sections throughout Australia.

General: Australia is committed to assisting Indonesia in the establishment of an animal husbandry research laboratory and associated buildings, conducive to problem-solving research of the highest standard, with the long-term view of staffing it fully with Indonesian scientists trained to carry out first-class research. The agreement extends over a period of ten years and has been in operation for four years. The Australian aid contribution is administered by CSIRO on behalf of the Australian Development Assistance Bureau.

Modern well-equipped laboratories are nearing completion at Bogor 70 km from Jakarta. Excellent facilities are available for research on problems related to the nutrition, growth, reproduction, management, physiology and breeding of chickens, ducks, sheep, goats, cattle and buffaloes. In the longer term a field station is expected to provide additional opportunities for ruminant research, particularly with cattle and buffaloes.

The Centre is giving considerable emphasis to studying (a) factors that limit animal productivity at the village level, (b) problems of utilizing local byproducts in intensive poultry rations, (c) problems of management, feeding and breeding of ducks under intensive conditions, (d) the biology and production potential of Bali cattle in comparison with other breeds of cattle and with buffaloes, (e) the growth and reproductive potential of Indonesian sheep and goats, and (f) the problems associated with enhancing the use of local byproducts for ruminant rations generally. The Centre recognises the overriding importance of agricultural byproducts such as rice, bran, rice straw, cassava, animal faeces, etc. as the major source of livestock feedstuffs in Indonesia, and aims to develop special research expertise in the efficient utilization of these byproducts.

Research scientists at the Centre are supported by Veterinary and Analytical laboratories and other well-equipped supporting services such as library and workshop.

Vacancies exist for five senior scientists viz: Research Leader, Ruminant Nutritional Biochemist, Microbiologist, Research Chemist, and Biologist/Ecologist (Village Production Systems). These new appointees will join a group of 10-12 Australian and Indonesian scientists who are supported by technical staff. They will have major roles in the planning and development of long-term research policies and projects for the Centre. In addition to personal research, these scientists will participate in the continued training of Indonesian scientific and support staff. All applicants will be expected to have had experience in the management of research staff.

1. Research Leader

Duties: The Research Leader will be responsible to the Officer in Charge for the planning and management of the Centre's research programmes, the organization of seminars and the planning and guidance of training for young Indonesian scientists and technicians associated with the Centre.

Qualifications: PhD or equivalent qualifications, and international recognition for research in animal science. A broad background in animal science and a wide knowledge of Australian teaching and research centres and of animal scientists in Australia and overseas in order to provide a strong link between the Centre and animal scientists, particularly in Australia, but also internationally. An understanding of tropical livestock production as applied to a country like Indonesia. Experience in Staff Management.

2. Ruminant Nutritional Biochemist

Duties: To plan and conduct research aimed at developing village level technologies for overcoming nutritional limitations of Indonesian agricultural byproducts for ruminant feeding, giving initial consideration to the study of factors that limit the nutritional value of rice straw, rice bran and tree legumes. To investigate simple methods for storage of crops and byproducts as part of the development of village level technologies for enhanced ruminant production.

Qualifications: PhD or equivalent qualifications and extensive research experience in ruminant nutrition and/or biochemistry.

An interest in tropical plants and animals is essential. Experience in staff management.

3. Microbiologist

Duties: To plan and supervise fermentation studies aimed at enhancing the nutritional properties of agricultural byproducts, giving particular attention to the development of village level technologies appropriate for the production of microbial protein from these substrates. To undertake studies aimed at preventing the proliferation of mycotoxic fungi during storage of conventional feeds in Indonesia.

Qualifications: PhD or equivalent qualifications, with extensive experience in the microbiology of food or feeds or in the microbiology of waste product fermentations. Experience with mycotoxin-producing fungi desirable. An understanding and/or interest in tropical biology. Experience in staff management.

4. Research Chemist

Duties: To plan and supervise research aimed at overcoming the anti-nutritional and toxic factors such as alkaloids, tannins, mycotoxins, phytic acid, etc., which limit the nutritional value of Indonesian feedstuffs, and defining the mineral, amino acid and vitamin status of these feedstuffs. To collaborate with other scientists at the Centre to develop technologies to overcome these nutritional limitations.

Qualifications: PhD or equivalent qualifications, with extensive research experience in the field of Analytical Chemistry as applied to feeds, food or agriculture in general. A general understanding of the tropics and an interest in the problems of developing countries. Experience in staff management.

5. Biologist/Ecologist (Village Production Systems)

Animals in Indonesian villages are a vital part of a delicately balanced ecosystem which has been developed over centuries. With the increasing population and the consequent pressure on available land, demand for meat etc., it is necessary to introduce technologies which will help the village farmers in their efforts to maintain the balanced ecosystem.

Duties: To lead a research programme which will relate current research projects designed to increase livestock production to village livestock husbandry systems. In addition to the development of new findings, to consider the introduction or adaptation of proven technologies, breeds or feeds that already exist in other countries. To initiate and supervise research projects, and to work closely with research staff in other programmes at the Centre.

Qualifications: The position calls for someone with a deep interest and understanding of ecology, agricultural and animal husbandry as applied to villages in countries like Indonesia. The appointee will be expected to have appropriate academic qualifications (PhD or equivalent) and research experience in tropical biology or agriculture. Experience in the development of appropriate technologies in developing countries desirable. Experience in staff management.

Salary: Research Leader:

Principal Research Scientist/Senior Principal Research Scientist \$A23247 — \$A30530 p.a.

Other appointments:

Senior Research Scientist/Principal Research Scientist \$A19572 — \$A26368 p.a.

Appointment at higher levels will be considered for outstanding applicants for all positions.

Tenure: The period for which these positions is available is subject to negotiation, but a minimum of 3-4 years is envisaged. The appointee may be offered an indefinite appointment within CSIRO.

Applications in duplicate, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting the reference number 1000/4S should reach: The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, LONDON WC2R 3EH, by 21st April 1979.

Applications in U.S.A. and Canada should be sent to: The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue N.W., WASHINGTON D.C. 20036

Carbohydrate Chemist

A vacancy exists for a carbohydrate chemist within the Biochemistry Department of The Lord Rank Research Centre at High Wycombe. The Biochemistry Department's activities particularly involve the biochemistry and chemistry of cereal crops and products.

Applications are invited from persons who are interested in basic research and in the structure and properties of natural polysaccharides and applications in the food and agriculture industries.

Applicants should have a strong background in carbohydrate research. A Ph.D. degree or equivalent research experience is a minimum requirement for this position and good career prospects exist for the person with the ability to lead others in a multi-disciplinary research environment. Publication of research work will be encouraged.

The Research Centre provides research and development facilities to the Ranks Hovis McDougall Group of Companies whose activities include the manufacture and distribution of a wide range of milling, baking, agricultural and convenience food products.

A competitive salary will be offered, dependent upon qualifications and experience, and the terms and conditions of employment are as expected of a major organisation. Please apply in writing with curriculum vitae, quoting reference CO1 to:

Personnel Administration,
RMH RESEARCH LIMITED,
The Lord Rank Research Centre,
Lincoln Road, High Wycombe, Bucks.



172(A)

LOTHIAN HEALTH BOARD SENIOR GRADE CYTOGENETICIST

Applications are invited from suitably qualified graduates for the above post, to be held in the Area Diagnostic Cytogenetics Laboratory, Royal Hospital for Sick Children, Edinburgh.

Experience in all branches of cytogenetics is essential, especially in relation to amniotic fluid cell analysis, tissue culture, and all types of banding techniques.

An interest in teaching could be helpful as the Department has a University teaching commitment.

Applications, which should be typewritten, giving particulars of age, qualifications and previous experience together with the names and addresses of two referees should be lodged with the Secretary, 11 Drumsheugh Gardens, Edinburgh EH3 7QQ not later than April 5, 1979.

143(A)



LECTURER IN PHARMACEUTICAL CHEMISTRY

Applications are invited for the post of Lecturer in Pharmaceutical Chemistry in the School of Pharmacy, Trinity College, Dublin.

Salary Scale: £4317-£8487. Appointment is likely to be made in the range £4317-£4774. There is a non-contributory pension scheme.

Interested persons should, in the first instance, telephone the Staff Office, Trinity College, on Dublin 772941 Ext. 1678/1775.

The closing date for receipt of applications will be 7th May 1979.

W7(A)

UNIVERSITY OF STRATHCLYDE DEPARTMENT OF MINING ENGINEERING PROFESSORSHIP AND HEAD OF THE DEPARTMENT OF MINING ENGINEERING

Applications are invited from appropriately qualified and experienced candidates for appointment to the Professorship and Head of the Department of Mining Engineering in succession to the late Professor J. Dewi Jenkins.

The post will be remunerated within the Professorial range for universities with U.S.S. benefits.

Application forms and further particulars (quoting 14/79) may be obtained from the Registrar, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW, with whom applications must be lodged by April 27, 1979.

170(A)

INSTITUTE OF TERRESTRIAL ECOLOGY HIGHER SCIENTIFIC OFFICER/ SENIOR SCIENTIFIC OFFICER

A vertebrate ecologist at Higher Scientific Officer/Senior Scientific Officer level is required to join a group studying the role of pollutants in the population dynamics of predatory birds, the work will involve research on sparrowhawks and kestrels.

The successful candidate will be based initially at the Institute in Edinburgh with the possibility of a move to ITE's Monks Wood Experimental Station near Huntingdon at a later date.

Qualifications

Candidates should have a good honours degree in zoology, biology or an allied subject and at least two years relevant post-graduate experience preferably in bird ecology. Applicants should be physically fit as duties include strenuous field work including tree climbing.

Salary and conditions of service

The salary will be within the following scales:-

HSO £4,101 to £5,448 per annum
SSO £5,154 to £6,898 per annum
with 22 days paid annual leave plus 10½ days public and privilege holidays. Starting pay according to age, qualifications and experience.

There is a non-contributory superannuation scheme.

The Natural Environment Research Council is not a Government Department but conditions of service are similar to those of the Civil Service. Application form and further details are available from:

Establishments Section
Institute of Terrestrial Ecology
68 Hills Road
Cambridge

CB2 1LA Telephone (0223) 69745

Please quote reference: 29.

Closing date for completed applications is: April 6, 1979. 164(A)

THE UNIVERSITY OF WESTERN ONTARIO LONDON—CANADA

Applications are invited for a one year limited term position from July 1, 1979 to June 30, 1980 at the rank of Assistant Professor to work with algae. Candidates will be expected to contribute to teaching in introductory biology in addition to teaching algology. Candidates will also be encouraged to pursue research in an area of algology of their choice. Post-doctoral experience would be an advantage. The salary will be not less than \$17,000 per annum, subject to the availability of funds. Please submit a curriculum vitae and the names of three referees to:

The Chairman
Department of Plant
Sciences
Biological and Geological
Building
University of Western
Ontario
London, Ontario
N6A 5B7. W10(A)

SALOP AREA HEALTH AUTHORITY ROBERT JONES AND AGNES HUNT ORTHOPAEDIC HOSPITAL Oswestry, Salop CHARLES SALT RESEARCH CENTRE BIOCHEMIST

Graduate Research Assistant required to take part in immunochemical investigations on human intervertebral discs in relation to Low Back Pain.

Salary scale (Basic Grade Biochemist with 1st or 2nd Class Honours Degree) £3,486 rising to £4,275 per annum.

Applications with names of two referees should be addressed to Mr N. W. Nisbet F.R.C.S., Director of Research, Charles Salt Research Centre, at the above hospital. 145(A)

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited for two
LECTURESIPS

in Microbiology in the above Department. No field of interest is excluded but it is hoped that one post will be filled by someone with an interest in microbial/molecular genetics.

The posts are permanent and superannuable under U.S.S. Salary scale (under review) £3,883 to £7,754 with entry at points appropriate to the ages, qualifications and experience of the successful candidates.

Applications (six copies, overseas applicants one copy) quoting Reference EST/32/79X and naming three referees should be lodged as soon as possible with The Secretary, The University, Dundee DD1 4HN, from whom further particulars are available. 103(A)

LUDWIG INSTITUTE FOR CANCER RESEARCH (London Branch)

(in association with the Royal Marsden Hospital and the Institute of Cancer Research, Sutton, Surrey)

UNIT OF MOLECULAR BIOLOGY (Technical staff appointments)

A new cell and molecular biology unit is to be established shortly. Its interests will centre around experimental models of breast cancer.

CHIEF TECHNICIAN

is required: a wide experience of methods of tissue culture and light microscopy is essential and some knowledge of immunological techniques is desirable. While the duties of the post are largely practical, the successful candidate will also be expected to teach and train junior staff. Starting salary will be in the range £5,472 to £6,192, plus London Weighting, according to age and experience. Duties for this post will commence in the early summer.

RESEARCH OFFICERS/ TECHNICIANS

are also required with some previous experience of techniques of tissue culture and of molecular biology or protein biochemistry. Starting salary, probably within current range of £3,582 to £4,902 plus London Weighting (Research Officer), or £3,261 to £4,680 plus London Weighting (Technician). Duties for these posts will commence in the summer and the precise grading will depend on age, experience and qualifications.

Apply with full particulars and giving the names of two referees to The Secretary, Ludwig Institute for Cancer Research (London Branch), The Haddow Laboratories, Clifton Avenue, Sutton, Surrey SM2 5PX, before April 20, 1979. 167(A)

KING'S HEALTH DISTRICT (TEACHING) DENTAL SCHOOL SCIENCE GRADUATE OR BIOCHEMIST

Required for research project to study the cellular reactions to metallic alloys. This project should be suitable for a Ph.D. thesis. Experience in macrophage tissue culture and atomic absorption analysis methods desirable.

This post is tenable for one year in the first instance (to March 31, 1980), and is subject to annual renewal.

For an informal discussion about the post contact Mr B. Eley on 01-274 6222, extension 2834 or bleep. For job description and application form please contact the District Personnel Department, King's College Hospital, Denmark Hill, London SE5 9RS. Tel: 01-274 6222, extension 2254.

Closing date: April 6, 1979. 109(A)

THE OPEN
UNIVERSITY
FACULTY OF
SCIENCE



RESEARCH
TECHNICIAN

BIOPHYSICS RESEARCH GROUP,
OXFORD RESEARCH UNIT

Applications are invited for the post of Research Technician to assist in the study of the structure and transparency of the corneal stroma by a variety of biochemical and biophysical methods, working under the supervision of Professor G. F. Elliott at the Biophysics Research Group, Oxford Research Unit. Techniques used in the laboratory include X-ray diffraction, microelectrode measurements and gel electrophoresis. Computer experience might also be useful. The successful candidate will probably have a minimum of four years experience in a biochemical/biophysical laboratory.

The post is available for three years from 1st April 1979. Salary within the Technician Grade 5 scale £3,186-£3,720 p.a. (under review) according to experience.

Application forms and further particulars are available from The Recruitment Office (JD3812/1), The Open University, P.O. Box 75, Walton Hall, Milton Keynes, MK7 6AL or telephone Milton Keynes 63404: there is a 24 hour answering service on 63868. Closing date for applications: 5th April.

101(A)

LUDWIG INSTITUTE FOR
CANCER RESEARCH
(London Branch)

(in association with the Royal Marsden Hospital and the Institute of Cancer Research, Sutton, Surrey)

UNIT OF MOLECULAR BIOLOGY
Applications are invited from suitably qualified and experienced

BIOCHEMISTS

to fill one or other of two appointments in the new Unit which will be established shortly under Dr P. S. Rudland. The principal focus of research will be on experimental aspects of mammary cancer.

For post (a), previous experience of protein and nucleic acid biochemistry is essential.

For post (b), previous experience of protein purification techniques and cell biology is essential.

Further particulars may be obtained from the address given below. Candidates for these appointments, which will commence in or before September 1979, should have gained appropriate postdoctoral experience which will be reflected by the salaries likely to be offered £5,129 to £6,080, plus London Weighting (equivalent to M.R.C. non-clinical Scientific Grade II). The appointments will be for an initial period of three years. University Superannuation Scheme available.

Apply with curriculum vitae and names of two referees to The Secretary, Ludwig Institute for Cancer Research (London Branch), The Hadow Laboratories, Royal Marsden Hospital, Belmont, Surrey SM2 5PX, before April 27, 1979. 166(A)

POSTDOCTORAL RESEARCH

Applications from Ph.D. graduates in Immunology or related area interested in studying regulatory mechanisms of the immune system and tumour immunity.

Salary is \$13,000 for the first year and \$13,800 for the second.

Send curriculum vitae and references to: Dr I. A. Ramshaw, Department of Microbiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0. W16(A)

CSIRO

AUSTRALIA

Postdoctoral Research Fellows

Fuel Geoscience Unit North Ryde, NSW

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

General: The Fuel Geoscience Unit conducts research relevant to exploration for the fossil fuels—coal, petroleum, natural gas and oil shale—and to their assessment and extraction.

Field: Coal Mine Geology—Ref. No. 602/063

Duties: To initiate and conduct research aimed at generating information relevant to coal mining prior to the construction of mine openings. The appointee will be, or should become, familiar with geological factors of importance in underground coal mining and will be required to apply some of the techniques of sedimentary (including coal) petrology and engineering geology to studies of drill core and other samples.

Qualifications: A Ph.D. or equivalent qualifications in a relevant area of geology. Experience appropriate to duties above, especially concerning the significance of the physical properties of rocks to mining practice would be an advantage.

Field: Gas in Coal Seams—Reference No. 602/060

Duties: To initiate and conduct research, within a team, into the nature and cause of gaseous outbursts in coal mines. The appointee will be required to investigate the physical and chemical constitution of coal and coal measure rocks as these relate to the occurrence and severity of outbursts and their prediction and possible prevention.

Qualifications: A Ph.D. or equivalent qualifications in a relevant field such as Geochemistry or Physical Chemistry. Familiarity with mining or geology of coal would be an advantage.

Field: Coal Utilization—Ref. No. 602/066

Duties: To initiate and conduct, within a team, research directed towards making the most

effective use of Australian coal resources, with special emphasis on conversion of coals to liquid fuels. The appointee will be required to use petrological techniques to study the properties of coals and of products obtained from coals after treatment.

Qualifications: A Ph.D. or equivalent qualifications together with interest and experience in the fields of coal petrology and coal chemistry.

Field: Coal Science—Ref. No. 602/067

Duties: The successful applicant will be required to initiate and conduct research into the physical and chemical character of coals and coal derivatives as part of a project on the fundamentals of obtaining liquid fuels from Australian coals. It is expected that this research will make considerable use of NMR studies. A Bruker CXP 100 NMR spectrometer, suitable for both high power and high resolution techniques is available for this work. A high temperature specimen probe is on order.

Qualifications: A Ph.D. degree or equivalent qualifications in physics or chemistry, with considerable experience in relevant fields of nuclear magnetic resonance spectroscopy. Experience with coal or related materials would be an advantage.

Salary: Research Scientist/Senior Research Scientist: \$A15,422—\$A22,405 p.a.

Tenure: Appointment to these positions will initially be for a period of twelve months with the possibility of extension.

Applications in duplicate, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting the appropriate reference number(s) should reach: The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, LONDON WC2R 3EH by 21st April, 1979.

Applications in U.S.A. and Canada should be sent to: The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue N.W., WASHINGTON D.C. 20036. 158(A)

THE
QUEEN'S UNIVERSITY
OF BELFAST
CHAIR OF ZOOLOGY

Applications are invited for the Chair of Zoology which will become vacant on October 1, 1979 on the appointment of Professor G. Owen as Principal of University College of Wales, Aberystwyth. The salary of the Chair is £10,575 per annum (under review) with contributory pension rights under F.S.S.U. or U.S.S.

Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. Closing date: April 27, 1979. (Please quote Ref. 79/N.) 104(A)

UNIVERSITY OF
BIRMINGHAM
DEPARTMENT OF
CANCER STUDIES
POSTDOCTORAL
RESEARCH FELLOW

is required to join a team of scientists studying the proteins of normal and transformed cells. The project will be of three years' duration and the successful candidate will be expected to have a good biochemistry background and a working knowledge of protein separation techniques. Salary will be in the range £3,883 to £6,555. Applications, giving a full curriculum vitae, a copy of the synopsis of their Ph.D. thesis and the names of two academic referees, should be sent to Professor D. G. Harnden, Department of Cancer Studies, Medical School, University of Birmingham B15 2TJ. 119(A)

UNIVERSITY OF
EDINBURGH
POSTGRADUATE
STUDENT

A vacancy will arise in October 1979 for a postgraduate interested in working in the field of mode of action of drugs and investigating the possibility of separating the contributions of efficacy and affinity to the action of drugs at H₁ and H₂ receptors.

Please send applications giving full curriculum vitae and names of referees to:

Professor E. W. Horton,
Department of Pharmacology,
1 George Square,
Edinburgh EH8 9JZ. 116(A)

**Forestry Commission, Northern
Research Station, Roslin,
Edinburgh**

Entomologist

... to join a small team conducting research into the relationship between the pine beauty moth *Panolis flammea* and its host lodgepole pine and the forest environment. The work will involve making a detailed study of the life cycle of the insect and its natural enemies, investigating the influence of lodgepole pine phenology and climatic variables in the development of populations, and examining the biological importance of host nutritional factors affecting the insect. In addition, the entomologist will study the results of these experiments to assess the factors responsible for population changes.

Candidates, normally aged under 32, should have a good honours degree or equivalent in Zoology or Forestry and at least 2 years' postgraduate experience in entomology.

Appointment as Senior Scientific Officer (£5150-£6900) or Higher Scientific Officer (£4100-£5450) according to qualifications and experience. Salaries under review. Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 12 April 1979) write to Personnel Branch, Forestry Commission, 231 Corstorphine Road, Edinburgh, EH12 7AT. Please quote ref: SA/9/DM.

Plant Pathology Laboratory

Entomologist

The Laboratory, at Harpenden, provides the scientific basis for the introduction and administration of legislation intended to prevent the start or further spread of dangerous plant diseases and pests.

This post is in the Entomology Department where the successful candidate will join a team working on the elimination of pests from imported herbaceous planting material and woody plants. This work will involve developing chemical and physical methods of treatment and liaison with importing firms.

Candidates, normally aged under 27, should have a good honours degree or equivalent in Biology, preferably including specialised studies of entomology or pest control.

Appointment as Scientific Officer (£2840-£4410; under review) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 17 April 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote ref: SB/23/AG.

**Ministry of Agriculture,
Fisheries and Food**

ALL THESE POSTS ARE OPEN TO MEN AND WOMEN.

**Ministry of Agriculture,
Fisheries and Food,
Food Science Division, London**

Food Scientists and Nutritionists

These posts are in the Nutrition Section which assists the Division in providing an advisory service on all aspects of food science, food technology and human nutrition, both at home and abroad.

Nutrition and Food Composition Studies

The scientist appointed will supervise the collection and interpretation of information on the nutrient content of food. This work will involve close liaison with statisticians, food manufacturers, caterers and dieticians to determine needs and priorities and the commissioning of analytical work on foods. In addition, the successful candidate will act as secretary to a committee on the composition of food and food products.

Candidates, normally aged under 32, should have a good honours degree or equivalent in an appropriate discipline, coupled with at least 4 years' relevant experience. The ability to evaluate information on nutrition and food composition and a familiarity with data processing is highly desirable.

Appointment as Senior Scientific Officer (£5675-£7420; under review) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme. Ref: SB/37/AE.

Analysis of Material for the National Food Survey

The successful candidate will join a team involved in analysing trends in food consumption and processing methods and assessing the effects on nutrient intake. In addition, the scientist will liaise with Government Departments, Research Councils and Industry to provide information for Ministerial briefs and the general public.

Candidates, normally aged under 27, should have a good honours degree or equivalent in human nutrition or related discipline. Experience of data processing is highly desirable. *Final year students may apply.*

Appointment as Scientific Officer (£3360-£4940; under review) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

Ref: SB/38/AE.

For further details and an application form (to be returned by 18 April 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote appropriate reference.

**Science
Group**
CIVIL SERVICE

**Tropical Products Institute
London**

Biochemist/ Plant Scientist

... to study the biochemical changes which occur in tropical products, particularly beverage crops, during handling, storage, processing and transformation. This work is undertaken with a view to improving product quality and efficiency of crop utilisation and will involve extended overseas assignments.

Candidates, normally aged under 30, should have a degree or equivalent in an appropriate discipline coupled with at least 2 years' relevant experience, preferably gained in a developing country.

Appointment as Higher Scientific Officer (£4620-£5970; under review) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme. For further details and an application form (to be returned by 17 April 1979) write to Miss C A Hall, Tropical Products Institute, 127 Clerkenwell Road, London EC1R 5DB. Please quote ref: SA/97/3D.

**Science
Group**
CIVIL SERVICE

180(A)

UNIVERSITY OF OXFORD

DEPARTMENT OF ZOOLOGY

EDWARD GREY INSTITUTE OF FIELD ORNITHOLOGY

Applications are invited for the post of Departmental Demonstrator in the above Institute from September 1, 1979. The successful candidate, who should recently have received a Ph.D. or be in his final year before submission, will be required to take part in departmental teaching and to undertake research in field ornithology. The salary will be within the University scale—£3,384 to £4,466 p.a. with membership of U.S.S.

Applications, including the names of two referees, should be submitted to the Director, Edward Grey Institute of Field Ornithology, Department of Zoology, South Parks Road, Oxford OX1 3PS by April 30, 1979. 152(A)

**UNIVERSITY OF READING
PROFESSORSHIP OF
SOIL SCIENCE**

Applications are invited for the Professorship of Soil Science, which is vacant as a result of the resignation of Professor D. J. Greenland.

Candidates should be sympathetic to the dual nature of the Department of Soil Science (which is concerned both with the fundamental aspects of Soil Science and with its relationship with agricultural, biological and other sciences) and be capable of maintaining and developing its work along these lines.

The appointment will be made from a date to be arranged with the successful candidate.

Further information may be obtained from the Registrar (Room 214, Whiteknights House), The University, Whiteknights, Reading RG6 2AH, by whom applications should be received not later than April 21, 1979. 113(A)

**UNIVERSITY OF LIVERPOOL
DEPARTMENT OF BIOCHEMISTRY**

Applications are invited for the

POSTDOCTORAL

SENIOR

RESEARCH ASSISTANT

in the Department of Biochemistry. The post is funded by a grant from the North West Cancer Research Fund to study the hormone responsiveness of a line of human breast cancer cells. Experience of tissue culture would be an advantage.

The initial salary will be within the range £3,883 to £4,882 per annum (Senior Research Assistant Ia). The appointment will be for one year in the first instance.

Applications, together with the names of two referees, should be received not later than April 13, 1979, by The Registrar, The University, Liverpool L69 3BX. Quote Reference RV/525/N. 110(A)

**UNIVERSITY OF
ASTON IN BIRMINGHAM
DEPARTMENT OF METALLURGY
AND MATERIALS**

ARCHAEO-METALLURGIST

Applications are invited for a Research post in archaeometallurgy from candidates with at minimum a good first degree in an applied physical science, together with some knowledge of microstructural methods of analysis and of archaeology. The appointment is supported by the Department of the Environment through its Ancient Monuments Laboratory and will involve:

- the investigation of archaeological finds of metallurgical significance;
- the development of suitable techniques for such investigation;
- research in the areas of provenance and technology in relation to early metallurgical operations.

Commencing salary will be within the range £3,883 to £4,631 per annum.

Application forms and further details may be obtained from the Staff Officer (quoting Ref. No. 346/N), University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET (Tel. 021 359 3611, Ext. 201) and applications should be returned not later than Friday, April 13, 1979. 111(A)

**CITY OF LONDON
POLYTECHNIC
DEPARTMENT OF BIOLOGICAL
SCIENCES
ELECTRONICS TECHNICIAN
GRADE 5**

The City of London Polytechnic require, as soon as possible, an Electronics Technician for the design and construction of electronic physiological and neurophysiological apparatus for teaching and research, together with day-to-day servicing of neurophysiological teaching laboratory, and supervision of the electronics workshop. Candidates should possess an H.N.C. in an appropriate field.

The vacancy exists in our Biological Sciences Department which is located at Calcutta House, Old Castle Street close to Aldgate and Aldgate East Underground stations.

Starting salary, which will depend on qualifications and experience, will be within the scale of £3,675 to £4,212 inclusive (under review).

Further details and an application form can be obtained from the Staff Records Officer, City of London Polytechnic, 117/119 Houndsditch, London EC3A 7BU. Please quote reference number 79/18. 148(A)

**UNIVERSITY OF BRISTOL
Long Ashton Research Station
ENVIRONMENTAL
PHYSIOLOGIST**

Required in the Microclimatology Section to study the effects of disease on the physiology of cereals in the laboratory and field. The post requires knowledge of whole-plant physiology (growth analysis, photosyntheses measurements etc.) and of micro-meteorology. Experience with instruments and a high degree of numeracy an advantage.

Appointment in the Scientific Officer grade (£2,893 to £4,415 per annum). Starting salary dependent on qualifications and experience. Non-contributory superannuation scheme.

Qualifications
Honours degree or equivalent in Botany, Applied Botany or Agricultural Botany, or an ordinary degree with relevant experience.

Applications
Written applications should be sent to the Secretary, Long Ashton Research Station, Long Ashton, Bristol BS18 9AF, by not later than April 12, 1979. 147(A)



**UNIVERSITY OF DUBLIN
Trinity College**

**Temporary Lecturer
in Physics**

Applications are invited for the above post for the academic year 1979/80.

Salary scale: £4,317 to £8,487. Appointment will be made within the range £4,317 to £4,774.

Interested persons should, in the first instance, telephone the Staff Office, Trinity College, on Dublin 772941 Ext. 1678/1775.

The closing date for receipt of applications will be April 16, 1979. W12(A)

**UNIVERSITY OF
NEWCASTLE UPON TYNE
BIOLOGICAL CONSERVATION
AND POLLUTION STUDIES**

Applications are invited for appointment to a newly established

LECTURESHIP

Applications are invited for a Science in the Faculty of Agriculture from well qualified persons with research experience in either biological conservation or pollution studies in the countryside. The main teaching duty will involve a contribution to courses in one of the above subjects: the interdepartmental Honorary Degree in Agricultural and Environmental Science.

Salary will be at an appropriate point on the Lecturers' scale: £3,883 to £7,754 per annum, according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

Further particulars may be obtained from the Senior Assistant Registrar, The University, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU, with whom applications (3 complete copies together with the names and addresses of 3 referees, should be lodged not later than May 1, 1979. Please quote reference N. 137(A)

**UNIVERSITY OF KENT
AT CANTERBURY
LECTURER AND
TUTORIAL FELLOW
IN BIOCHEMISTRY**

Applications are invited for appointment to a Lectureship and Tutorial Fellowship in Biochemistry, both of which become vacant on October 1, 1979, the former because of the appointment of the holder, Dr C. Pegg, to a Chair. Preference will be given to candidates whose research interests and experience are in mammalian biochemistry.

It is expected that the Lecturer will be appointed within the first six points of the scale £3,883 to £7,754 and the Tutorial Fellowship, which is for a fixed term of three years, will be filled within the first three points of the scale £3,883 to £6,555.

Application forms and particular may be obtained from the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT7 7NH, to whom completed application should be returned by April 13, 1979. Please quote ref. A21/79. 140(A)

TECHNISCHE HOGESCHOOL-DELFT

The Faculty of Chemistry and Chemical Engineering of the Delft University of Technology invites applications for the position of

FULL PROFESSOR OF MICROBIOLOGY

Microbiology, biology and technology are being integrated to establish a single group for biotechnology. The successful candidate will be expected to direct his/her teaching and research towards both applied and fundamental aspects of microbiology. Close cooperation with other colleagues concerned with biotechnology will be required.

Teaching: The professor will be responsible for lectures and courses in General Microbiology, Industrial Microbiology, Microbiology of Water and Wastewater, possibly Cell Biology and Microbial Genetics. The supervision of experimental work carried out by undergraduate and graduate students will also be involved.

Research: Experimental work will be concerned with microbiological systems related to industrial processes and environmental control. In addition the task includes the supervision of basis research by some staff members.

Administrative duties: The successful applicant will be expected to participate actively in the administrative tasks of the Microbiology Group and the Faculty. Additional information will gladly be supplied by the Chairman of the Appointing Committee.

Applications, including curriculum vitae, giving full details of qualifications, experience and names of at least two referees should be addressed to the Chairman of the Appointing Committee, Prof. N. W. F. Kossen, c/o Bureau van de Afdeling der Scheikundige Technologie, postbus 5045, 2600 GA Delft, The Netherlands.

W3(A)

THE QUEEN'S COLLEGE, GLASGOW SENIOR LECTURESHIP IN MICROBIOLOGY AND BIOCHEMISTRY

Salary Scale: £7,155 to £7,962 (Bar) to £9,042 per annum

Applications should have an Honours Degree in Microbiology, Biology or Chemistry, and a Higher Degree, preferably in Microbiology.

The successful candidate would be expected to play a major role in course development, research and administration within the Department of Science, as well as teaching on a wide range of courses.

Further details and application forms may be obtained from:

The Principal,
The Queen's College, Glasgow,
1 Park Drive,
Glasgow G3 6LP.

Closing date for applications: April 20, 1979.

118(A)

THE CITY UNIVERSITY DEPARTMENT OF CHEMISTRY Applications are invited for the newly established CHAIR OF INDUSTRIAL CHEMISTRY

The salary will be in the range for professorial appointments and there will be a London Allowance of £450 per annum. Superannuation will be in accordance with the provisions of U.S.S.

Further particulars may be obtained from the Academic Registrar, The City University, Northampton Square, London EC1V 0HB. The closing date for applications is May 3, 1979. Please quote ref. C/235/N. 106(A)

UNIVERSITY OF OXFORD M.R.C. UNIT FOR MOLECULAR HAEMATOLOGY. Research Assistant is required to join a newly formed M.R.C. Unit working on hereditary disorders of Human Haemoglobin Structure and Synthesis. The work involves isolation and assay of mRNA, and the characterisation of mutant globin genes by restriction endonuclease mapping and recombinant DNA techniques. Candidates should possess a degree in Biochemistry, Chemistry or a Related Scientific subject. Experience in nucleic acid research desirable but not essential. Salary on the Oxford University Research Staff Grade 1a (at present under review) £3,383 to £6,555 per annum depending upon age and experience. Application forms and further details may be obtained from: The Departmental Administrator, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford. 181(A)

UNIVERSITY OF THE WITWATERSRAND, Johannesburg, South Africa DEPARTMENT OF BIOCHEMISTRY SENIOR LECTURER/ LECTURER

Applications are invited from suitably qualified persons for appointment to the above post. Applicants are expected to have a Ph.D. or equivalent and appreciable research experience.

The salary ranges are as follows:

Senior Lecturer:

R10,144 to R13,489 per annum

Lecturer:

R7,554 to R11,007 per annum

A salary review is expected in April, 1979.

The initial notch and level of appointment will be determined in accordance with the qualifications and experience of the successful applicant.

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion or colour. Further particulars relating to this practice and the above post are included in an information sheet obtainable from the London Representative, University of the Witwatersrand, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, 2001 South Africa, with whom applications should be lodged by April 12, 1979. 128(A)

MEDICAL RESEARCH COUNCIL NATIONAL INSTITUTE FOR MEDICAL RESEARCH DIVISION OF BIOCHEMISTRY A POSTDOCTORAL

position will be available in the autumn to work with Dr Ian Kerr on the control of macromolecular synthesis and cell growth with particular reference to the antiviral and antitumour effects of interferon. Current interests are centred on the role of protein kinases and the oligonucleotide effector pppA₅p₅A₂p₅A in interferon-treated cells and the possible wider significance of the latter in normal cell growth and development.

Applicants should have either a Ph.D. or complete the requirements for one in the near future.

The appointment will be for up to three years and will be in the salary range £4,408 to £6,080 per annum according to age and qualifications, plus £450 per annum London Allowance. Superannuation provision.

Applications, giving details of qualifications, experience and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA, before April 30, 1979, quoting reference B/1. 171(A)



UNIVERSITY OF DUBLIN
Trinity College

LECTURER IN PHARMACEUTICS

Applications are invited for the above post in the Department of Pharmaceutics, School of Pharmacy, Trinity College, Dublin.

Salary Scale: £4317-£8487. Appointment is likely to be made in the range £4317-£4774. There is a non-contributory pension scheme.

Interested persons should, in the first instance, telephone the Staff Office, Trinity College, on Dublin 772941 Ext. 1678/1775.

The closing date for receipt of applications will be 7th May 1979.

W8(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF ORGANIC CHEMISTRY

Applications are invited from suitably qualified candidates for the post of LECTURER in the above department, available from April 1, 1979. Candidates should have special research interests in the chemistry of natural products, particularly in bioactive molecules.

Salary on the scale for lecturers: £3,883 to £7,754 (under review), according to age, qualifications and experience.

Applications (quoting reference number 45/8/D), giving details of research interests and an account of the chemistry that the candidate would like to pursue, with a curriculum vitae and the names and addresses of three referees, should be made no later than April 30, 1979 to the Registrar, The University, Leeds LS2 9JT (from whom further particulars may be obtained). 144(A)

UNIVERSITY OF NATAL DEPARTMENT OF BIOLOGICAL SCIENCES DURBAN

Applications are invited from suitably qualified persons regardless of sex, religion, race, colour or national origin for appointment to the post of

LECTURER

in the Department of Biological Sciences.

The present salary scale attached to the post is: R6,300 by R30 to R9,180, plus a pensionable allowance of 19.9% of basic salary and it is expected that salaries will be increased from April 1, 1979.

Preference will be given to candidates with research and teaching experience in the field of quantitative plant ecology, with emphasis on plant/herbivore interactions and the assessment of habitat condition and trend. As well as participating in the normal teaching programme, the incumbent of the post will be expected to engage and supervise research in one of the above fields.

The commencing salary notch will be dependent on the qualifications and/or experience of the successful applicant. In addition, an annual vacation savings bonus is payable, subject to Treasury regulations.

Application forms, further particulars of the post and information on pension, medical aid, group insurance, staff bursary, housing loan and subsidy schemes, long leave conditions and travelling expenses on first appointment are obtainable from the Registrar, University of Natal, King George V Avenue, Durban, 4001, with whom applications, on the prescribed form, must be lodged not later than April 30, 1979, quoting the reference Adv. D13/79. W4(A)

UNIVERSITY OF GLASGOW RESEARCH ASSISTANT

Applications are invited for the post of research assistant in the Department of Veterinary Pathology, University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, to undertake studies on immunity in the respiratory tract of the dog with special reference to the prophylaxis of canine respiratory disease. Applicants should have, or expect to gain, a good honours degree in immunology or another relevant biological science. The successful applicant may apply to study for a postgraduate degree.

The appointment, which is funded by the Wellcome Trust, will be for three years and will be superannuated. Salary will be within the range £3,883 to £4,382 of the scales for Research and Analogous Staff.

Applications with the names of two referees should be submitted to Dr I. A. P. McCandlish at the above address, from whom further particulars can be obtained. Closing date for applications: March 30, 1979.

In reply please quote Ref. No 4405M. 175(A)

GEOSCIENTIST

The Whiteshell Nuclear Research Establishment, Pinawa, Manitoba has a vacancy for a Geoscientist to join a multi-disciplinary group verifying the concept of deep underground disposal of nuclear wastes. The job will involve specifically ensuring that backfilling, chemical buffering and sealing of a vault in hard rock are given appropriate attention within an overall research and development programme. This will include (1) supervising contracts with outside organizations such as government agencies and departments, consultants and universities; (2) setting up an experimental programme within AECL; (3) providing the necessary geotechnical expertise to ensure that all laboratory scale tests and experiments are representative of the geologic reality; (4) help to design tests of a scale appropriate for the demonstration of safe disposal of nuclear wastes in appropriate geologic formations.

The successful candidate should have a PhD or MSc degree in one of the Geosciences and a good background in Chemistry. Experience with clay minerals, radioactive tracers, computer modelling techniques, mining engineering, backfilling or sealing of mines or boreholes are all desirable but not necessarily essential. Preference will be given to candidates with the personal qualities and experience necessary to both supervise contracts and work in a team or group situation.

Starting salaries will be commensurate with qualifications and experience. AECL has a comprehensive benefits package and relocation assistance is provided.

The Whiteshell Nuclear Research Establishment employs approximately 800 people and is located about 105 km northeast of Winnipeg near the Whiteshell resort area. Pinawa is a modern townsite about 15 km from WNRE.

Men and women who may be interested in this position and possess the necessary qualifications should apply in writing giving full particulars to Personnel Supervisor, Atomic Energy of Canada Research Company,

WHITESHELL NUCLEAR RESEARCH ESTABLISHMENT PINAWA, Manitoba, ROE 1LO.



L'Énergie Atomique
du Canada, Limitée

Atomic Energy
of Canada Limited

W15(A)

UNIVERSITY OF EDINBURGH DEPARTMENT OF BOTANY Applications are invited for a POSTDOCTORAL RESEARCH FELLOW

to work with Dr. A. Trewavas on a three-year project supported by the A.R.C. The work will involve preparation of cDNA to cellulase mRNA to investigate the mechanisms of transcriptional control induced by auxin. Experience of techniques in this area of work would be an advantage but is not essential.

Salary is on the scale £3,883 to £6,555 per annum.

Applications (C.V. and two referees) may be made to Dr A. Trewavas, Department of Botany, The King's Buildings, Edinburgh, EH9 3JH, from whom further details may be obtained. Please quote Reference 5011. 115(A)

UNIVERSITY OF LONDON GOLDSMITHS' COLLEGE New Cross, London, SE14 6NW SCHOOL OF SCIENCE AND MATHEMATICS DEPARTMENT OF GEOLOGY Applications are invited for a LECTURERSHIP IN GEOLOGY

which is available from September 1, 1979. Applicants should be currently carrying out research work in one or other of the following fields: geochemistry/economic geology or economic stratigraphy.

Salary on scale £4,575 by 12 increments to £7,539 per annum inclusive of London Allowance.

Write for further details, to the Personnel Officer, to whom applications should be sent by April 12, 1979. 176(A)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF GENETICS
Applications are invited for the post of Grade 2B Laboratory Technician in the Department of Genetics to help with laboratory classes and assist generally as required. Applicants should possess ONC/OND qualifications or 2 'A' levels in appropriate science subjects or Part I City & Guilds Science Laboratory Technician Certificate. Experience of working in either school or University laboratories would be an advantage. Preference given to candidates experienced in Microbial techniques.

Salary in the range of £2,529 to £2,880 per annum (under review).

Application forms can be obtained from the Establishment Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Telephone Nottingham 56101 extension 3397. Please quote reference No. GN. 3/79. 161(A)

UNIVERSITY OF EDINBURGH RESEARCH ASSOCIATE

Applications are invited from Honours graduates in Botany or Microbiology to work on a project investigating the efficiency of nitrogen fixation in legume root nodules. The project is funded by the A.R.C. and is under the supervision of Dr R. O. D. Dixon. The post will be tenable for three years from May 1, 1979. The salary is in the range £3,384 to £4,133 per annum.

Applications should be submitted by April 10, 1979 to Dr R. O. D. Dixon, Botany Department, The King's Buildings, Mayfield Road, Edinburgh EH9 3JH. Please quote Reference 5012. 114(A)

Recombinant DNA Research

Genentech, the leading company in the application of recombinant DNA techniques to problems in human health care, is expanding its research program and has opportunities for outstanding scientists.

Qualified individuals will be joining an innovative research team working in a unique scientific environment. Genentech offers the scientific freedom to develop and direct your own independent research program and the intellectual stimulation of interacting with quality associates while contributing to the understanding and expansion of this new technology.

Senior Protein Recovery Scientist/Bioengineers. Opportunities exist to participate in the development of new procedures for the recovery and purification of polypeptides from microorganisms. A Ph.D. or equivalent in biochemistry or bioengineering plus at least three years relevant post-doctoral or industrial experience is required.

Senior Level Positions. Openings are available for biochemists, microbiologists, molecular biologists, molecular immunologists, and protein chemists. Applicants should have a Ph.D., postdoctoral research experience, and an outstanding research record.

Postdoctoral Position. Genentech has a postdoctoral research program. Opportunities exist for participation in ongoing projects or for initiation of new basic research projects. The two year appointments carry a first year salary of \$15,000.

Support Positions. B.S. or M.S. level biochemists with expertise in nucleic acids or protein chemistry are invited to apply.

Genentech offers an exceptional working environment and an outstanding compensation and benefits package. Please send curriculum vitae, reprints of pertinent publications, and a list of three references to Personnel Department, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080.

We are an equal opportunity employer.

W11(A)

CSIRO AUSTRALIA

Postdoctoral Research Fellow

Division of Food Research North Ryde NSW

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: Plant Physiology

General: The research programmes of the Division include many aspects of the physical and biological sciences related to the handling, processing and storage of foods and to the needs of the food industry and the consumer. The Plant Physiology Group within the Food Research Laboratory at North Ryde, NSW, is conducting major investigations into the pre- and post-harvest physiology and biochemistry of plants and their fruits.

Duties: The appointee will join a group of plant biochemists and physiologists who are studying the effects of temperature, particularly in the chilling range, on plants and their fruits. The appointee will develop methods of determining chilling injury in plants suitable for application in plant breeding programmes to increase chilling resistance.

Qualifications: A PhD or equivalent in an appropriate field and proven research ability. It is expected that the appointee will be a plant physiologist/biochemist with a demonstrated interest in genetics or plant breeding, or a geneticist/plant breeder with a strong background in plant physiology or biochemistry.

Salary: Research Scientist: \$A15,422—\$A18,904 p.a.

Tenure: This position is available for a period of one year with the possibility of an extension to two years.

Applications in duplicate, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting reference number 300/716 should reach: The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by April 21, 1979. Applications in U.S.A. and Canada should be sent to:—The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A. 160(A)

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(UNIVERSITY OF LONDON)

Keppel Street, WC1E 7HT

Lecturer/Senior Lecturer in Virology

Applications are invited for the above post in the Arbovirus unit of the Departments of Entomology and Medical Microbiology. Candidates should have gained a Ph.D. in Virology or a related discipline, or a medical degree, and should have at least 5 years' experience in different aspects of virus research such as tissue culture, serology and animal models. Field experience, although not essential, is desirable, and the successful candidate will be expected to undertake visits abroad at short notice if necessary.

The appointment will be made within the salary scales for university Lecturers (£3,883 to £7,754) or Senior Lecturers (£7,506 to £9,263) or the appropriate scale for clinically qualified staff. The post will be tenable at the Field Station at St Albans. Application forms, which should be returned by May 17, and further information can be obtained from F. C. Ball, Assistant Secretary at the School. 146(A)



UNIVERSITY OF DUBLIN
Trinity College

Environmental Sciences Unit

LECTURESHIPS

The Environmental Sciences unit has the function of encouraging interdisciplinary research and postgraduate teaching especially between the Departments of Botany, Geography, Geology, Microbiology and Zoology.

Applications are invited for two Lectureships in Environmental Sciences. Ideally, candidates should have research or applied experience in one of the following areas:

Whole Ecosystem field studies: Microbial aspects of Ecology:

Applied Pollution Ecology: Ecological Systems Analysis:

Geochemistry of Contemporary Sediments.

Salary Scale: £4,317 to £8,487 p.a. Appointment, in the case of each lectureship, is likely to be made within the range £4,317 to £4,774 p.a. There is, in addition, a non-contributory pension scheme.

Persons interested in making application for these posts should, in the first instance, Telephone the Staff Office, Trinity College, on Dublin 772941 ext. 1678/1775.

The closing date for receipt of completed applications will be April 16, 1979. W14(A)

UNIVERSITY OF BRISTOL

Applications are invited for the post of **LECTURER IN ANATOMY**

A vacancy exists for a lecturer with a primary responsibility to the teaching of medical students. As well as being responsible for the teaching of gross anatomy to medical, dental, veterinary and science students, the Department offers courses in neuro-anatomy, endocrinology, reproduction, embryology, dental anatomy and physical anthropology, with specialised teaching in each of these topics to an honours degree level.

The Department has excellent facilities for research and is presently organised into a number of research groups working in the following areas: biochemistry and physiology of peptide-secreting neurones, neuroanatomy of dopamine pathways, electrophysiology of the C.N.S., control of parturition, bone mechanics, tooth eruption and physical anthropology.

Salary will be on the scale £3,883 to £7,754 (under review) and letters of application including the names and addresses of three referees should be sent to the Registrar and Secretary, University of Bristol, Senate House, Bristol BS8 1TD, before May 18, 1979 (quoting ref. TLJ). Interested persons are welcome to visit the department by arrangement with the Head of Department's secretary (Tel: 0272-24161, Extension 367). 141(A)

UNIVERSITY OF BRISTOL RESEARCH ASSISTANT

Applications are invited from graduates or students about to graduate in Physiology, Zoology or a related science who would like to join an active research group using electrophysiological methods to investigate the role of the nervous system in labour, lactation and other aspects of mammalian reproduction. A detailed knowledge of electrophysiological recording methods is not essential.

The appointment is funded by the Medical Research Council for three years at a commencing salary of £3,384 (Range 1B) (scale under review).

Applications giving details of age, qualifications and the names of two referees should be sent by April 20, to Dr D. W. Lincoln, The Medical School, University Walk, Bristol BS8 1TD, from whom further particulars are available (Tel: Bristol 24161, ext. 557). 154(A)

UNIVERSITY OF CAMBRIDGE/ ROYAL BOTANIC GARDEN EDINBURGH RESEARCH ASSOCIATE IN PLANT TAXONOMY

Applications are invited for a Research Associate to help in the preparation of a new Flora of Cultivated Plants. This post is funded by a grant from the Stanley Smith Horticultural Trust and will be for two years with a possible extension for a third year. Applicants should have a Ph.D. in higher plant taxonomy or equivalent experience. Salary, based on the S.R.C. 1A scale, ranging from £3,883 to £4,882 per annum. The successful candidate will work for the most part at the Royal Botanic Garden Edinburgh.

Send curriculum vitae (three copies) and names of two referees to: Dr S. Walters, University Botanic Garden, Cambridge CB2 1JF. Closing date: April 16, 1979 132(A)

UNIVERSITY OF SURREY DEPARTMENT OF METALLURGY AND MATERIALS TECHNOLOGY CHEMIST or PHYSICIST

A vacancy has arisen in the Department of Metallurgy and Materials Technology for a chemist or physicist to work on the surface chemistry of carbon fibres with Dr W. Watt, F.R.S.

The work will involve the desorption of surface species with increasing temperature, their identification and quantification with mass spectrometry and interpretation of results. High vacuum, mass spectrometry and surface chemistry techniques are involved. The work should be of interest to a graduate or post doctorate wishing to gain experience in these important fields and their application to the surface chemistry of high strength high modulus carbon fibres.

The apparatus is fully functional. Many results have already been obtained and the work has reached a very interesting stage. Liaison will be maintained with Materials Department, Royal Aircraft Establishment.

The appointment will be for one year with starting date as soon as possible.

Salary will be in the range £3,384 to £4,133 for graduate or £4,382 to £5,122 for postdoctorate, with starting point dependant on qualifications.

Applications, with full curriculum vitae, to:

Professor J. E. Bailey,
Department of Metallurgy
and Materials Technology,
University of Surrey,
Guildford, Surrey GU2 5XH.

126(A)

THE UNIVERSITY OF MANCHESTER EXPERIMENTAL OFFICER DEPARTMENT OF PHARMACY

The person appointed will be responsible for the development and co-ordination of analytical techniques in research dealing with pharmacokinetics, drug metabolism and toxicology. The techniques primarily involve the use of chromatography, spectroscopy and radioactivity. The successful candidate will also help plan and run kinetic experiments in animals and man, and help in processing the data using computational techniques.

Applicants must be graduates in either chemistry, biochemistry, or a related science, with a minimum of two years' relevant experience post degree.

Initial salary in the range per annum £3,384 to £5,604, superannuation under U.S.S.

Particulars and application form (returnable by April 20) from the Registrar, The University, Manchester M13 9PL. Quote reference: 38/79/N 177(A)

**QUEEN ELIZABETH
COLLEGE**
Kensington
(University of London)
DEPARTMENT OF
MATHEMATICS
Part-time
LECTURERS

required, in session 1979-80 only, to teach two courses to first year students. One course is **CALCULUS AND ALGEBRA** and the other is **MECHANICS**. Each involves 40 lectures plus some tutorials, marking and examining. Fee £700 for each course. Apply to Professor W. B. Bonnor or Departmental Secretary at Queen Elizabeth College, Campden Hill Road, London W8 7AH. Tel: 01-937 5411. 131(A)

**MOLECULAR BIOLOGY OF
ONCOGENIC VIRUSES
ASSISTANT OR
ASSOCIATE MEMBER**

The Division of Virology, St Jude Children's Research Hospital, invites applications from individuals with promise (assistant) or evidence (associate) of outstanding research abilities. We favour candidates with interests in nuclear DNA viruses (papova, adeno, herpes) and experience in recombinant DNA technology. However, exceptional candidates with other research interests will be considered. This is a full-time research position with no teaching or service responsibilities. St Jude Hospital is an equal opportunity employer. Please submit curriculum vitae, summary of research plans, and names of three referees to: Dr Allan Granoff, Chairman, Division of Virology, St Jude Children's Research Hospital, Memphis, TN 38101. W1(A)

**UNIVERSITY OF
DUNDEE
DEPARTMENT OF
BIOCHEMISTRY
POSTDOCTORAL
RESEARCH FELLOW**

Applications are invited for an M.R.C.-supported postdoctoral fellowship to continue highly-productive work on the perinatal triggering and development of enzymes, particularly those in the endoplasmic reticulum and concerned with drug metabolism. The successful applicant would join another Postdoctoral Fellow and a technical team utilising a wide range of techniques, including hormone assay and cell culture, but proved biochemical research ability and imagination are more important than experience in these particular techniques.

The appointment will be available from June 1, 1979 until September 30, 1981. Renewal for a subsequent period may prove possible. Salary within the range £3,883 to £6,555 (under review) with initial placing dependent on age, qualifications and experience. Further information is available from Professor G. J. Dutton.

Applications, quoting Reference EST/39/79K and containing full curriculum vitae and the names of two referees, should be lodged by April 16, 1979 with The Secretary, The University, Dundee DD1 4HN. 135(E)

**UNIVERSITY OF
SOUTHAMPTON
SCHOOL OF BIOCHEMICAL
AND PHYSIOLOGICAL
SCIENCES
POSTDOCTORAL
RESEARCH FELLOWSHIP
PHYSIOLOGY/
PHARMACOLOGY**

Applications are invited for a POSTDOCTORAL RESEARCH FELLOWSHIP IN PHYSIOLOGY. Candidates should have a scientific and/or medical qualification and research experience, preferably with publications, in an aspect of Physiology and/or Pharmacology. It would be helpful if the applicant had particular interest in cardiovascular and/or renal physiology and in the mechanism of action of angiotensin. The successful applicant will join research teams actively involved in transport studies renal physiology and hypertension and/or studies into the physiological effect of burns. The Research Fellow may be called upon to participate in research supervision and from time to time in a small amount of teaching of courses in both the Faculty of Science and in the Faculty of Medicine. Excellent facilities for research in all aspects of physiology, pharmacology, histology and histochemistry are available. The appointment is for one year in the first instance, is renewable and will commence as soon as possible. Further particulars are obtainable from Professor K. A. Munday.

Salary Range: £3,883 to £4,882 per annum (under review). USS benefits. Initial placing according to qualifications and experience. Applications giving date of birth, curriculum vitae and the names and addresses of three referees, should be sent by April 20, 1979 to: Mrs P. Vaughan-Smith, The University, Southampton SO9 5NH, quoting reference 1061/R. 162(E)

COURSES

**University of London
ROYAL POSTGRADUATE MEDICAL SCHOOL
ADVANCES IN IMMUNOLOGY
14-18 MAY 1979**

Recent Advances in Immunology will be presented in small symposia, by experts in their particular fields, followed by panel discussions. There will also be presentations of clinical material and current research at the Royal Postgraduate Medical School. The course is intended for those who already have a background in Immunology and will be of interest to both clinicians and to non-clinical immunologists.

Contributors include:

Professor J. R. Batchelor	Dr. M. J. Crumpton
Professor A. J. S. Davies	Dr. A. Feinstein
Dr. S. Gordon	Dr. G. R. V. Hughes
Professor J. H. Humphrey	Dr. A. B. Kay
Dr. G. G. B. Klaus	Professor P. J. Lachmann
Dr. W. E. Parish	Professor J. Pepys
Dr. M. B. Pepys	Professor D. K. Peters
Dr. M. C. Raff	Professor I. M. Roitt
Professor J. Soothill	Dr. C. J. F. Spry
Dr. M. J. Taussig	

Special Guest Lecturer: Professor R. R. Porter

Course fee: £90 (including catering)

Course organisers: Dr. M. B. Pepys and Dr. C. J. F. Spry

Application forms available from:

The School Office (SSC)
Royal Postgraduate Medical School,
Hammersmith Hospital,
Du Cane Road,
London W12 0HS.
Telephone: 01-743 2030 ext 351

130(D)

**THE ANIMAL
HEALTH TRUST
WOOLDRIDGE FARM
LIVESTOCK FELLOWSHIPS**

Applications are invited from veterinarians and scientists with postgraduate research experience who will design and undertake studies of the major factors influencing the health and productivity of cattle, sheep and pigs.

The fellowships are tenable for up to three years at an agreed place of work commencing October 1, 1979.

Stipends, depending on age and experience, are within the range £3,500 to £5,000 per annum. A reasonable sum will be allowed for approved expenditure.

Applications which must be sponsored by the head of the department in which the candidate proposes to work, should include the candidate's formal curriculum vitae, full details of the proposed research programme, and the names and addresses of three referees.

Further details and application forms are obtainable from the Director, The Animal Health Trust, Lanwades Hall, Kennett, Nr. Newmarket, Suffolk CB8 7PN. Closing date April 20, 1979. 121(E)

**UNIVERSITY OF
NOTTINGHAM
DEPARTMENT OF CHEMISTRY
TEACHING FELLOW IN
INORGANIC CHEMISTRY**

Applications are invited for a University Teaching Fellowship from candidates who have recently obtained, or expect shortly to obtain, a Ph.D. degree. The person appointed will be required to help with the teaching of Inorganic Chemistry and will also be expected to join a research group with Professor J. J. Turner and Dr M. Poliakoff working in the areas of laser photochemistry and matrix isolation of inorganic species.

The appointment will be for three years and within the salary range £3,883 to £4,631 p.a. (under review).

Further information can be obtained from Professor Turner (Department of Chemistry); applications enclosing a curriculum vitae and the names of two referees should be sent to the Staff Appointment Officer, University of Nottingham, University Park, Nottingham NG7 2RD. 163(E)

FELLOWSHIPS

**UNIVERSITY OF
ABERDEEN
DEPARTMENT OF
MICROBIOLOGY
POSTDOCTORAL
RESEARCH FELLOWSHIP**

Applications are invited for an S.R.C. Research Fellowship working with Dr W. A. Hamilton and Dr I. R. Booth on the role of ion transport systems in energy transduction in bacteria.

Appointment will be from October 1, 1979 and will be for one year in the first instance with possible extension up to a maximum of three years.

Salary within Range 1A, £3,883 to £4,631 per annum, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by April 13, 1979. 117(E)

**UNIVERSITY OF
ABERDEEN
DEPARTMENT OF BIO-MEDICAL
PHYSICS AND BIO-ENGINEERING
Summer School in Medical
Physics for Schools'
Physics Teachers**

From 20-24 August, 1979, the above Department will be running a Summer School in Medical Physics to show how current ideas and practices in medical physics may be used to illustrate fundamental principles.

The course is intended primarily for schools' sixth form physics teachers but is also open to members of other educational establishments.

Further details and application forms from the Course Organiser, Dr P. P. Dendy, Department of Bio-Medical Physics and Bio-Engineering, Foresterhill, Aberdeen. 151(D)

**UNIVERSITY OF
THE WITWATERSRAND,
Johannesburg, South Africa
SOLID STATE PHYSICS
RESEARCH UNIT
SENIOR
RESEARCH FELLOW/
RESEARCH FELLOW**

Applications are invited for appointment to the above two posts. Duties are to be assumed on a date to be negotiated.

Research Fellowships carry very light teaching responsibilities, an appointments are normally for a period of two to three years, although short-term contracts may be considered. The interests of the Unit include low temperature physics, E.S.R., N.M.R., X-ray diffraction, optical and other studies of defects in solids and theoretical solid state physics.

The salary ranges are:

Senior Research Fellow:

R10,144 to R13,489 per annum

Research Fellow:

R7,554 to R11,007 per annum

A salary review is expected in April 1979.

The initial salaries for both posts will be determined according to the qualifications and experience of the appointees. A grant towards removal expenses is normally made.

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion or colour. Further particulars relating to this practice and to the above posts are included in an information sheet obtainable from the London Representative, University of the Witwatersrand, Chichester House, 27 High Holborn, London WC1V 7HE, or from the Registrar, University of the Witwatersrand, Jan Smuts Avenue Johannesburg, 2001 South Africa, with whom applications should be lodged by April 12, 1979. 127(E)

FELLOWSHIPS—continued

UNIVERSITY OF
DUNDEE

Candidates with appropriate post-graduate qualifications in Biochemistry are invited to apply for appointment to a

POSTDOCTORAL

RESEARCH FELLOWSHIP

to work with Dr J. L. Young (Dept. of Obstetrics and Gynaecology) and Dr D. A. Stansfield (Dept. of Biochemistry) on a M.R.C.-funded study of the hormone receptor/adenylate cyclase complex of the corpus luteum.

The appointee will be based in the Department of Obstetrics and Gynaecology at Ninewells Hospital and Medical School, Dundee, but close co-operation with the Department of Biochemistry will be maintained.

The appointment is available for approximately three years. The initial salary will be dependent of qualifications and experience but within the range £3,883 to £6,555.

Applications in writing, quoting Reference EST/41/79J and containing the names of two referees, should be lodged as soon as possible with The Secretary, The University, Dundee DD1 4HN. 134(E)

UNIVERSITY OF
ABERDEENDEPARTMENT OF CHEMISTRY
POSTDOCTORAL
RESEARCH FELLOWSHIP

Applications are invited for an R.C. Research Fellowship working with Dr A. R. West on novel, solid state energy storage systems.

Appointment will be from April 1, 1979, or as soon thereafter as possible, and will be tenable for two years. Commencing salary within Range 1, £3,883 to £4,382 per annum, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen with whom applications (2 copies) should be lodged by April 27, 1979. 136(E)

THE UNIVERSITY
OF SUSSEX
SCHOOL OF MOLECULAR
SCIENCES

RESEARCH FELLOWSHIPS

Two Research Fellowships available for up to three years' research on M.R.C. projects related to the mechanism of action of metallo-enzymes. Ph.D. in chemistry or biochemistry required with experience in one or more of the following fields: e.p.r. of free radicals or transition metals; molybdenum chemistry; enzyme mechanisms or enzyme isolation; cryobiochemistry; fast reactions. Salary on the scale £3,883 to £6,555 p.a. (under review) with superannuation benefits. Open to men and women. Full details of experience and the names of two referees should be sent to Dr R. C. Bray, School of Molecular Sciences, University of Sussex, Falmer, Brighton, BN1 9QJ. 105(E)

RESEARCH FELLOWSHIP
INSTITUTE OF CELL
BIOLOGY

Swiss Federal Institute of
Technology (ETH)
Zürich—Switzerland

A
POSTDOCTORAL FELLOW

is required from August 1, 1979 to join a group working on structural aspects of chromatin and DNA using biophysical (electron microscopy, laser light scattering) and biochemical techniques. Experience in molecular biology or physico-chemistry of macromolecules desirable.

Salary in accordance with age and experience on ETH Grade I (Sfr. 41'600.— to 48'900.—).

Enquiries or applications with curriculum vitae and names of two referees to:

Dr Th. Koller,
Institute for Cell Biology,
ETH-Hönggerberg,
CH-8093 Zürich,
Switzerland. W18(E)

THE QUEEN'S
UNIVERSITY OF BELFAST
ROBERT W. WALLACE
MEMORIAL
STUDENTSHIP

This Studentship is open to graduates in Agriculture, Agricultural Science or Science for research in the Department of Mycology and Plant Pathology of the Faculty of Agriculture and Food Science on gangrene and related tuber diseases of potatoes. The holder will be required to study for a higher degree of the University. The value of the Studentship, which is tenable for three years from October 1979, is £1,650 p.a. (subject to review), plus fees and a sum of up to £150 per annum will be available for travelling and other expenses.

Candidates should apply to the Dean, Faculty of Agriculture and Food Science, Newforge Lane, Belfast, BT9 5PX, for an application form which should be returned by April 27, 1979. 155(F)

EDINBURGH
UNIVERSITY
SCIENCE STUDIES
UNITPostgraduate
Studentships

available for research in social and historical aspects of science and technology, including Appropriate Technology for the Third World. Further details from: Secretary, Science Studies Unit, 34 Buccleuch Place, Edinburgh EH8 9JT. 142(F)

THE UNIVERSITY
OF MANCHESTER

Applications are invited from graduates or those expecting to graduate in 1979 for:

RESEARCH STUDENTSHIP
IN SCIENCE AND MEDICINE

Value as for S.R.C. studentships, renewable after first year.

Particulars and application forms (returnable by June 1) from the Registrar, The University, Manchester M13 9PL. Quote ref: 55/79/N. 108(F)

AWARDS

IMPERIAL COLLEGE
London, S.W.7

S.R.C. C.A.S.E. AWARD

Applications are invited for an S.R.C. C.A.S.E. Studentship to investigate Combustion in Heat-recirculating Burners.

Candidates should be graduates, or expecting to graduate in 1979, with a good honours degree. The research project, concerned with pollution-free burning of poor fuels and lean mixtures in spouted beds and heat exchangers, will be carried out in collaboration with the British Petroleum Research Centre at Sunbury.

In addition to the standard S.R.C. rates (currently £1,990 per annum) the bursary will include a financial contribution from British Petroleum Limited. Additional allowances apply in the case of married students and those with industrial experience.

The successful candidate will be expected to proceed to the Ph.D. and D.I.C. degrees.

Applications, including the names of two referees, should be sent as soon as possible to Professor F. J. Weinberg, Department of Chemical Engineering and Chemical Technology, Imperial College, Prince Consort Road, London SW7 2BY. 133(N)

GRANTS & SCHOLARSHIPS

THE ANIMAL
HEALTH TRUST
RESEARCH TRAINING
SCHOLARSHIPS

Scholarships for veterinary or science graduates who are under 30 years of age and wish to pursue a veterinary research career.

Value £1,500 per annum plus a reasonable contribution towards essential fees.

Tenable at appropriate U.K. institutions for up to three years.

Applications which must be sponsored by the head of the department in which the candidate proposes to work, should include the candidate's formal curriculum vitae, full details of the proposed research programme, and the names and addresses of three referees.

Further details and application forms are obtainable from the Director, The Animal Health Trust, Lanwades Hall, Kennett, Nr. Newmarket, Suffolk CB8 7PN. Closing date April 20, 1979. 120(H)

ASSISTANTSHIPS

UNIVERSITY COLLEGE
LONDONDEPARTMENT OF CHEMISTRY
POSTDOCTORAL RESEARCH
ASSISTANT

Applications are invited for a post-doctoral research assistantship tenable from about October 1, 1979, for one year with the possibility of extension for a second year.

Initial salary in range £3,909 to £4,408 (under review) depending on age and experience, plus £450 London Allowance. The research assistant will work with Professor F. Sondheimer on the synthesis of nonbenzenoid aromatic compounds and related substances.

Applications (one copy) with a brief curriculum vitae and names and addresses of two referees should be sent as soon as possible to Professor F. Sondheimer, Chemistry Department, University College London, 20 Gordon Street, London WC1H 0AJ. 173(P)

UNIVERSITY COLLEGE
LONDONDEPARTMENT OF PHYSICS
AND ASTRONOMYApplications are invited for a
POSTDOCTORAL RESEARCH
ASSISTANTSHIP

tenable for up to two years from October 1, 1979. This is funded as part of an S.R.C. grant for theoretical research on Interactions of Hadrons, Nuclei and Quarks.

Initial salary will normally be in the range £3,909 to £4,408 plus £450 London Allowance and the post carries U.S.S. benefits.

Applicants are requested to send curriculum vitae including list of publications, and also to arrange for three references to be sent direct to Professor L. Castillejo, Department of Physics and Astronomy, University College London, Gower Street, London WC1E 6BT. 178(P)

SEMINARS & SYMPOSIA

THE 27th SYMPOSIUM OF VERTEBRATE PALAEONTOLOGY AND COMPARATIVE ANATOMY is to be held at Oxford from September 24 to September 28, 1979. Anyone wishing to attend may obtain further particulars and a booking form from Dr T. S. Kemp, University Museum, Parks Road, Oxford OX1 3PW. 139(M)

September 23-29, 1979. International Symposium on E.S.R. in Chemistry. Thessaloniki (Prof. A. Anagnostopoulos, Inorganic Chemistry Laboratory, School of Chemical Engineering, Faculty of Technology, Aristotle University of Thessaloniki, Greece). W13(M)

CONFERENCES

AN INTERNATIONAL CONFERENCE ON
CELL PROLIFERATION IN THE
GASTROINTESTINAL TRACT

Will be held in Newcastle upon Tyne, England
on September 10, 11 and 12, 1979

Speakers include: N. A. Wright, R. P. C. Rijke,
R. F. Hagemann, C. S. Potten, J. P. Sunter and M. Lipkin

Contributions are invited.

Further particulars may be obtained from Dr D. R. Appleton,
Department of Medical Statistics, University of Newcastle
upon Tyne, NE2 4AA, England. 153(C)

STUDENTSHIPS

MEDICAL RESEARCH COUNCIL

Clinical and Population Cytogenetics Unit

Applications are invited for graduates with good honours degrees and from prospective graduates for

MRC RESEARCH STUDENTSHIPS

that have been requested for October 1979. Awards will be in the fields of meiosis immunoglobulin production *in vitro* and computer pattern recognition. Studentship will be for a maximum period of 3 years and successful applicants will be expected to register for the degree of Ph.D. at the University of Edinburgh.

the Administrative Officer, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU. 174(F)



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THURSDAY, NOVEMBER 4, 1869	
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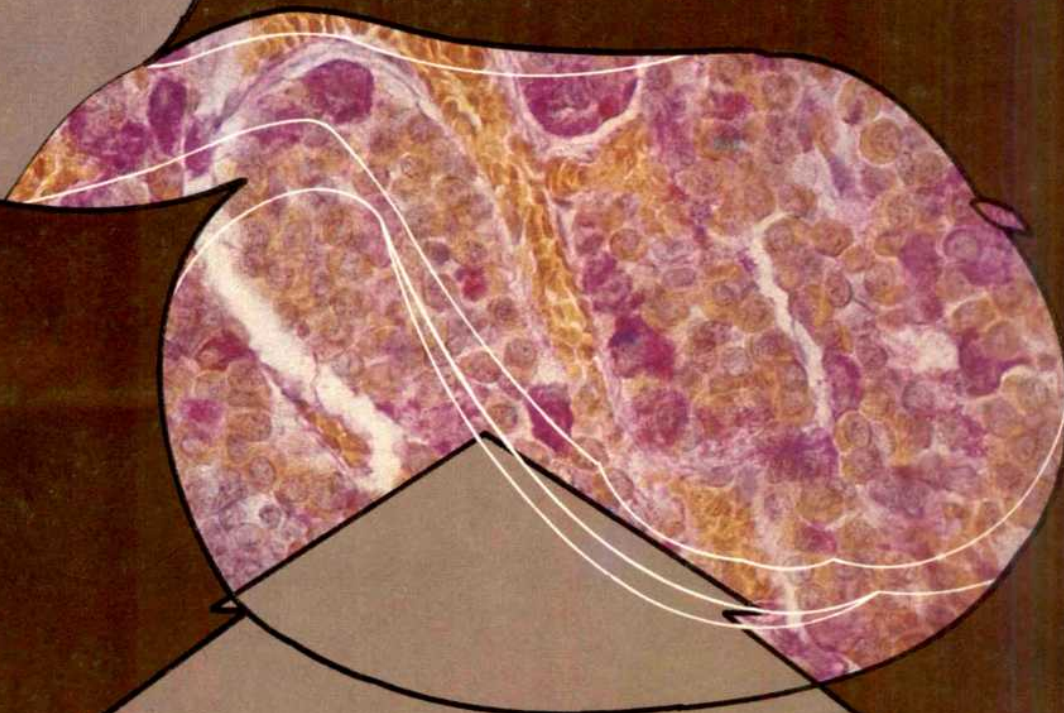
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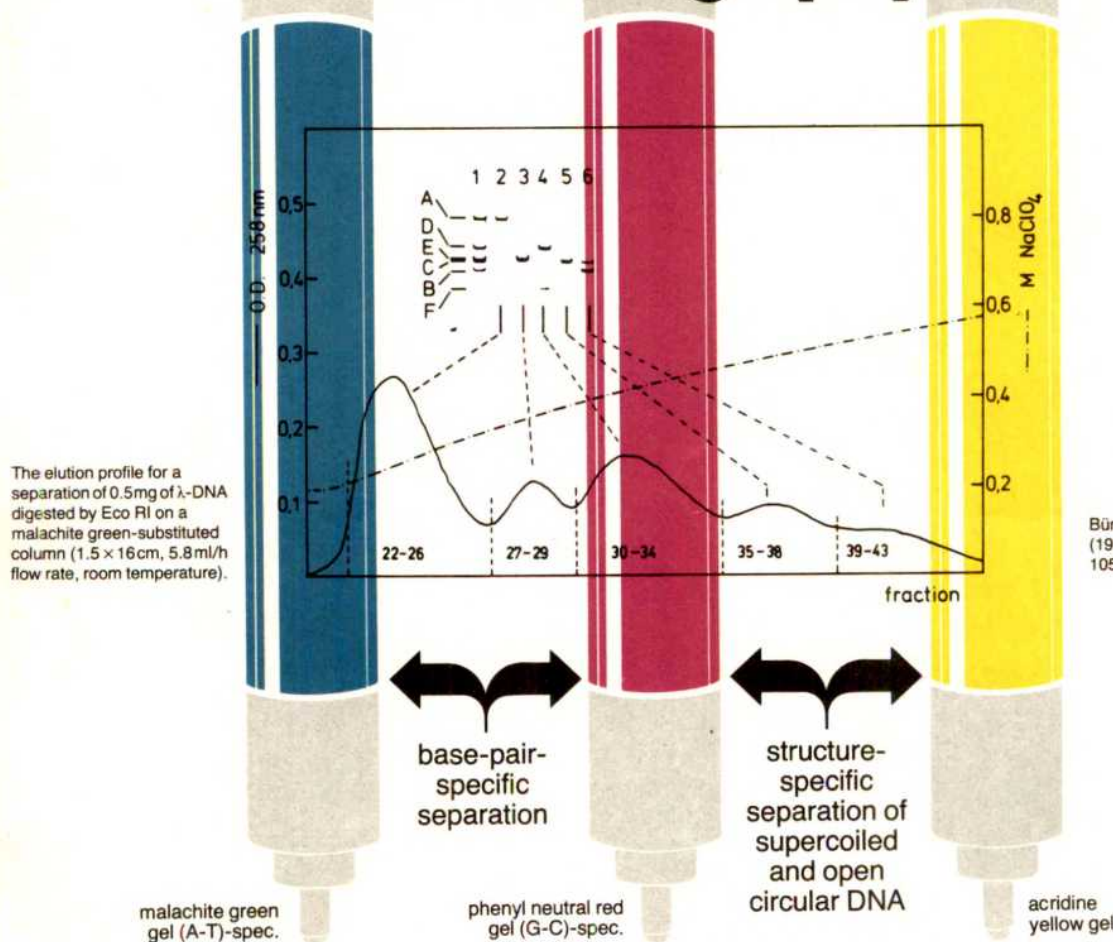
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● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

● Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.

● 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

Articles should be accompanied by an abstract of not more than fifty words. Letters should begin with a paragraph giving the background and main conclusions in terms intelligible to as wide a readership as possible.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; $\exp(a)$ is preferred to e^a if 'a' is more than one character.

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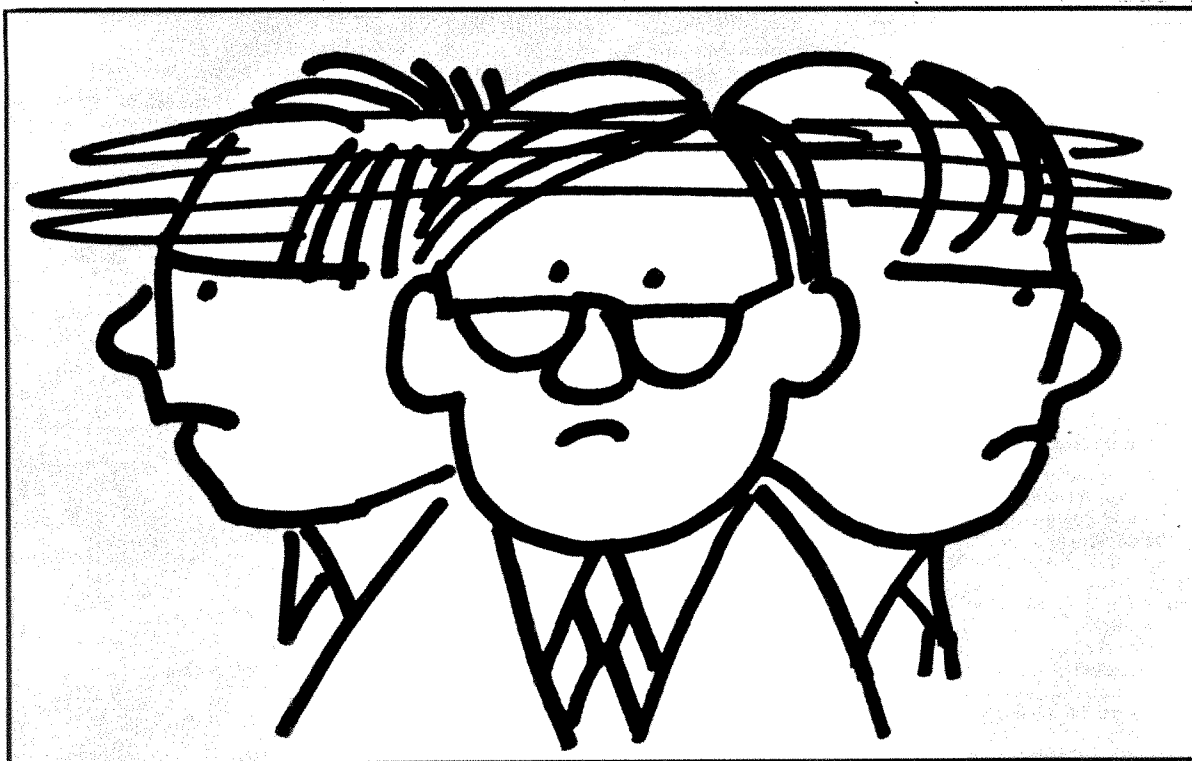
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29 March 1979

Recombinant DNA—how public?

A PROBLEM increasingly facing scientists is the pressure to become involved in public discussion of the assessment of risk. Although initial fears about potential health hazards of recombinant DNA research have proved to be exaggerated, the public discussion has required scientists to take a stand on the risks involved—just as public policy decision in other fields, such as the control of chemical carcinogens, has needed assessments that can be backed up by scientific data. In both instances there are two, related, requirements: the first is a reasonable interpretation of available data, and the second is that the public should have confidence in this interpretation.

Much thinking has, of course, been taking place on both sides of the Atlantic on how best to relate the scientific to the policy aspects of risk assessment (the need for which is now generally accepted even by those who continue to dispute whether such assessment can be reduced to cost/benefit analysis). Some have argued strongly for a clear separation of the scientific and regulatory aspects. The US National Academy of Sciences, for example, in a report on food additives published in Washington last month, suggested that it should be a scientific task to divide potentially harmful food additives into three categories of risk, and then a regulatory task to decide how substances in each category should be dealt with. And staff members of the President's Science Adviser's office have suggested a broadly similar, interagency approach to all areas of regulation of carcinogenic substances (page 388).

Such an approach has obvious attractions but in practice it is often difficult to draw the dividing line between the scientific and the political judgement. Frequently there is a lack of adequate scientific data to reach a firm conclusion on the subject under debate—the effects of low level radiation, or even the health hazards of recombinant DNA research, are two obvious examples. In such cases, a variety of interpretations can be compatible with such data as are available. And the choice between different interpretations is one that expresses social or political interests (those who wish to pursue a certain line of research, whether the nuclear industry or DNA research workers, will tend to favour the interpretations that minimise the hazards—their critics will tend to take the opposite view).

One should not be surprised at this situation, merely aware of the bias that will inevitably creep in. But one

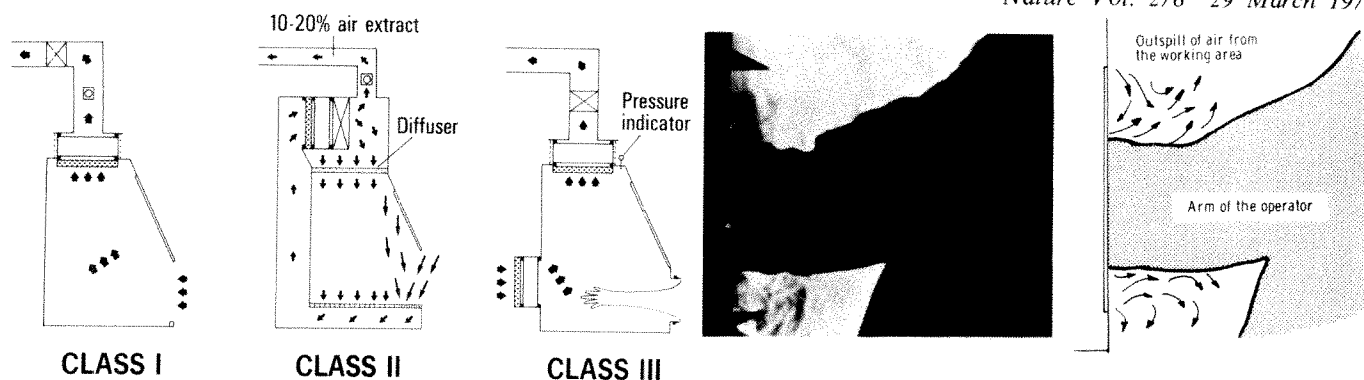
thing that is certain is that to achieve a reasonable and acceptable policy position, the public debate on such risks must be as open as possible.

What then are we to make of the restrictive notice from the organising committee of the forthcoming international scientific meeting on recombinant DNA at Wye College, Kent, which puts the whole meeting 'off the record'? The purpose of the meeting, supported by ICSU and the Royal Society, is 'to review the current status of DNA research and technology, and to discuss matters of concern such as support for the research, its regulation through guidelines and legislation, and the eventual practical application of the technology'; widespread participation is called for. And yet participants have now been told that for 'free and frank discussion usual to scientific meetings' the proceedings will be strictly off the record, and that everyone should respect the 'established convention of private communication between scientists, and not contribute to any reviews for publication other than . . . the official proceedings'. Originally the press was to be excluded entirely, but now three science writers will attend (from *Nature* and *New Scientist*) provided they refrain from recording the meeting, or writing reports for general publication directly from the proceedings.

Of course scientists working in this field may at times have felt hard done by the media. And of course it is open to journalists to get the material by the alternative route of dogged pursuit of participants for on-the-record comment. But the committee has already clearly set the tone—the discussions are too sensitive to be widely disseminated, except in an official version. Others may conclude—perhaps quite wrongly—that there are other things to hide.

Moreover, it is never possible for all those who wish to attend to find the time and money to do so. Sober reporting of the proceedings fulfils an important function of allowing the whole community, not just those able to get to a particular place, to gain at least some impression of what went on.

Science, we are continually reminded, is public knowledge, freely available. Anyone lecturing to a large audience accumulated by advertisement as well as by invitation participating in this tradition, not conforming to the established convention of private communication. They should think again.



'Safety' cabinets are not safe, says UK study

Some of the most popular makes of safety cabinet used for biological research in the UK have containment factors 1000 times lower than the new British Standard, writes **Judy Redfearn**.

WORK sponsored by the UK Medical Research Council has shown that the vast majority of 'safety' cabinets currently being used for microbiological work in hospitals, universities, and research establishments throughout the UK offer so little protection to those working with them that the term safety cabinet is a misnomer. An important and positive outcome of this work, however, has been the preparation of the first British Standard (BS 5726) for safety cabinets, which is due for publication "any time now". The standard will define three classes of cabinet (see figure) and recommend minimum containment levels for each.

The cabinets which are causing most concern have a linear downflow of air at the opening. Of the dozen or so makes of this type (class II) currently available in the UK, only two, the UK-manufactured Howarth and the Italian Gelaire, have been found to come up to the new BS recommendation for containment.

Some popular makes of cabinet to have containment factors up to 1,000 times less than the recommended minimum. According to Dr R. P. Clark of the Clinical Research Centre, Harrow who has done much of the work for the MRC, in some cases and for some uses a microbiological worker could be at greater risk when using a cabinet than when working on the open bench—because the air flow created by the cabinet could cause infectious material to be blown directly at his or her face.

Class II cabinets are very popular for work where total containment is not thought necessary, because they are had been thought to offer some protection both to the worker (from inhaling infectious particles) and to the work (from contamination from the environment). The latter can be important during work with tissue cultures.

The cabinets operate by drawing air through the front opening and down through a grille in the floor. The air

then moves behind the cabinet and through high efficiency filters before re-entering the cabinet through the roof. During normal operation, about 80-90% of the air is recirculated back into the cabinet and the remaining 10-20% is filtered and discharged to the atmosphere. Drs Clark and B. J. Mullan used Schlieren photography to look at the air flow in and around cabinets running with and without an operator. They also examined the effect on the air flow of a person walking past the front of the cabinet or of a door opening and closing near it, of talking or coughing towards the cabinet and of a lighted Bunsen burner inside it. Behaviour of the air flow was also examined after switching the cabinet off.

For all the cabinets tested they found that in the first 3s after switch-on a "large rolling eddy" of air spilled out of the front opening. The flow pattern then settled down and a substantial bulge of air began to circulate regularly outside the cabinet. Some air was convected away from the bulge to mingle generally with the air of the room.

The effect of an operator working with the cabinet was to cause turbulence in the air bulge outside. When a smoke pellet was ignited in the cabinet, smoke was clearly seen emerging through the front opening above the operator's hands and moving towards the face. The effect of someone coughing or speaking towards the cabinet or walking past the front of it, or of a door opening and closing near it, was to disturb the air bulge and cause it to mingle with the air in the room. After the air flow through the cabinet was switched off, the warm air inside moved upwards and outwards from the front opening and continued to do so for 10-20 min.

Drs Clark and Mullan have tried to quantify these disturbing observations by measuring the containment of various makes of cabinet. They created an aerosol of potassium iodide solution inside each one and measured the

number of droplets contained per unit volume of air just outside the front opening. They then performed the same experiment on the open bench and calculated the ratio of the measurement made on the open bench to that made with the cabinet.

This work is the basis of the new British standard. The standard will recommend that Class II cabinets (with a dummy arm inserted in the front opening) offer 10^5 times more protection to the operator than the open bench.

When tests first started at the Clinical Research Centre no UK-manufactured cabinets came up to this specification and some had containment factors of only 10^2 . In the light of this work, however, one UK manufacturer has designed a new cabinet, the Howarth, which meets the BS specification. The cost of this model compares well, according to Dr Clark, with the cost of other less adequate cabinets. However it is disturbing, says Dr Clark, that some manufacturers are now rushing to sell off their old stock. He is aware of two firms taking orders in the last two weeks and threatening cancellation charges if the orders are withdrawn when the standard is announced.

But if—against this trend—the publication of BS 5726 goes hand in hand with a willingness of other manufacturers to substitute better designs, then the biologists will at least have started to tackle their major safety problem. Fume cupboards for chemical work, however, are likely to remain without a British Standard for some years yet. □

Figure: cabinets in Class I (left) are designed to protect the worker but not the work. Those in Class II (second left) are for protecting both the worker and the work—as are those in Class III (centre). The latter are for work with highly infectious or pathogenic organisms where total containment is needed. Right: what happens when an operator disturbs the air curtain in Class II cabinet.

US drug companies push for changes in recombinant DNA guidelines

David Dickson reports that fears of industrial espionage are making drug companies reluctant to comply with rules covering genetic engineering research

US pharmaceutical manufacturers are challenging a key section of the National Institutes of Health's guidelines covering recombinant DNA research, claiming that compliance under present procedures could lead to the disclosure of proprietary information to potential competitors.

The section in question is that which specifies the conditions under which certain experiments can be exempted from a prohibition on research involving more than 10 litres of culture—a situation which many companies are rapidly approaching as recombinant DNA technology moves from the research to the production stage.

Under an interim procedure agreed by the NIH's Recombinant DNA Advisory Committee (RAC) last month, applications for such exemptions have to be circulated to all members of the committee, and approved by a three-person subcommittee, before they can be granted by the director of NIH, Dr Donald Fredrickson.

Although legal provisions exist covering what may or may not be divulged by RAC members as "special government employees", Dr Fredrickson has said that he cannot guarantee the complete confidentiality of information submitted to the RAC as part of this approval procedure.

On the other hand, regulations covering the use of recombinant DNA techniques in private industry which are currently being drawn up by the Food and Drug Administration—and which will provide statutory protection for proprietary information—are not at present scheduled to be on the statute book before February 1980.

In the interim, unless an alternative way is found of protecting such information, pharmaceutical companies and other groups pursuing recombinant DNA research who need to go beyond the 10-litre limit as a step towards full-scale production, but do not want to divulge too much information to a potential competitor, have been faced with some hard choices.

Some, such as the Berkeley-based Cetus Corporation which is required by local laws to observe the NIH guidelines, plan to hold back on any experiments for which providing full details to the NIH would, they feel, prejudice their proprietary rights.

In contrast, the San Francisco firm Genentech is planning to take what one pharmaceutical industry official calls a "calculated risk" in conducting scaled up experiments according to the technical criteria that have been laid down by NIH, but without seeking the NIH's formal approval (although NIH will be informed when such experiments take place).

"To get the full benefits of this technology out, it is important to go rapidly to volumes greater than 10 litres", Mr Robert A. Swanson, president of Genentech, told *Nature* last week. He added, however, that there was no mechanism for doing this through NIH at present "that would take into account the necessity to protect proprietary information".

(Eli Lilley, the pharmaceutical company which has entered an agreement with Genentech to develop techniques for producing insulin, has suggested to the FDA that its proposed regulations—which are likely to require companies to show that a product has been developed under the NIH guidelines—"should include provisions for exempting studies commenced prior to the effective date of the guidelines or regulations".)

Issues related to the confidentiality of information, although they have been heatedly debated over the past two years in Britain's Genetic Manipulation Advisory Group, have tended to remain on the sidelines within the US debate, where greater attention has been given to public concern about the potential health hazards of the research.

As the production of substances such as insulin, growth hormone and interferon using recombinant DNA techniques comes closer to reality, however, companies which have agreed to comply voluntarily with the NIH guidelines are becoming increasingly concerned about being required to divulge commercially valuable information.

"There are not many companies wanting to do so at the present time, but within the next year or two you will have a lot of companies wanting to scale-up their research. And how you deal with proprietary information in such circumstances is causing some concern", says Dr Herbert Weissbach of the Roche Institute of Molecular Biology.



Dr Donald Fredrickson, Director of the NIH: under pressure on confidentiality

These sentiments are echoed by Dr John Adams, vice-president of the Pharmaceutical Manufacturers Association in Washington. "We do not feel that the NIH guidelines in their current form adequately address the problems that we have with regard to protecting the confidentiality of information. The problem is that there is nothing in the statutes of NIH that covers trade secrets."

At the same time, however, trade union and public interest groups are arguing that the more recombinant DNA techniques become part of standard production techniques, the greater the potential for serious health effects—and hence the greater the need for information about the processes involved.

Under the terms of the guidelines published by NIH last December, experiments requiring more than 10 litres of culture are prohibited unless the recombinant DNA's are rigorously characterised and "the absence of harmful sequences established". In such cases, the guidelines state that the experiments can be authorised by the director of NIH "under procedures specified by the RAC".

Exactly what these procedures should be is now under debate. At a meeting of the advisory committee held in Bethesda last month, Dr William Gartland, head of the NIH's Office of Recombinant DNA Activities (ORDA) suggested that as an interim procedure, applications for scaled-up experiments should be reviewed by the chairperson and two other members of the RAC.

This group, he suggested, would recommend whether the request should be approved by ORDA or whether it required review by the full committee, in which case a subcommittee of the RAC would review the application prior to being passed to the full committee.

Various members of the committee objected to this procedure, claiming that all members of the full committee should be involved in the decision.

receive details of all applications for scale up. "Otherwise the sub-group could theoretically be given a blank cheque to pass experiments without reference to the full committee" says committee member Dr Karim Ahmed.

After a certain amount of debate, it was agreed that until the next meeting of the advisory committee in May, any application for experiments over 10 litres would be examined by a three-person subgroup, but would also be circulated to all members of the committee one week prior to the subgroup's meeting (so far no such application has been received by NIH).

The next RAC meeting will also consider the report of a subcommittee set up by chairperson Dr Jane Setlow on how the phrase "the absence of harmful sequences" should be interpreted. Some have argued that even an insulin producing gene might be described as potentially harmful, depending on where it was functioning; and Dr Setlow is keen to ensure a reasonable uniformity of interpretation between different institutional biohazard committees (IBCs), which are already required to make such judgments in certain circumstances.

The major question regarding procedure, however, is whether the pharmaceutical companies, if they are required by the FDA to comply with the NIH guidelines, are prepared to accept the proposed arrangements as adequate to protect proprietary information; and conversely whether an alternative arrangement more to their liking will be acceptable to all members—and in particular the "public interest" representatives—on the advisory committee.

Already at least one company has approached Dr Fredrickson to see if he could give his approval to certain scaled-up experiments without consulting the RAC. But Dr Fredrickson has informed them that, under his interpretation of the current guidelines, there is no way this can be done.

The pharmaceutical companies are now focusing attention on the form of regulations which the FDA is likely to bring in, following its announcement last December that it was proposing to require that any firm seeking approval of a product "requiring the use of recombinant DNA methods in its development or manufacture" demonstrate its compliance with the requirements of the NIH guidelines.

According to FDA Commissioner Dr Donald Kennedy, the agency is confident that it can find some way to protect proprietary information as it does now with other commercial products, even if this information has to be submitted to NIH for approval.

But the drug companies are not so confident, and indeed are challenging

whether the FDA has the right to regulate the process, in addition to the product, of research.

"It may be difficult to show that any particular research protocol involves a specific trade secret", says Dr C. W. Pettinga, executive vice president of Eli Lilly which has been saying for some time that the need to conduct experiments involving more than 10 litres of culture is imminent, and is keen to press ahead with its own insulin research. "Also it may be possible for a competitor to determine from a series of protocols the general thrust of research activity that in part or in whole might be valuable proprietary information."

In comments to the FDA which Dr Adams of the Pharmaceutical Manufacturer's Association says are broadly supported in the industry, Dr Pettinga warns that unless information can be submitted in confidence to the members of the RAC, there is the danger of the premature disclosure of a potentially patentable idea "which would almost certainly preclude patent protection in most foreign countries".

Scale-up a local problem

He suggests that authority to increase the 10-litre limit, in a manner consistent with appropriate containment standards, be given to local institutional biohazard committees (which would also be empowered to approve the utilisation of host-vector systems and research protocols for all except experiments requiring the highest level of containment).

In broader terms, Dr Pettinga, with the support of other members of the PMA, says that "the regulation of basic research by FDA is not desirable". And he suggests a mechanism should be developed for voluntary compliance with NIH guidelines, and that FDA should remain involved solely

with procedures regarding the safety and efficacy of drugs, and not the regulation of research.

How FDA and NIH respond to these recommendations remains to be seen. Already there are disagreements over what constitutes adequate protection of potentially valuable information. NIH, for example, has suggested that to defend proprietary rights, institutions should apply for a patent before submitting information to the RAC; the pharmaceutical companies have replied that in most cases this is unlikely to work, since a potentially patentable discovery will be based on work that requires registration and/or approval before it can be carried out.

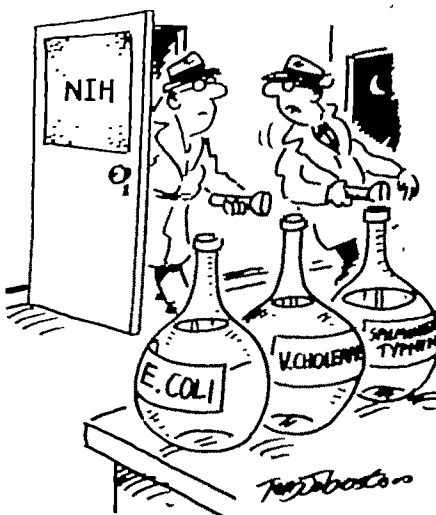
All sides are agreed, however, that the current situation is unclear. "We are hoping that somewhere down the road the rules of the game will be clarified and that reason will prevail. At present it is total confusion as far as we are concerned", Mr Peter Farley, vice-president of Cetus, said last week.

"Within the year we will be very concerned about the precise scale-up requirements. We will then have to pick and choose on a case by case basis whether to disclose full information of our plans to the NIH; if we were doing something very clever, the details of which we did not want others to know about, then for the time being we would do nothing."

One issue to be decided is how much information would need to be divulged to the NIH before a certain experiment would be approved as safe. "The characterisation of the gene presents no problem at all, since we would want to do this for our own safety. But describing specifically what one has done or providing the justification for going to larger volumes could involve sensitive information" says Dr Joseph Grady, of Upjohn Co.

A further matter of concern is that if private companies find they are unable to operate with the desired secrecy under NIH guidelines, then they may carry out their research in other countries with less stringent requirements. Already some US corporations with interests in recombinant DNA research are supporting the activities of foreign companies.

Any attempt to exploit loopholes in current legislation, however, is not likely to be looked upon kindly by Congress, which last year held back from legislating on recombinant DNA research in the private sector largely on the basis of companies complying voluntarily with the NIH guidelines. No company has refused to comply with the technical aspects, covering for example biological and physical containment levels; but neither has any said that they can work with the complete guidelines as they now stand. □



"Be careful! This place may be bugged!"

Britain's shadow science minister believes in experts

With the present government in Britain under extreme pressure to call an election,

Robin McKie interviews the Conservative spokesman on science and education, who feels that the major research companies should be more open about the research they undertake.

But expert committees should be left to experts

Ask a group of working scientists to react to the name of Mark Carlisle and it is likely that their responses will include a fair number of vague and inaccurate replies. This may not be particularly surprising but one cannot avoid feeling there should be a little more interest and reaction to a man who could shortly become overall leader of Britain's science effort.

In a surprise Shadow Cabinet reshuffle late last year, UK Opposition leader Mrs Margaret Thatcher appointed Mr Carlisle Shadow Secretary of State for Education and Science—in place of the previous, ebullient incumbent, Norman St John Stevas. Of course, the move does not automatically guarantee him the post of Education and Science Secretary in the increasingly likely event of the Conservatives bringing down the present Labour Government—but one only has to look to the example of Mrs Thatcher who held the same Shadow Cabinet post in 1970 and was appointed Secretary after Ted Heath won the general election that year. And it would also be strange to appoint another person just as Mr Carlisle had achieved some understanding of the complexities of the job.

So it seems timely to focus attention on the views of this 50-year-old barrister who may shortly influence so much of Britain's science policy. However, perhaps not surprisingly, Mr Carlisle is still reluctant to commit himself to particular doctrines or attitudes, stressing frequently that he will be guided throughout by the professional scientists and the various research advisory bodies. It is a natural reaction of a man new to the science arena, although one can already discern a less piercing or forceful interest in particular scientific topics than that shown by the present Secretary Mrs Shirley Williams, who has occasionally revealed great interest in subjects of special public concern such as genetic engineering.

However, some attitudes do become clear. In particular, Mr Carlisle shows a keen concern that science must be more closely tailored to suit the needs of British industry. In educational terms, this is reflected in a desire to increase emphasis on sandwich courses,

part-time education and polytechnic training.

And he particularly believes that greater encouragement must be given to further education at non-advanced, technician levels to provide necessary skilled manpower. "I think that one can foresee for the universities a period of consolidation, rather than expansion, and that any shift within the whole of education must go to scientific education and education in practical skills beyond the age of 16".

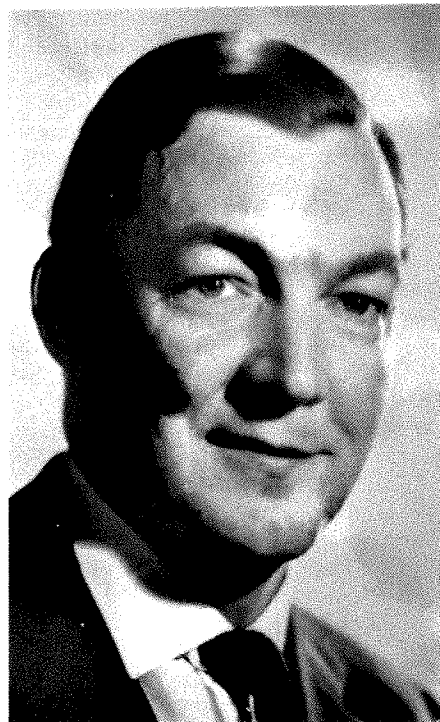
"The polytechnics have tended to move away from their original design and become less areas of technical education and more areas of general education. I think we have got to put the emphasis back on training the engineers and scientists that industry needs", he stated.

Mr Carlisle also stresses that more encouragement must be given to industry to carry out its own research rather than rely on strong Government backing all the time. "The Conservative philosophy is that Government should get off industry's back and leave it enough money for expansion which will allow more research. This will come from improving the tax structure and economic atmosphere of the country".

But if he is keen to tie science more closely to industry, then Mr Carlisle is less willing to have interference in the actual work of scientists. For instance he is quite adamant in his opposition to lay or union representation in bodies such as the Dangerous Pathogens Advisory Group or even the Genetic Manipulation Advisory Group.

"These are advisory boards on highly technical subjects and have to advise Government. If you are deciding on the appropriate degree of safety for testing then it seems to me that this is an area only in which the qualified are able to speak with authority", he added.

Instead Mr Carlisle believes, rather naively it may seem, that public opinion—"the most vociferous lobby in the country"—provides the best form of surveillance of the scientists' activities. However, on the subject of the Press, the major shapers of public opinion, he is less enthusiastic. "The newspaper reporting of science is probably no worse than the newspaper reporting of



Mark Carlisle: opposes union representation on GMAG and DPAG

anything else. It is only the bad news that makes the headlines".

But he does maintain that there should be greater openness in allowing more reporting of scientific matters. "I am quite sure that the major research companies have nothing to be ashamed of and should always be willing to speak to people and make it clear that they have nothing to hide."

On the more vexed problem of staff stagnation and ageing populations at universities, Mr Carlisle—in keeping with most other public figures—offers little in the way of comfort or solutions for the future.

"I am conscious that there is a feeling among younger members of university departments that today an awful lot of them are waiting to fill dead men's shoes. That is the root cause of the feeling of frustration among younger university lecturers.

"Sadly I don't think there is any simple solution. However, I think we have to invite the University Grants Committee completely to review the whole situation, particularly the question of levels of senior staff, and also encourage the setting up of fellowships, like the present SRC initiatives, to take up some of the strain".

And it is to be hoped that these measures, and the others outlined by Mr Carlisle, will prove in some way to be effective—for in a very short period they could be put into rapid practice. When, and if, that happens, we can then perhaps expect a little more reaction to the name of Mark Carlisle.

US urged to centralise scientific assessment of cancer risks

A SINGLE scientific organisation responsible for identifying and characterising potential carcinogens across a range of government agencies has been recommended in a report prepared by the staff of the US Office of Science and Technology Policy.

Such an organisation, which it is suggested should be based on an expansion of the national toxicology programme established last year by the Department of Health, Education and Welfare, would have two main responsibilities: to identify those substances which pose a potential carcinogenic risk to humans, and to characterise in quantitative terms the nature of the risk and the degree of certainty of current estimates.

The report is part of an attempt by the administration to improve the scientific base of the regulatory process, a need which President Carter was expected to refer to briefly in a speech on regulatory principles which he was due to give this week.

Addressing a senate subcommittee last week, Dr Frank Press, the President's science adviser, said that one of the current needs in science policy was to carry out an analysis of the research base of the regulatory agencies to see if it was adequate. "If more resources are needed, I cannot think of a higher

priority area than this one," he said.

Responding to a number of recent issues, such as heated debates over occupational exposure levels for lead and air pollution standards, the OSTP report says that in order to help develop a uniform decision-making framework for controlling potential chemical carcinogens, it is necessary to start by distinguishing scientific from regulatory decisions.

The first stage would involve scientific data collection and analysis, with risks expressed in comparative and quantitative terms, while the second would reflect a complete cost benefit analysis, taking into consideration human, economic and social factors—including the societal distribution on benefits and costs.

Priority for scientific analysis should be given to chemicals for which cost-benefit analysis performed with existing data suggests that regulation be indicated, but leaves too much uncertainty to justify immediate action.

"Priority setting and coordination of testing ideally should be done by a single group with input from all relevant agencies," says the report, recommending this approach in contrast to one in which assessment would be assigned to various scientific organisations on an agency-by-agency basis. □

UK ministry gives green light to 2,4,5-T

A REVIEW of the herbicide 2,4,5-T was published by the Ministry of Agriculture, Fisheries and Food (MAFF) last week. The document compiled by the Ministry's advisory committee on pesticides outlined the reasons for the MAFF announcement in July 1978 that herbicides containing 2,4,5-T were safe "if used in the recommended way for the recommended purposes" and that approval had been given for their continued use in the UK.

This is the advisory committee's 8th review of 2,4,5-T since 1967 and it points out that the decision it reached was based on certain premises. These are that "in general the dose makes the poison", that there can never be "total proof of safety", that where reasonable doubt about the risk of a product exists the advisory committee's policy is to "err on the side of safety".

The review points out that it is the presence of the toxic contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin)—a known teratogen and carcinogen—which is central to the concern over the safety of 2,4,5-T, some 3 tons of which are used annually in the UK—1/15 of the 1970 figure. Pure 2,4,5-T, the advisory committee

concludes, offers no hazard to users. And it adds that the dioxin itself would "ensure no hazard" if it was present in the trichlorophenol used to manufacture 2,4,5-T in quantities less than 0.1 ppm.

In arriving at its conclusions, the committee reviewed several major studies on the herbicide, including a New Zealand Department of Health Safety Report which could find no link between human birth defects and 2,4,5-T spraying; an Australian National Health and Medical Research Council report refuting that birth defects in the state of Queensland were associated with the presence of dioxin caused by burning sugar cane stubble sprayed with 2,4,5-T; a West German Federal Health Office statement and Review each approving the use of the herbicide in the Federal Republic; and reports from Vietnam that 2,4,5-T spraying caused an increase in the incidence of malformation in children, but which were inconclusive owing to the inevitable inadequacy of the data from a war zone.

The advisory committee points out, however, that decisions such as this one on 2,4,5-T are never final. □

MRC unit finds UK suicide rate falls

THE MRC Clinical Psychiatry Unit in Chichester has released the results of a major study showing that from 1962-1974 suicide rates in the UK have declined in all age groups except young women of age 15-24. Cautioning against simple interpretations, Jim Jenkins, co-author of the report said that an "entire constellation" of factors must be taken into account in the case of the female figures including family size, number of children and patterns of female employment.

The overall decline in suicide rate could be attributed to the increasing social cohesion in Great Britain in the face of a declining standard of living and increasing unemployment. Ironically, the MRC unit itself is slated for unemployment. Following the Medical Research Council's usual policy of closing units when their directors retire the Chichester Unit will be closed in 1982.

An MRC site visit three years ago found that "suicide research had no future" and a petition to the MRC with the support of the MRC's National Staff Side Committee failed to re-open the case. "We tried to get the MRC to look at their decision again," says Jenkins, "but we were given no scientific explanation why the unit should be closed." □

Misha Voikhanskii finally permitted to leave Leningrad

DR MARINA VOIKHANSKAYA, the former Leningrad psychiatrist, was forced to emigrate from the Soviet Union in 1975 after she had repeatedly refused to administer neuroleptic drugs to political "patients". Her nine-year-old son, Misha was to have followed her, but his promised emigration visa was withheld until a few days ago.

Since coming to the UK in 1975, Dr Voikhanskaya has been an active campaigner against political misuse of psychiatry in the USSR. In 1977 she addressed the Honolulu meeting of the World Psychiatric Association on this subject. Meanwhile as Misha grew up he too became interested in psychiatric abuse. In December 1976, it was he who notified campaigners abroad that activist Vladimir Borisov had been taken to a psychiatric hospital.

Dr Voikhanskaya told *Nature* this week that she felt sure that the long delay in Misha's visa was an act of reprisal against her for having spoken out against Soviet abuses of psychiatry. "It is only due to constant pressure from the scientific community and the public at large that the Soviet authorities have finally given way." □

AGE SPECIFIC DEATH RATES FROM DIARRHOEA

Place & year	Age specific death rates per 100,000	
	0-11 months	1-4 years
New York, 1900	5603	398.7
New York, 1961	45	2.4
Punjab, India, 1959	3446	312

(From Gordon et al, (1963),
Amer.J.Sci. 245 : 345)



A cure for a killer—but how to deliver it?

Millions of Third World children die of diarrhoea each year, although there is a simple cure available: oral rehydration. Discovered by scientists a decade ago, it has still not reached the people most in need. **Anil Agarwal** reports

THE possibility of oral rehydration has provided Third World health authorities with a very valuable breakthrough in the treatment of diarrhoeal diseases. Loss of fluids and electrolytic salts is the major complication in acute diarrhoeas, and if uncorrected, the continuing loss results in shock and eventually death. Until now rehydration has been possible only by administering fluids intravenously, as the small intestine loses its ability to absorb sodium during an attack of acute diarrhoea. Oral rehydration has now become feasible, however, with the observation that glucose absorption remains largely intact, and that sodium is carried along with it.

The application of this discovery—largely the result of work carried out at the Infectious Disease Hospital, Calcutta, the Cholera Research Laboratories, Dacca, Johns Hopkins University and the US Centre for Disease Control, Atlanta—now gives mothers the opportunity to treat their children suffering from diarrhoea themselves, by making a simple solution of glucose and electrolytic salts and feeding it to them.

Millions of deaths

Mothers no longer have to take their suffering children to health centres and hospitals tens of kilometres away, merely to seek the qualified nursing abilities required for intravenous treatment. For the majority of Third World mothers, who still do not have any reasonable access to health centres and hospitals, the necessity for intravenous treatment has really meant no treatment at all, and hence death for their children—a report from a research project situated in the relatively well-served region of Punjab, India, recently pointed out that though medical orders to village health workers called for children with clinical signs of dehydration to be immediately referred to the physician at the health centre, many children still died at

home of dehydration after they had been seen by the health worker. Many other children died without being seen at all. In this region, diarrhoeas were found to be responsible for 44% of all deaths amongst children less than three years of age. Considering that in many parts of the Third World, deaths amongst children constitute nearly half of all deaths in the population, the control of diarrhoeal diseases is of vital importance for the improvement of Third World health. It is estimated that there are some 500 million episodes of diarrhoea every year amongst children below the age of five. Experts differ, but it is widely accepted that these attacks result in the death of some 5-20 million children every year.

It is ironic that though diarrhoeal diseases have been reduced to a mere inconvenience in the developed countries, they still constitute the biggest cause of death and ill-health in the Third World. The primary cause of diarrhoeas is, of course, lack of clean water and generally insanitary conditions. But unfortunately these conditions are quite unlikely to change for at least another decade or two. WHO surveys show that as much as 78% of the Third World's rural population is still without clean community water supplies and about 85% is without adequate excreta disposal facilities.

Given these dismal conditions, oral rehydration has certainly opened up a glorious opportunity to take treatment, if not prevention, of diarrhoeal diseases to every doorstep in the Third World, however remote it might be, and control their worst consequences, dehydration and shock. In fact, oral rehydration can render an even more valuable service by helping to overcome the debilitation caused by the overwhelming majority of diarrhoeal attacks, which are mild and moderate, and do not lead to acute complications like dehydration. Together with mal-

nutrition, these diarrhoeas combine to form a vicious, mutually reinforcing complex of disease and ill-health. Malnutrition increases susceptibility to diarrhoeal attacks and persistent diarrhoeas increase malnutrition. If a child somehow survives the combined influence of these two, then a minor disease like measles often proves sufficient to kill him. However, studies now show that a child regularly treated with oral fluids should be able to maintain relatively good health. Trials in the Philippines for seven months, and in Turkey for 16, revealed that children who were given oral fluids had much higher monthly weight gains than those children who were left untreated: the mean weight gain of the non-treated children was significantly below that of the average growth curve.

Tests

The first serious test of oral rehydration took place in 1971 when cholera epidemics were taking a heavy toll of millions of Bangladeshi refugees who had poured into India. Two or three physicians, assisted mainly by the illiterate family members of the patients, were able to treat with oral fluids 3,700 severely ill refugees in less than two months. It was a state of total confusion, with up to 200 new patients being admitted daily; even space on the mud floor was full of purging patients. But only 125 patients (3.6%) died—and half of these before they could be given any fluids. By contrast, 9% of cholera cases in Italian hospitals died in 1973.

WHO and UNICEF are now spear-

Above: left, the death rate tells its own story. Right, the malnourishment of this child obscures diagnosis. The combination of symptoms is not rare in cases of diarrhoea, where repeated attacks worsen the condition. The majority of cases occur within the age group 6 to 18 months.



Measuring bottles and clean glassware are available in hospitals, as here in Papua New Guinea, but not in villages.

heading an international campaign to persuade Third World governments to introduce oral rehydration work in their primary health care activities. Numerous WHO-sponsored field studies are in progress, or have been completed in Egypt, El Salvador, Guatemala, India, Iran, the Lao People's Democratic Republic, Liberia, Nigeria, Turkey and the Philippines. Through these studies, WHO hopes it will be possible to convince local health administrators of the practicability and benefits of the new method. Many Third World hospitals have already adopted oral rehydration. The great majority of cholera patients at the Infectious Diseases Hospital in Calcutta, for instance, are now being treated in this way. The switchover from intravenous to oral fluids has meant a saving of about 50,000 dollars every year for the hospital in the cost of the fluids alone. More than a third of the beds in children's hospitals and wards in developing countries are usually occupied by diarrhoea cases receiving expensive antibiotics and intravenous fluids, thus putting a heavy load on their limited budgets. These units will greatly benefit from oral rehydration.

Delivery

Exactly how this new revolutionary therapy should be delivered to the Third World's million villages is, however, a matter of controversy amongst child health experts. WHO believes that the ideal course of action, in the interest of standardisation and quality control, is to prepare packets of oral rehydration salts in a standard mixture (3.5 g sodium chloride, 2.5 g sodium bicarbonate, 1.5 g potassium chloride and 20 g glucose) that would make a litre solution, and then sell these packets widely through commercial channels or distribute them through rural health centres. UNICEF is helping developing countries not only to distribute these packets but also to set up units to produce them. The packet approach is gaining acceptance. UNICEF has already distributed more than a million packets in developing countries, while Royal Drugs Ltd in Nepal has marketed over a quarter of

a million packets through commercial channels in the Kathmandu Valley. Since 1974, Indonesia has been running a national rehydration campaign with provision of locally packaged glucose-electrolyte mixture to its more than 2,000 rural health centres, and large numbers of commercial firms also retail the packets in the open market place. Costa Rica has instituted a national programme using packaged solutions, with the help of the Pan-American Health Organisation. The Cholera Research Centre in India has launched a drive to popularise oral fluid therapy among doctors and the lay public. Under contract from the Indian Council of Medical Research, a reputable drug firm has been entrusted with the large-scale production of oral rehydration packets.

WHO's "appropriate technology for health" programme is simultaneously trying to find cheap and simple packaging methods, in the interest of the poorer consumers: packaging constitutes as much as 50-75% of the price of producing a pre-packaged mixture. Machine-packaging is one possibility, using a vacuum sealed aluminium packet, coated with a plastic membrane inside. This type of packaging would minimise the moisture content, thus increasing the shelf-life of the powder. The price of a 1-litre machine-packaged UNICEF packet is currently estimated at 6.5 US cents. Hand-packaging would be cheaper and would create more employment, though the shelf-life of the powder would be less. In many rural areas of the Third World there already exist small packaging workshops where household articles and some drugs are packaged into polythene bags, and such workshops would be ideal for the hand-packaging of glucose-electrolyte mixtures. The cost of such mixtures is estimated to be about 5 US cents. All the ingredients could be put together in one polythene packet, or, because hand-packaging retains some moisture, the salts could be packaged separately and then added to a packet containing glucose. The cost of the latter technique would be higher, but the shelf-life would be increased.

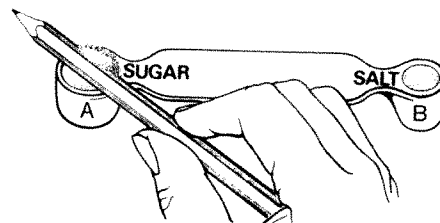
The major advantage of pre-packaged solutions is that mothers would get the quantity of each ingredient correctly measured. An excess of potassium salts can be toxic. Large excess quantities of sodium can cause hypernatraemia, a condition that affects the heart.

Critics

There are, however, several critics of the WHO-UNICEF approach. Their arguments are largely based on the grounds that this approach has not adequately taken into account the extent of poverty of a large percentage

of the rural population and on the logistic difficulties of distributing centrally-produced packets to every village in the Third World. Packaging, which only adds to costs, can make even this simple and cheap oral rehydration therapy virtually prohibitive for millions of mothers. Distribution through commercial channels increases the price further by adding profits for manufacturers, distributors and retailers. Although these packets will eventually be required in hundreds of millions, manufacturers have not leaped into production, because, it seems, of the low profits at which they would have to sell them to reach the mass of the consumers. Distribution through health centres would also not reach the majority of the Third World rural population. In Indonesia, for instance, it has been estimated that distribution of packets through health centres has reduced mortality to less than 5% in dehydrated children, but only 10% of the total cases actually use health centres.

Another problem with the WHO standard 1-litre solution is that in most villages of the Third World mothers will not find it easy to measure a litre. Instead, every culture has some standard measure, based on a readily available container: in India, for instance, a 300 ml beer bottle can be found almost everywhere. But packaging in smaller quantities will only increase the cost of packaging, and hence the total cost of treatment. Moreover, keeping a litre of aqueous solution



This simple measuring spoon was developed for home use in Indonesia.

containing glucose for more than a few hours is not advisable in unhygienic rural conditions, as it would provide an excellent culture for several pathogenic organisms. Instead, it would be better to make the solution every time it is needed.

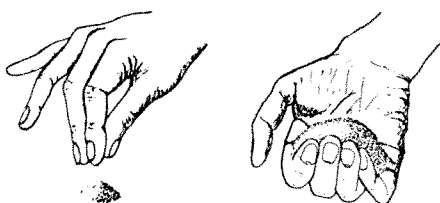
Mother the focus

The alternative approach being suggested is to make the mother the focus of oral rehydration treatment—to enable her to make and administer the solutions herself. Jon Rohde, who is working with a Rockefeller Foundation project on oral rehydration in Indonesia, argues that dehydration is just a form of malnutrition: fluid-electrolyte malnutrition. The key role of the mother in the treatment of

protein-calorie malnutrition is widely accepted. "Her role in replacing fluid and electrolytes is just as crucial," says Rohde. "perhaps more so considering the acute course of fluid-electrolyte malnutrition."

To enable the mother to treat her child herself, the WHO standard mixture can be considerably simplified. One problem is the availability of glucose, which has to be imported by most developing countries. Rural mothers will find it very difficult to obtain it. Glucose could therefore be replaced by the easily available white sugar (sucrose) which breaks down to glucose in the intestine. Sugar-salt solutions have indeed been tested by several researchers and have been found to be almost as effective as the WHO standard mixture in helping acute diarrhoea patients to recover. The simplicity of the sugar-salt solutions makes it possible for virtually every mother to become self-reliant. If nothing else is available, Dr William Cuttings of the London School of Hygiene and Tropical Medicine even recommends that mothers would do very well to use brown sugar or honey, which is available in many rural areas where beekeeping is a traditional practice. For potassium a mother could possibly squeeze in some orange or any other citrus fruit.

Researchers at the Institute of Child Health, Lagos, have found that poor mothers adopt the sugar-salt solution with great alacrity. All the



Fingers alone can measure quantities roughly. Left, 1.5 g, right, 30 g.

women attending the Institute's clinic are being taught oral rehydration therapy. In a recent survey, even women expecting their first child and others who had never used the service were able to describe the treatment. The survey however revealed that nearly half of the 217 women interviewed were planning to use more than four times the quantity of salt suggested to them (one-quarter teaspoon in a standard local beer bottle filled with water) and three cases were going to use sixteen times as much. Even very educated Western mothers have the tendency to believe that if something is good, more of it is even better.

To solve the problem of accurate measurement, a special plastic spoon has been developed in Indonesia which

has two separate indentations for measuring salt and sugar. This plastic spoon costs just 0.5 US cents. Such spoons are not only cheap, they could easily be made to suit local measures. Jon Rohde is using these spoons to teach children in primary schools and groups of mothers to make sugar-salt solutions. Dr Zafarullah Choudhury, a leading primary health care innovator from Bangladesh, is also teaching schoolchildren to make their own solutions. Because of its simplicity, Rohde strongly argues oral rehydration should be made everybody's business: "Teachers, agriculture extension workers, government employees, boy scouts, women's groups and even schoolchildren should be taught to mix rehydration fluids and use them."

No global method

Dr Christopher Lomax of the London-based Appropriate Health Resources and Technologies Action Group argues that "as in most 'appropriate technologies' it is difficult to recommend a single method to be used on a global scale, for each method will be viewed from a different perspective in different parts of the world".

Dr David Morley of the Institute of Child Health, London, who also strongly supports the use of spoons for measuring, believes that it would be best if national oral rehydration programmes were designed to operate at different levels of sophistication. At the level of the health centre, where even seriously dehydrated cases can be expected to turn up, both oral and intravenous therapy should be provided using the WHO standard mixture. But at the level of the individual mother, who has to treat most of the time mild and moderate diarrhoea cases, health authorities should promote the use of simple sugar-salt solutions made with the help of spoons.

WHO's Director-General, Halfdan Mahler, has in his speeches favoured the mother-based approach as best both in the long and short term. For WHO's new community-based approach to primary health care to succeed, Mahler has argued that a demystification of medical technology is needed. Oral rehydration, he believes, is an extremely good example where the responsibility of the community's health can be put into the hands of the community itself.

Maintaining standards

WHO's officials, however, who are very conscious of the organisation's reputation for medical standards, find it necessary to be cautious. WHO does not oppose any studies to test the efficacy of home-made sugar and salt solutions. But as all the major studies that have been published to date on the



The trend to bottle-feeding among the urban poor increases risk. Poverty means the mixtures are over-dilute, leading to malnourishment, and sterilisation of the bottles is impossible.

efficacy of oral rehydration have used the complete formula, WHO is making all attempts to promote widespread distribution of packets containing the standard mixture. It is felt that with sufficient education by health auxiliaries the complete formula can be mixed by mothers at home. With recurrent use of this solution, less education would be needed, and therefore hopefully there will soon be a minimum need for home-made sugar-salt solutions.

Dr Richard Cash of Harvard University also opposes attempts to simplify the oral rehydration therapy too fast. He believes that the medical community should not be bypassed. It is possible to go to the people directly and teach them self-help, but local doctors too must be adequately convinced of the effectiveness of a therapy before it is widely promoted. Otherwise, they will only discourage people from using them.

Furthermore, Dr Cash argues, the real problems in reaching the people is not scientific—whether one solution should be used or another—but lack of political will. It has taken ten years for governments to wake up to the possibility of oral rehydration since it was first indicated by scientific research. Producing packets and selling them is a far simpler job than undertaking a massive programme to educate every mother about oral rehydration. Governments, which still have to pick up the courage to provide more than half their population with adequate health services, are more likely to adopt the easier alternative.

The debate about oral rehydration does show that it is often easier to generate new medical knowledge that meets the needs of the neglected, underserved poor of the world, than it is to find ways to enable them to benefit from that knowledge. The debate about the delivery of oral rehydration parallels virtually every issue that has been raised in the more general primary health care debate about delivering health services to the rural Third World.

news in brief

France and Germany launch millimetre radio telescope project: The final go-ahead of the joint Franco-German millimetre radio telescope project was announced by Dr Kjell Johnson at the Rome meeting of the European Physical Society on 26 March. Conceived in 1973 the project was approved by the Centre National de la Recherche Scientifique (CNRS) and the Max-Planck-Gesellschaft zur Förderung der Wissenschaften (MPG) at the end of last year. CNRS and MPG will share the initial construction expenses of 160mFF equally with much of the MPG contribution coming from the Volkswagen Foundation. The facility will consist of a single fully steerable 30 m dish located at an altitude of 3,000 m in the Sierra Nevada in the south of Spain near Granada and an interferometer of three 12–15 m fully steerable and moveable antennae with a baseline of 1.5 km x 0.8 km located at 2,500 m in the Plateau de Bure near Grenoble. In exchange for offering a site and a local headquarters in Granada, Spanish astronomers will get about 10% observation time at both observatories. The main office of the Institut de Radio-astronomie Millimetrique (IRAM) in Grenoble will house 20–25 people by the end of 1979. The construction order for the large dish is ready to be placed and design of the interferometer is in the initial stage with the dish expected to be operational in 1982 and the interferometer in 1986. Millimetre range astronomy covers cold thermal sources (a 10° blackbody peaks at 1 mm wavelength) including planets, asteroids, and dense interstellar molecular dust clouds.

Costs may boost UK energy conservation: An inter-Departmental report by officials under the chairmanship of the Department of Energy declares that higher prices will "provide scope for considerable further savings" as the price of oil in particular is expected to double by the end of the century. Higher prices form a principal part of the government's recommended strategy and it stresses that economic energy pricings should "reinforce conservation measures and not work against them." This is believed to be a reference to the relatively low price of petrol in the UK which many politicians would like to see raised. A Department of Energy spokesperson said that "the Government should not take measures to cut fuel prices." Other measures in the government's strategy include: information; "a good example from Government"; and specialised advice and training. Research and development, expected to rise to £30m per year, is believed to be adequately funded at present. There are to be no mandatory controls on industry, which consumes the largest share (39%) of the UK's energy budget. The large energy users (50% of industrial energy is consumed by 14 firms and 70% by less than 200 firms) are believed to have an incentive to save energy voluntarily and the report argues that "mandatory measures would increase the burden on industry and would operate against the immediate need to increase industrial efficiency and competitiveness." Energy Conservation: scope for new measures and long term strategy Energy Paper Number 33. HMSO £2.

Europe moves further towards independent space capability: The remote sensing board of the European Space Agency (ESA) approved, on 15 March, the start of a remote sensing preparatory programme to develop the technological systems necessary to monitor both land and ocean surfaces. The preparatory programme will be for two years with the sizeable budget of nine million accounting units. (The 1979 ESA budget is 573 MAU with large individual programmes such as Exosat and the space telescope costing 25 MAU

and 15 MAU respectively.) Denmark, France, Italy, Netherlands, Sweden, and the UK have already subscribed and other member states are expected to join shortly. The programme will depend heavily on independent national development programmes as well, such as the multi-mission platform being developed in France and the synthetic aperture radar system being developed in Germany, both of which are being prepared for use in Spacelab flights in 1983.

Moscow Radio criticises US scientists: US scientists participating in the growing "SOS" (Scientists for Orlov and Shcharanskii) campaign has been severely censured by Moscow Radio's English-language service to North America. The 2,000 or so signatories of the SOS protest pledge themselves to refrain from taking part in certain specific forms of exchange programme with the Soviet Union until Orlov and Shcharanskii are released and cleared of the charges of anti-Soviet activity for which they were sentenced last year.

According to Moscow Radio, the SOS participants are misinformed and chauvinistic—the latter term since they are acting on the "assumption that only the Soviet Union will suffer" from such severance of ties. The commentator stated that, on the contrary, research from Tokamak and "many other achievements of Soviet science" are used in US laboratories, and that in any case, all blockades—political, economic and diplomatic—of the Soviet Union have failed in the past. This broadcast took place on the same day that a group in Britain announced the formation of a "Lerner Circle" to campaign for Aleksandr Lerner, the cyberneticist and a "refusnik" of eight-year's standing to be granted an emigration visa. Among the founding members of the "circle" are Max Perutz, Nevill Mott and Rodney Porter.

US to increase scientific cooperation with Brazil: The United States is to expand its scientific cooperation with Brazil as part of an effort to increase the relations between the two countries, Vice-President Walter Mondale said on a visit to the country's capital, Brasilia, last week.

Mr Mondale said that the Brazilian president, General Joao Baptista Figueiredo, had accepted an invitation to send the president's science advisor, Dr Frank Press and a team of specialists to consult with Brazilian scientists on the form that this cooperation might take.

Little was said during the Vice-President's visit of previous US criticism of Brazil's nuclear power programme, in particular its plan to import reprocessing facilities from West Germany as well as nuclear plants.

New Windscale leak discovered: Borehole sampling in the vicinity of a temporary storage tank at British Nuclear Fuel Ltd's reprocessing plant at Windscale, Cumbria has shown a marked increase in radioactivity. The temporary storage tank holds highly active liquid wastes dissolved in nitric acid for concentration by evaporation after which the waste is transferred to highly active waste storage silos. A BNFL spokesman said that the activity in the borehole was 1% of that in the temporary tank which in turn was 1% of that in the highly active tanks but said that the precise level of activity was "unavailable." Borehole monitoring was initiated two years ago when 200 gallons per day of liquid waste was found seeping into the soil around one of the concrete storage silos. This leak has not been located but the area has been isolated and the radioactivity is believed to be retained in the soil, as tests of ground water have shown no increase in activity.

correspondence

Nuclear decisions

SIR,—In a recent article (22 February, page 594) Peter Taylor calls for changes in the way in which decisions are taken on nuclear energy matters. The article contains two straightforward issues and two more complex ones of interest to the Health and Safety Executive. Let me first discuss the straightforward points.

The first is the reference to the alleged assumption that the issues are "primarily 'scientific' and 'technical', and thus... the exclusive field of experts". I doubt if anyone really makes this assumption these days. It is certainly not a view held in the Health and Safety Executive.

The second issue relates to the Hinkley Point incident, which Peter Taylor has seriously misunderstood. The ministerial statement, to which he refers, makes it quite clear that the coolant involved was not the core coolant, which is carbon dioxide, but the sea water coolant for auxiliary equipment. Cooling was maintained by a jury-rigged system involving a fire pump. The reactor coolant was unaffected.

The most important of Peter Taylor's points relates to risk assessment. He casts quite legitimate doubts on the accuracy with which quantitative probability estimates can be made for various scales of accidents and their consequences. He goes on, however, to say that "probabilistic presentation obfuscates rather than illustrates the potential effects". On this I think he is wrong. The study of the range of accidents and consequences, including very serious ones, is helpful and is standard practice. The search for a worst case is less useful because of the difficulty of defining "worst". The worst case owes more to the imagination and inventiveness of its postulator than to reality. More seriously, the discussion of a worst case diverts attention and effort away from the more likely accidents. Moreover, a study of very serious accidents without reference to their likelihood does not provide any basis for decisions. Thus, for example, the worst possible accidents in many forms of transport, chemical processing and gas distribution would be unacceptable if they occurred frequently; it is the very low probability of these serious accidents that makes these industries acceptable in a society which relies heavily on the benefits they bring. It would be wrong to ban large passenger aircraft just because one might, one day, crash into a crowded stadium. It would be wrong precisely because the probability is very low, even though the consequences would be very severe.

Of course the probability and scale of accidents do not alone provide a definitive basis for decisions on, in particular, the licensing of nuclear installations. They do, however, provide an essential input.

The final point of importance relates to the supply of information. Peter Taylor writes of "a retreat into secrecy" but the alternative, moving towards greater openness, is not without difficulties. Much of the information in the detailed safety

assessment of a plant is owned by the designer and is of real commercial value to him. So long as we expect to have a commercial structure, nationally or internationally, to our nuclear industry this point will limit disclosure. I believe, and have said so in public, that there is too much use of the claim for commercial value and that we ought to move towards a more open situation. But this requires additional resources, both in the industry and in the Executive, and my hand is not strengthened when published information is misquoted and misused. The "selective use of the results of scientific research" is not confined to any one group.

H. J. DUNSTER

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SIR,—Peter Taylor (22 February, page 594) refers to the safety target which I proposed in 1967. He is right in quoting my view that the setting of a target in no way gives assurance that the target is or will be achieved.

Both the Flowers Report and the recent report of the Lewis Commission (USA) strongly support the use of fault trees and event trees for licensing purposes and to direct the research programme. Lewis also supports the Rasmussen methodology. Use of these techniques necessarily requires an adequate data base.

The Safety and Reliability Directorate (SRD), originally under my direction, have worked extremely hard during the last 13 years to collect relevant data and develop the highly disciplined and critical assessment techniques. These are now used in the nuclear industry and are being more widely used in the chemical and allied industries in association with over 60 agencies and major companies in over 16 countries.

I know that numerical assessment is not perfect and is not enough in isolation to determine design and operation but, rather than obfuscate, the need to obtain data and analyse quantitatively wonderfully sharpens the mind and illuminates the problem, even if to show up its difficulties.

In my view the use of emotive words such as credible, incredible, maximum credible, did much to confuse safety issues in the first 10–15 years of reactor design.

Taylor asks that the worst condition should be stated. There is seldom a "worst". By assuming a combination of more and more unlikely events I am sure that I could develop a hypothetical situation to beat Mr Taylor, but of what value would this be?

When referring to the AGR and the NRPB/NII study Mr Taylor quotes 100,000 and 600,000 casualties. The reader might reasonably believe this to be deaths, but paragraph 266 of the Flowers Report clearly states that the major effect would be thyroid damage. The number of deaths were estimated to be around about 300/400 although this number could be an order of magnitude higher or lower.

As to the fast reactor, the Flower's Report (paragraph 304) states, "The consequences of such accidents would be so severe that the FBR could scarcely

be contemplated on an extensive commercial basis unless it is established with a very high degree of confidence that reactivity accidents can be controlled so as to prevent gross fuel vaporisation." It is still my view and that of my colleagues that gross fuel vaporisation is highly improbable, and many international research programmes still continue to provide further evidence on this point.

I support Mr Taylor's desire to ensure a highly critical approach on safety matters. We have done much to improve the standard of objective criticism in the UK, but I invite Mr Taylor to be equally concerned about the safety of man and the environment in all our industry.

F. R. FARMER

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United Kingdom

Atomic Energy Authority

SIR,—Peter Taylor (22 February, page 595) states "... We were able to run NRPB calculations (which were for a notional site) for a specific locality (we chose Kalkar in Wes. Germany). We discovered that NRPB had chosen not to present their own work calculations—the results of one weather category which led to 600,000 casualties. Our site-specific study, having altered only the population data on the programme, produced over 900,000 casualties". Perhaps this concise account disguises the actual situation and I should like to reply in the absence abroad of Dr G. N. Kelly, the principal author.

When NRPE published its initial study of consequences of notional fast reactor accidents (Kelly, G. N., Jones, J. A. and Hunt, B. W., An estimate of the radiological consequences of notional accidental releases of radioactivity from a fast breeder reactor, NRPB-R53), it stated that "further analyses are being undertaken to assess the sensitivity of the results to variations of the more important parameters". At that stage no calculation of the type referred to by Mr Taylor had been done. In fact, the first sensitivity analysis has just been published (Kelly, G. N., Jones, J. A. and Simmonds, J. R., The influence of the physico-chemical form of the aerosol on the radiological consequences of notional accidental releases of radioactivity from a fast breeder reactor, NRPB-R73). Further studies are in hand which will include variation in meteorological conditions and population densities, from the likely parameters used in the initial study to the remote but nevertheless possible extremes. It is notable that the Political Ecology Research Group itself showed (RR-1, A study of consequences to the public of a severe accident at a commercial FBR located at Kalkar, West Germany) that the increased magnitude of the consequences in the worst conditions is broadly compensated for by the lower frequency of occurrence, giving comparable risks.

When NRPB has completed its investigations they will be published through HMSO.

M. J. GAIN

National Radiological Protection Board

news and views

Opioid peptides and their relatives

from John Hughes

MORPHINE appears to produce its analgesic pharmacological effects by mimicking the action of natural endogenous opiate peptides. Recent research has illuminated various aspects of the origin and physiological relevance of these peptides.

The critical structure responsible for opiate peptide activity is Tyr-Gly-Gly-Phe(X) where X is a hydrophobic amino acid such as methionine or leucine in Met and Leu-enkephalin. Either N or C-terminal extension of these sequences preserves opioid activity to some degree although an N-terminal block, acylation, for example, abolishes activity. H. R. Morris originally noted the homology between the β -lipotropin (LPH) sequence 61–65 and Met-enkephalin, and the relationship between LPH, Met-enkephalin and β -endorphin (LPH 61–91) has since received considerable attention. All fragments of LPH commencing with residues 61–65 have opioid activity to some extent although peak activity is seen with Met-enkephalin and the C-terminal fragment of β -endorphin. This has posed problems in determining the possible biological significance of a given sequence, particularly since a number of active fragments of LPH have been isolated from pituitary extracts. However, it now seems likely that proteolysis during extraction accounts for the presence of most of the α and γ -endorphin (LPH 61–76 and 61–77) in pituitary extracts (Rossler *et al. Life Sci.* **21**, 847; 1977). LPH 61–77 (γ -endorphin) may in fact be in the first metabolic product of β -endorphin allowing the final inactivation by amino peptidase which does not attack intact β -endorphin.

There is now overwhelming evidence that a family of related lipotropin/corticotropin intermediate lobe peptides are derived from a common glycoprotein precursor of molecular weight ~30,000 (Mains, Eipper & Ling *Proc. natn. Acad. Sci. U.S.A.* **74**, 3014; 1977; Roberts & Herbert *Proc. natn. Acad. Sci. U.S.A.* **74**, 4826; 1977). Bio-

synthesis of β -endorphin from β -LPH and its precursor (pro-opiocortin) has been demonstrated with radiolabelled pulse chase experiments in the pars intermedia (Crine *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 4719; 1978) and there is evidence that corticotropin and lipotropin/endorphin activity is present in the same secretory granules (Weber, Voigt & Martin *Brain Res.* **157**, 385; 1978).

The mRNA encoding for pro-opiocortin has now been purified from bovine pituitary intermediate lobe (Nakanishi *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 6021; 1978) and a further report in this issue of *Nature* (Nakanishi *et al.* page 423) now describes the full nucleotide sequence of this mRNA following cloning of the cDNA species in *Escherichia coli*. Inspection of the corresponding amino acid sequence reveals a number of interesting features. The molecule itself seems to consist of four repetitive units based on the MSH/ACTH core sequence, these units are separated by paired basic residues. ACTH and β -lipotropin exist as contiguous peptides at the C-terminal and are separated by the Lys-Arg sequence. Thus, in common with other protein precursors, the biologically active sequences are flanked by paired basic residues at which proteolytic processing takes place. An exciting and novel aspect of this study is the recognition of a new MSH sequence termed γ -MSH, this sequence is also flanked by paired basic residues and by implication could be of physiological significance. Nakanishi *et al.* postulate that the structural gene for pro-opiocortin may have evolved through a series of sequence duplications of certain common ancestral DNA segments, other modifications occurring through substitution, addition or deletion of some regions.

The existence of so many potentially active molecules in one precursor is a subject of much interest. The question of how pro-opiocortin cleavage is regulated is thus an intriguing and important one. Are all the possible

peptide sequences processed and released or do controlling and inactivating mechanisms exist? Relatively little is known of these mechanisms and the enzyme(s) responsible for sequence cleavage remain to be isolated. There is evidence that these control mechanisms may alter during development (Silman *et al. Nature* **276**, 526; 1978). Thus α -MSH, CLIP (ACTH 18–39), β -MSH and β -endorphin predominate in the fetal pituitary of the rhesus monkey whilst ACTH, γ -LPH and β -LPH predominate in the adult. Similar changes in these patterns of peptide production may occur in the adult in response to pathological states or changes in hormone balance. The identification of N-acetyl forms of β -endorphin (Smyth *et al. Nature* in the press) may reflect one controlling mechanism whereby acetylation inactivates the opioid peptide whilst leading to activation of α -MSH. The activity of the acetylating system could thus determine the balance of biological activities between these two peptides and could provide another point of disturbance leading to altered neuronal activity and mental states.

Pro-opiocortin is a glycosylated peptide and glycosylation may be important for protection of the prohormone from nonspecific proteolysis during packaging and could also direct cleavage of specific sequences through conformational effects. A recent paper by Loch and Gainer (*FEBS Lett.* **96**, 769; 1978) illustrates this view. Tunicamycin inhibits glycosylation and although it does not prevent synthesis of un-glycosylated pro-opiocortin there is a rapid degradation of this un-glycosylated species with the production of atypical peptides. Conceivably a disturbance of glycosylation in pathological states could lead to the production of 'abnormal' neuropeptides with undesirable side-effects on neuronal function.

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Despite the presence of the Met-enkephalin sequence in β -LPH there is no evidence that β -LPH acts as a precursor for methionine-enkephalin. Neurones immunoreactive to β -endorphin, β -LPH, ACTH and α -MSH have been found in the brain (Bloom *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 1591; 1978; Watson *et al. Science* **200**, 1180; 1978; Jacobowitz & O'Donohue *Proc. natn. Acad. Sci. U.S.A.* **75**, 6300; 1978). It is not clear whether this is one single neuronal system, although the localisation of cell bodies in the hypothalamus and their projections to other brain areas are very similar; however these neurones may be clearly distinguished from the more diffuse enkephalin immunoreactive neurones (Watson *et al. Nature* **275**, 226; 1978). As β -endorphin/LPH antibodies also recognise pro-opiocortin but do not react with enkephalin cell bodies or neurones, it seems unlikely that a common precursor is involved. Studies on the incorporation of ^3H -Tyr into the enkephalins have shown that these peptides are locally synthesised in gut and brain and that synthesis may be blocked by protein synthesis inhibitors (Sosa *et al. FEBS Lett.* **84**, 195; 1977; McKnight *et al. Proc. Roy. Soc. Ser. B* in the press). The nature of the enkephalin precursor(s) is therefore of considerable interest. Recent work has thrown

some light on this problem (Lewis *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 4021; 1978). Extracts of striatum contain proteins (molecular weight 40,000 and 100,000) that yield opioid peptides on tryptic digestion, these peptides can be distinguished from LPH fragments by high pressure liquid chromatography. These findings may explain the presence of unidentified opioid peptides in the brain and striatum (Hughes *et al. Br. J. Pharmac.* **61**, 639; 1977; Yang *et al. Neuropharmacology* **17**, 433; 1978) since they could be intermediate products resulting from the conversion of pro-enkephalin to enkephalin.

Kangawa *et al. (Biochem. biophys. Res. Commun.* **86**, 153; 1978) have identified a new peptide containing leucine-enkephalin in pig hypothalamus. This 15-residue peptide, rather unfortunately in my view termed α -neo-endorphin, contains the leucine-enkephalin sequence followed by the basic sequence -Arg-Lys-Arg-, an observation which supports the concept of a separate enkephalin precursor. Studies in our laboratory indicate the presence in brain of other opioid peptide sequences in much greater amounts than those found for α -neo-endorphin and the relationship between these peptides will be of considerable interest. Also, their existence sounds a cautionary note for those of us using immuno-

assay and immunocytochemical techniques in view of possible cross-reactivity and misinterpretation.

Enkephalin immunoreactivity has also been detected in cells of the anterior pituitary producing growth hormone (Weber *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 6134; 1978). Could it be that growth hormone and methionine or leucine-enkephalin share a common precursor? A common precursor for both enkephalins is also a possibility and this would explain the apparent coexistence of these peptides in the same neurones although this has yet to be demonstrated conclusively.

The application of recombinant DNA techniques is relatively new to this field but it is likely to prove a powerful and original approach. Nakanishi *et al.* suggest that the nucleotide coding regions for ACTH/LPH may have evolved by a series of genetic duplications since the cloned DNA insert for pro-opiocortin is characterised by unusually high C+G nucleotide base contents and also by numerous DNA sequence duplications. Other neuropeptides also probably evolved in this fashion although nothing is known at present as to the origin of molecules such as substance P or neurotensin. The further unravelling of the various protein-peptide relationships is likely to prove both fruitful and exciting. $\square$

The representation of three-dimensional objects

from N. S. Sutherland

THE tasks carried out by the human brain are so complex that the only way in which to specify precise theories of how they are executed is to construct computational models. It is at present not possible to construct formal proofs to demonstrate what a complex but well specified series of information processing operations will accomplish. The number of operations needed to perform such tasks as the recognition of visual objects is so vast that we can only be sure that a given model actually accounts for successful performance if we translate it into a computer program and demonstrate that the program when run executes that task. Few, if any, such programs run successfully at the first attempt, but the discipline imposed by writing them and the study of the ways in which they fail generate insights into the structure of the task and the structure of the operations needed to carry it out.

A working computational model of task performance becomes a candidate for a theory of how the brain itself performs that task. Because of their complexity it is sometimes difficult to test such models experimentally, but in principle and with sufficient ingenuity they can be subjected to the same experimental tests and their plausibility evaluated in exactly the same way as the vague theories normally proposed by psychologists or the even vaguer ones sometimes advanced by physiologists.

In 1976, David Marr¹ proposed a computational model of how the brain recovers from the grey scale data present in the retinal image a two-dimensional description in which lines and edges are explicitly represented: he called this description 'the primal sketch'. The processes suggested by Marr are extremely complex, but his theory is both compatible with physio-

logical findings on the early stages of analysis in the visual system and also enables us to understand why the system is organised in the way that it is, given the requirements of the task it has to carry out.

The problem of three-dimensional vision

Virtually nothing is known about the physiological and anatomical arrangements that mediate the next stage of vision, that is the mechanisms that take as input the primal sketch containing information about the presence of lines and edges in the picture and compute a three-dimensional representation of the objects towards which the eyes are directed. In the 'sixties and early 'seventies, however, some programs²⁻⁴ were developed that successfully recovered a three-dimensional description of a scene from a line drawing. Most of this work was restricted to scenes containing only plane-faced polyhedra. Moreover, the 3-D descriptions formed were for the most part very impoverished. They usually

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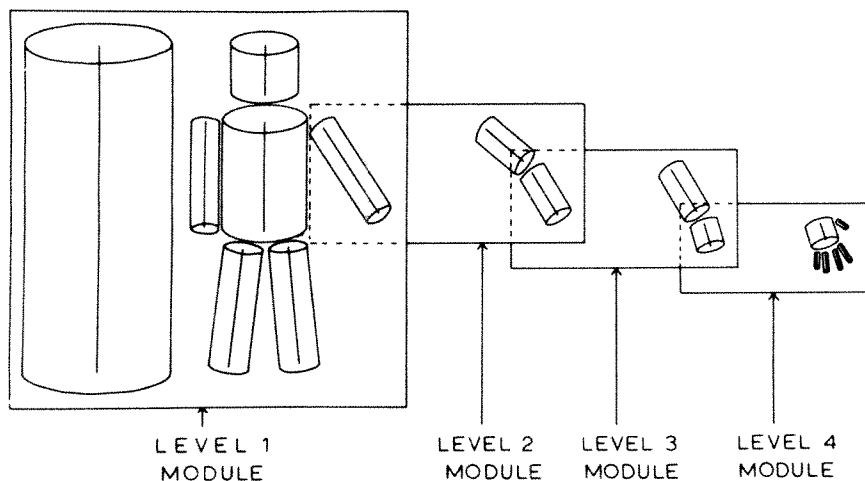


Fig. 1 Within each rectangle is shown on the left the 'model axis' for that level of description and on the right the way in which the model is broken up into component parts. The level 1 module represents the whole object, and the finer details of the arm, forearm and hand are represented respectively at levels 2, 3, and 4. At each level other than level 1, there would of course be many other modules representing for example the decomposition of the head, the nose and so on. Note that the body or component part represented at each level is decomposed into sub-components that are all of approximately the same size. (This figure is based on ref. 5.)

specified only the local relationships between pairs of surfaces in the scene: for example, two surfaces can be described as joining at a concave or convex edge or one surface may lie in front of another and partially obscure it. Even if additional information (such as the precise angle between two surfaces joined across an edge) is recovered and recorded, this kind of description is clumsy: considerable additional computation would be required to specify explicitly the different parts of an object and how they are related to one another. Moreover, anyone viewing an object has a strong impression of the volume of space it occupies and such information is not explicitly provided by a description that specifies only the relationships between neighbouring surfaces.

The construction by the brain of 3-D representations of objects serves two functions. First, in order to manipulate objects and to avoid bumping into them, organisms must be able to perceive the disposition of their surfaces in space. Second, in order to recognise an object and evaluate its significance for action, it is necessary to build a 3-D representation from the retinal image and then to match that description to a stored 3-D representation of the object. To facilitate the recognition process, it is likely that the representation constructed in immediate perception is similar in form to the stored representations with which it has to be matched in order for recognition to occur. In practice, the two processes—construction and matching—cannot be rigorously separated since, as will be

shown below, the construction of a perceptual representation may itself be guided by stored models of what is likely to be present in the environment.

The Marr and Nishihara model

Marr and Nishihara⁷ have recently made a fresh attack on the problem of how the brain represents information about 3-D objects: their proposals overcome some of the limitations of previous work. They lay down three criteria that such representations should satisfy in order to account for the efficiency with which the human visual system recognises 3-D objects.

Criterion (1) The representation should be easy to compute from the pictorial image.

Criterion (2) It should provide a unique description of any object which we are able to distinguish and the description should be canonical in the sense that the same object seen from any point of view will always yield the same unique description.

Criterion (3) Different representations should reflect the similarity between different objects whilst also preserving the differences: it is important to be able to recognise both that a shape is a man and that it is Jones or Smith.

One way of describing an object in three dimensions would be to compute the slope relative to the line of sight at each point on the visible surface of the object and the physical distance

between different points on its surface. Such a representation would be extremely cumbersome and would not satisfy criterion (2) since it would vary with changes in the observer's viewpoint. Marr and Nishihara argue for a volumetric description that is independent of viewpoint. To obtain such a description, some axis intrinsic to the object has to be selected to which the rest of the description can be referred: Marr and Nishihara term such an axis 'the model axis'. If the description of the same object seen from different points of view is to be canonical (criterion (2)), it is essential that when the same object is viewed on different occasions and from different viewpoints, the same model axis must be selected. For an elongated object, its longest dimension yields such a canonical model axis, and Marr and Nishihara only consider in detail objects of this sort. They suggest, however, that for some objects that are not elongated, like typewriters or armchairs, symmetry could be used to yield a canonical model axis. It is worth nothing parenthetically that for many objects commonly found in the same orientation, the visual system may select as a model axis the gravitational vertical.

Marr and Nishihara propose that objects are decomposed into their component parts. This proposal accords with our intuitions and has been previously made by others including myself⁸: some of my own experiments^{7,8} have indeed demonstrated that object recognition even in rats and goldfish is based on decomposing objects into their component parts. The originality of this aspect of Marr and Nishihara's work lies in their attempt to specify rigorously how such decomposition is effected, how the parts and their relationship to one another are described, and how the decomposition into parts can be used to facilitate the process of visual recognition.

In their theory the shape of each part is described as a generalised cone, that is, in terms of the surface swept out by a cross section moving along an axis and smoothly varying in shape and size. Binford⁹ was the first to suggest the use of generalised cones in the representation of 3-D objects, but Marr and Nishihara carry his suggestion much further by showing how it is possible to specify uniquely and simply the dispositions of the component parts of an object in terms of the relationships between the axes of the generalised cones by which they are represented. An important feature of their theory is that the descriptions are hierarchical: an object is first broken down into its main components and each of these is broken down into successively smaller sub-components.

Figure 1 illustrates such a representation. For an elongated form like that of a man, the longest axis is picked as the model axis and information is added about its length and the shape of the generalised cone that best approximates to the volume of space occupied by the whole shape. The object is next decomposed into its largest components and the component to which most other components are joined is then selected as 'the principal axis' for that stage of the description. Note that although in Fig. 1 the orientation of the principal axis of the whole form happens to be the same as that of the model axis, the two axes need not necessarily have the same orienta-

tion. Whereas the model axis depends on the direction of elongation of the whole body, the principal axis is the direction of elongation of the major component to which most other major components are joined. The orientation of the principal axis is specified in relation to the model axis: the angles and positions at which all other components join the principal axis are also recorded. The human form is most naturally decomposed initially into trunk, head, arms and legs with the trunk forming the principal axis. Where, as in the case of the arms and the trunk, the angle formed between one component axis and the principal axis is variable, information about the range of possible angles would also need to be stored. Figure 1 shows how in further modules the left arm can be decomposed into upper arm and forearm, with the forearm in turn decomposed into forearm and hand, and the hand into palm and fingers.

The resulting representation of a three-dimensional object is independent of viewpoint (criterion (2)), volumetric and modular. Marr and Nishihara call attention to three important advantages of modularity. First, it satisfies criterion (3): the similarity between objects can be extracted by examining the coarse representation of their major components, whilst small differences can be read off from modules that provide finer detail. Second, the model's modular construction makes it possible to effect changes in one module without disrupting others. Third, it facilitates recognition both of whole objects and of their parts.

Marr and Nishihara suggest a method of computing this type of representation from the visual image (criterion (1)). They consider primarily the construction of 3-D representations from silhouettes of objects: further details of the computational processes that effect the decomposition into parts and the assumptions underlying them can be found in Marr¹⁰. Figure 2 shows one way in which the main component axes may be recovered from the outlines of a donkey by segmenting the figure at its principal inflection points. Although this decomposition into component parts each with its own axis was achieved by a computer program, it is hard to evaluate how far the program would succeed with a range of other objects and how far it would succeed in producing the same segmentation with different viewpoints.

Even if the same segmentation is achieved, the initial description in terms of generalised cones and the relation between their axes will differ depending on viewpoint. In viewing real 3-D scenes, this problem can largely be overcome by taking into ac-

count cues (such as stereopsis and motion parallax) to the distance of different parts of the figure from the eye: such cues make it possible to compute the real length of the different axes and the real angle between one axis and another. Difficulties arise when the observer is presented with a two-dimensional picture of an object seen from a viewpoint that results in the foreshortening of the model axis. Marr and Nishihara consider a number of ways of dealing with this problem. If any part of an object can be recognised (for example, the foot of a horse), it would be possible to recover the stored representation of the whole object, and use that representation to guide the interpretation of the remainder of the picture. Moreover, the relative positions at which different parts of a body are joined to a principal axis are little affected by foreshortening and this information could again be used to retrieve the correct stored representation. Once the stored representation has been accessed, it is possible to estimate the orientation of the model axis with respect to the observer's viewpoint and to compute whether given this orientation the details in the image correspond to the model held in memory. Marr and Nishihara specify how this process may be carried out: it involves computing a 3-D representation of the object in viewer-centred coordinates, and adjusting the estimate of the orientation of the model axis by moving backwards and forwards between this representation and the representation stored in memory in object-centred coordinates.

Evaluation of the model

There are several difficulties in Marr and Nishihara's theory as developed so far, the first of which they note themselves. First, it is not clear that it would be possible to pick a model axis which remained the same for any given object regardless of viewpoint. As already noted, apart from the difficulties with objects that are not elongated, problems arise with elongated objects that are very foreshortened. This apparent drawback may in fact constitute one of the theory's strengths. Foreshortened pictures of common objects, for example, a pail or a telephone seen from directly above, are in fact very difficult to recognise (see Fig. 3). Second, it is not clear that it will always be possible to decompose the same object seen from different positions in the same way. Although at any level of modularity, the system is supposed to recover components of roughly the same size, decisions must be to some extent arbitrary. In Marr and Nishihara's own example (Fig. 1) an arm could be as readily decomposed

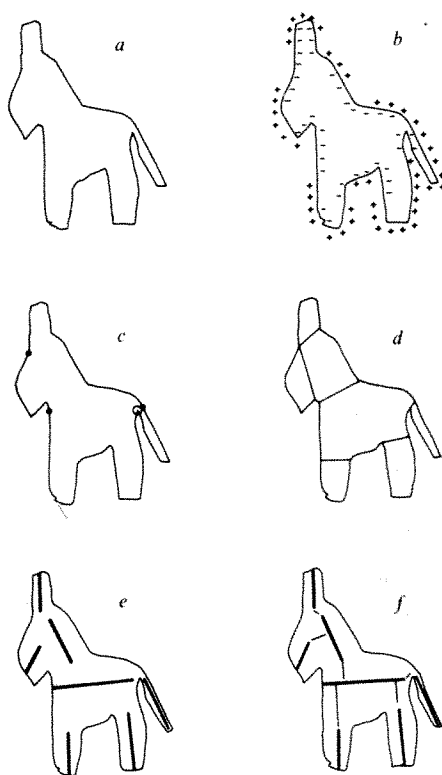


Fig. 2 The contours formed by the silhouette of a body composed of generalised cones can be used to locate the projections of its natural axes provided that they are not severely foreshortened. One method of decomposing the body and recovering the axes of the parts is shown in this example from a programme written by P. Vatan. The initial outline (a) was obtained by applying local grouping processes to the 'primal sketch' of an image of a toy donkey. This outline was then smoothed and divided into convex (pluses) and concave sections (minuses) (b). Next, strong segmentation points, like the deep concavity circled in (c), are identified and a set of heuristic rules are used to connect them with other points on the contour to produce the segmentation shown in (d). The component axes shown in (e) were then derived. The thin lines in (f) indicate the junctions of the head, leg and tail components to the torso axis, and of the snout and ear components to the head axis (figure and legend based on ref. 5).



Fig. 3

upper-arm, forearm and hand as into upper-arm and forearm (with the latter being decomposed in a further module into forearm proper and hand). The problem of decomposition into components of approximately the same size becomes particularly acute if some of the components are very foreshortened. Failure to decompose an object in the same way would make it difficult to match the initial representation of the object to the representation stored in memory. Third, the model seems in some ways to exceed the capacities of human vision. Rock¹¹ and others have found that elongated 2-D shapes are not readily recognisable when rotated in the picture plane unless the observer has had experience of seeing them in several orientations.

The proposed model does, however, have many new and useful features. As stressed above, the theory facilitates the computational processes underlying object recognition. It is virtually the first attempt to specify a general theory of the form in which visual representations of 3-D objects are stored. It is an advance on most previous theories in that it handles objects with curved surfaces and silhouettes of objects: although there is less difficulty in constructing unambiguous representations when viewing the real world directly, people can form 3-D representations from silhouettes and it is necessary to account for this capacity.

Moreover, in addition to its computational power, the model has in my opinion considerable plausibility on psychological grounds. First, in looking at an object we are simultaneously aware both of its visible structure with respect to our viewpoint and also of the volume of space it occupies: Marr and Nishihara compute both of these representations and give good reasons why it is necessary to do so. They show

how recognition can be facilitated by adjusting a representation in viewer-centred coordinates by fitting it by successive approximations to a stored model which is independent of viewpoint. It might be added that no matter how much constructing a description that is independent of viewpoint facilitates recognition, it would still be necessary to build a description in terms of viewer-centred coordinates in order to manipulate objects. Second, their model explains the difficulty experienced in recognising pictures of foreshortened objects. Third, although Rock¹¹ found that recognition of elongated objects rotated in the fronto-parallel plane was difficult, they were easier to recognise under rotation than objects that were not elongated. Fourth, as Marr and Nishihara note, there is evidence that people sometimes refer the description of the parts of an object to its longest axis. One instance, to which Mach¹² was the first to draw attention, is shown in Fig. 4. The single large diamond is seen as a diamond—its description is referred to its vertical axis; the diamonds forming a diagonal line are, however, usually seen as squares—their description is referred to the orientation of the model axis defined by the direction of elongation of the whole line. Finally, the theory captures our intuition that in viewing an object we see it (and verbally describe it) as composed of discrete parts each occupying a volume of space. Moreover, if it is assumed that it is possible to be conscious of the details of only one of the modules at a time, the theory satisfies our intuition that we cannot simultaneously form an image of the general shape of a man and of the details of the shape of his left ear.

The problem to which Marr and Nishihara address themselves is how

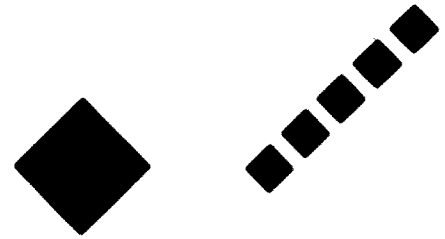


Fig. 4 The single diamond is seen as a diamond because its orientation is perceived with respect to the vertical; but the diamonds in the oblique line tend to be seen as squares because their perceived orientation is determined by their relationship to the diagonal line of which each is a part.

we recognise a given object as a member of a class of objects all of which have the same basic physical shape. It is worth noting that there is a further and even deeper problem of visual perception to which neither they nor anyone else have provided any solution. This problem is how we recognise the category to which a new object belongs when the new example has little or no similarity of shape to examples previously encountered, but does have the same function. Chairs come in a great variety of shapes and sizes and all they have in common is that they are man-made objects having a surface suitable to receive the weight of the human bottom and another fitted to support the human back. Nevertheless, we have little difficulty in recognising, even when first encountered, bizarre new chairs thought up by futuristic designers: in order to do so, we must integrate our knowledge of the function of chairs with the 3-D description formed of the new example.

It has of course been impossible to present here more than an outline of Marr and Nishihara's paper which contains many subtle points of detail. As with all Marr's work, the decisions he has taken in theory construction are always motivated: there is none of the 'I put that in because it seems to work, goodness knows why' attitude that characterises the *ad hocery* of some practitioners of artificial intelligence. Marr and Nishihara's paper provides a starting point, not a finished theory. Nonetheless, it contains some brilliant insights into a deep, complex, and much worked over problem, and it is possible that in a few years' time it will be regarded as a classic. □

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The Ice-age Amazon

from Paul Colinvaux

A MAP has been published by Ab'Saber (*Paleoclimas* 3, 1; 1977) which shows the Amazon basin to have been largely without closed forest during the last ice age. Wet forest was restricted to the eastern flanks of the Andes, to the mountains of Guiana in the north, along the coastal escarpment of eastern Brazil, up which there now climbs the spectacular highway linking São Paulo with its port of Santos, and in scattered patches along the Amazon river system. This map is the most definite statement yet of a growing consensus that equatorial South America has been subjected to major climatic change. The importance of this to both climate dynamics and ecological theory is very great.

The most persuasive direct evidence for environmental change in the Amazon basin is the existence of dissected and gullied land surfaces now covered by closed forest. These surfaces were revealed by side-scan radar from aircraft in surveys made by the Brazilian government. Complete maps from the side-scan survey do not seem to have been published but I was assured in Brazil that the coverage is extensive. Both Tricart (*Rev. Geomorph. Dynamique* 23, 145; 1975) and Bigarella & Becker (*Bol. Paranaense Geosci.* 33, 171; 1975) have given accounts of the fossil land forms, showing that at times in the Pleistocene wide stretches of the Amazon cannot have supported closed forest. Ab'Saber's map is a further compilation of these data, coupled with the results of many years of geological and soil survey. Dating of the dissected surface is still weak but Bigarella & Becker show that outside the Amazon basin near São Paulo, the last episode preceded a radiocarbon date of 10,000 b.p., thus allowing the event to be synchronous with northern glaciations.

The ice age climate of equatorial America is also charted in the high Andes and in the Galapagos Islands. Andean lakes from Titicaca in Peru to Bogota in Colombia have retreated from shorelines of much larger lakes in the recent geological past. The shorelines themselves have not yet yielded radiocarbon ages of their occupancy, but a powerful argument suggesting that the larger lakes were associated with glaciations comes from studies of end moraines abandoned by the Quelccaya ice-cap in Peru. The Quelccaya is the world's third ice cap, perched improbably on the mountains within 14° of the equator. Continuing mass-balance studies of the ice-cap by

Thompson *et al.* (*Science* 203, 1240; 1979) show that precipitation must be heavy to offset the high annual ablation and that an ice-cap can only be maintained when clouds shield the ice for many months each year. Extension of the ice-cap in the past, therefore, implies increased precipitation and cloud, conditions which would favour enlargement of the lakes and suggesting that their growth was synchronous with Andean glaciations.

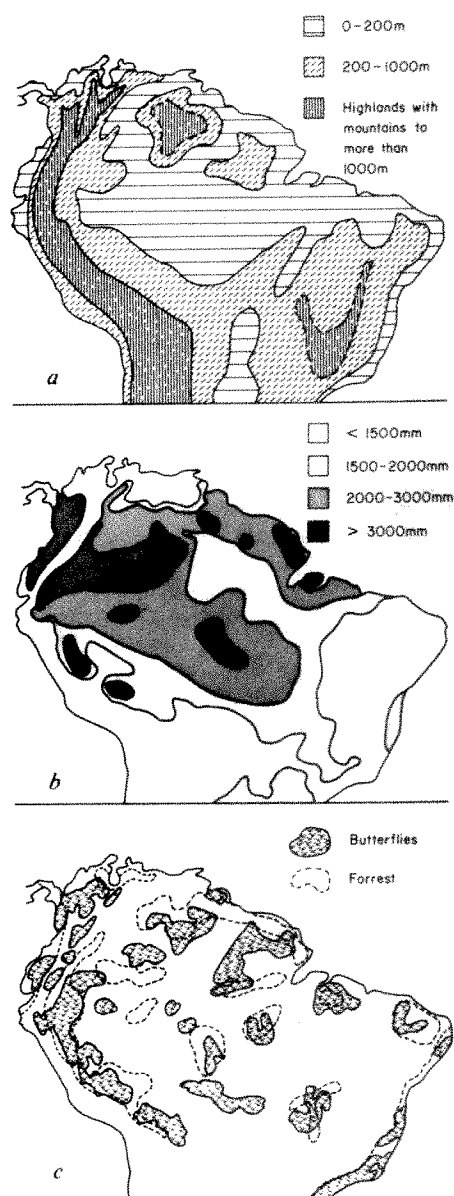
Finally there is well-dated sediment sequence from the Galapagos Islands which shows that the moist highlands, which are now shrouded by stratus cloud and fog-drip, were dry from 10,000 b.p. to a time beyond the reach of radiocarbon dating (see *Nature* 240, 17; 1972). The Galapagos record has the securest dating, encouraging the impression that climate along an equatorial strip across the continent suffered many changes during the last glaciation of the northern hemisphere. There was increased dryness at the Galapagos to the west, increased wetness in part of the high Andes, and a lack of forest cover in the Amazon basin (suggesting seasonal rain and drought) all the way across the continent to the edge of the coastal escarpment in the east. A possible implication for climate dynamics is that the Intertropical Convergence Zone (ITCZ) was largely obliterated in glacial times, as it is in Africa. At least there seems good reason for thinking that the zone was neither to the south nor to the north of its present position (see *Nature* 240, 17; 1972; 245, 91; 1973). But the more exciting implications are for ecological theory. A review of the latest data on the distribution of modern taxa by Simpson and Haffer (*Ann. Rev. Ecol. Syst.* 9, 497; 1978) is thus of great interest.

There are many Amazon data about butterflies compiled from the very large collections which have been continually growing since the first of their kind by Waterton and Wallace in the last century. Bird data are also more complete than one might expect and have been supplemented by less complete collections of lizards and some plants by Simpson and Haffer. The data show that there are many, highly local, definite, disjunct distributions. What is more, the disjunctions can be shown, particularly with the butterfly data, to be bordered by zones where arrays of hybrids are normal. Turner (*Evolution* 25, 471; 1971) compared the marking of modern hybrid butterflies with those illustrated in Dutch books of the 18th century and showed that the same hybrids have been produced for 200 yr, or 2,000 generations, making plausible the postulate that the present patterns of distribution are not changing rapidly. As these patterns of distri-

bution define patches within continuous rain forest today, Simpson and Haffer seek their origin in the past. They postulate that each centre of distribution represents a forest refuge in the more seasonal or drier climates of the last ice age. In a footnote to their paper they suggest that the forest refuges shown on the Ab'Saber map can be reconciled with these modern centres of distribution.

The figure shows sketch-maps of the region for modern topography, modern rainfall, and a summary of dispersal data for butterflies. Very approximate boundaries for the ice-age forests of Ab'Saber are added to the butterfly map.

There is a strong and obvious synchrony between many butterfly distributions and the postulated forest



a, Modern topography; b, modern rainfall; c, distributions of modern butterfly populations (shaded) and ice age forest refuges estimated from fossil land forms (dashed lines).

refuges but these tend to be along the mountains or escarpments that ring the Amazon Basin. On the lowlands themselves correlations are scarcely conclusive. It is probable that traces of the ice age Amazon are indeed stamped on modern distributions as Simpson and Haffer suggest, yet it may still not be the most parsimonious of hypotheses to say that the past has been decisive. The way must be open to ecological hypotheses explaining the patterns of distribution from modern rainfall, modern topography and the channels of modern rivers. These hypotheses may well be the more parsimonious, at least without detailed mapping of the ancient vegetation by such means as pollen analysis, something on which no start has yet been made.

Nevertheless, it is clear that all hypotheses of the high diversity of tropical biota must now reckon with the fact that the equatorial lowlands are not immune to climatic change, being in

fact subject to environments with each northern glaciation sufficiently different to replace one biome with another. One of the most attractive of the hypotheses for high diversity has been that of Sanders (*Amer. Nat.* **102**, 243; 1968) who showed that long physical stability on ocean floors is associated with high diversity, in spite of low productivity. The suggestion that high stability in equatorial latitudes accounted for the high species list in a similar way has been attractive to many of us. We now have to modify our views. Possibly, the important part of the stability of tropical systems is still that areas must always have persisted in which seasons were minimal, because without them the biota of the wet tropics as we know them cannot have survived. The areas may have moved around a bit, but they had to be there. In these places we can still postulate lower extinction rates, higher speciation rates, or more niche-overlap as our fancy dictates. □

'robust' australopithecines and have, in fact, been placed with that group by some workers. A further problem with White and Johanson's comparative group is that they appear to have included dental measurements from Taung for teeth (4th premolars and 3rd molars) which the child did not possess (*Science op. cit.*).

White and Johanson recognise that it is the total pattern of characters which must define a taxon and set it apart from other species. While bearing this in mind, it must be pointed out that very few characters of *A. afarensis* are not also found in *A. africanus*. For example, important distinguishing features of the new species are said to occur in the anterior premolars of the mandible. In *A. afarensis* this tooth has a large outer cusp and a very small inner cusp; the long axis of the tooth is rotated 45 degrees or more to the long axis of the tooth row. Yet a similar pattern of morphology is seen in the Miocene genus *Ramapithecus* (regarded by some workers as an ancestral hominid) and is also found in the Sterkfontein specimens (Robinson *Transvaal Museum Memoirs* No. 9, 72; 1956). Similarly, the mandibular canines of *A. afarensis* are said to be large, yet in both length and breadth means this tooth is larger in the Sterkfontein sample (Robinson *op. cit.*). Other features, such as three-rooted upper premolars and an increase in molar size from front to back can also be duplicated in the South African sample. Morphological congruence, rather than distinctiveness, can also be demonstrated in the post-cranial remains. One of the wrist bones, the capitate, of *A. afarensis* is described as "waisted" and the third metacarpal, with which it articulates, is said to lack a styloid process. A capitate from Sterkfontein has a similarly constricted shape on its lateral surface (Le Gros Clark *J. Anat.* **81**, 300; 1947; Lewis, *J. Hum. Evol.* **2**, 1; 1973). Moreover, while a third metacarpal is not known from this site, the articular area on the Sterkfontein capitate may indicate that the styloid process of the metacarpal was also missing (Le Gros Clark *op. cit.*). Finally, the innominate of one member of *A. afarensis* (AL 288-1 or 'Lucy') is extremely similar in both size and morphology to one from Sterkfontein.

It is also true, however, that the *A. afarensis* specimens do show characters not known to be represented in *A. africanus*. For example, the upper central incisors are said to be very broad in the earlier group and the lateral incisors relatively small. However, the incisors are very poorly known for both *A. afarensis* and *A. africanus*. While the known central

Difficulties in the definition of new hominid species

from our Palaeoanthropology correspondent

As a group, palaeontologists have a notable tendency to propose new names for the fossils they find. Whether from scientific profundity or other motives, new binomials seem to appear with an almost greater frequency than the fossils themselves. Historically, the same has been true of human palaeontologists; proposals of five or six different binomials for the same fossil hominid are not unknown. Paradoxically, however, the enormous number of new hominid fossils that have appeared in this decade has generated very little taxonomic activity. One exception, 'Homo ergaster', has been permitted to enter an unmentioned and well-deserved obscurity.

Now, Don Johanson and Tim White have proposed a new species for the Plio-Pleistocene hominid genus, *Australopithecus* (*Kirtlandia* **28**, 1, 1978; *Science* **203**, 321; 1979). The species, *A. afarensis*, is based on specimens found at Laetoli in Tanzania and in the Hadar area of Ethiopia; they appear to date between 2.9 and 3.8 million years ago. While the majority of this material has been recovered very recently, the 'Garusi' maxilla was recovered at Laetoli in 1939 by Kohl-Larsen.

A workable definition of a fossil species continues to elude palaeontologists. While recognising, as do neontologists, that genetic isolation is the prime criterion for species formation,

the way such isolation can be recognised in the fossil record is unclear. Most definitions centre on the demonstration of morphological distinctiveness; a fossil species should therefore have a clearly defined and distinguishing set of morphological characters. This is apparently the sort of definition that Johanson and White have worked with. However, their success in demonstrating the morphological distinctiveness of *A. afarensis* is by no means clear. Their difficulty, and the difficulty of those who try to evaluate their classification, lies equally in the incompleteness of the fossil specimens and in the wide morphological variability of early (as well as recent) human populations. Moreover, the type specimen of the most appropriate comparative taxon, *Australopithecus africanus*, is a juvenile. The fact that this specimen, the Taung child, has only the first molars of its permanent dentition in place has long caused taxonomic difficulty. For this reason, the gracile australopithecines from Sterkfontein, in South Africa, are usually regarded as representatives of this species. White and Johanson have also included the australopithecines from Makapan, along with those from Sterkfontein, in their comparative group. However, the gracile status of the Makapan hominids is not certain. They do demonstrate several cranial and dental characters more often found in the heavily built

incisors are, indeed, very large in *A. afarensis* they are known from only two individuals. The comparative sample from Sterkfontein consists of a single individual. The very limited sample of lateral incisors from Sterkfontein ($n=2$ individuals) shows this tooth to be smaller than the very "reduced" *A. afarensis* tooth. In the *A. afarensis* maxilla the canine characteristically projects below the level of the other teeth and a diastema is present in the tooth row. Although this is often regarded as a primitive feature, at least one *Homo erectus* individual, from Java, also shows a similar pattern. It should be pointed out that in the original reports on the Hadar material, specimens showing these features were placed not in a primitive hominid group as they now are, but in the more advanced genus, *Homo*.

Thus, there are very few features said to be characteristic of *A. afarensis* which are, in fact, distinctive of that group. Perhaps a major feature of the specimens now grouped into *A. afarensis* is the amount of variability present. This variability originally led to the informal proposal that as many as three taxa were present at Hadar. Therefore, while the original reports maximised the taxonomic importance of this variability these current reports have, in effect, minimised it to the level of sexual dimorphism. Undoubtedly, considerable sexual dimorphism was present in hominids of the Plio-Pleistocene but can such dimorphism explain all the variability present at Hadar and Laetoli? Perhaps, but both the recovery of additional material from this important time period as well as more careful comparative studies of existing specimens are required before the validity of *A. afarensis* can be affirmed. □

Death of a schistosome

from F. E. G. Cox

Schistosoma mansoni in laboratory rodents is a useful model for the study of schistosomiasis and much of what we know about the biology of the parasites that cause the human disease has been derived from studies on rats and mice. The infection in a mammal begins when free-swimming cercariae, released from a snail intermediate host, attach to and penetrate the skin. While this is happening, the cercariae rapidly transform into schistosomula by discarding the glycocalyx required during their sojourn in freshwater and reveal-

ing the characteristic double bilayer outer membrane. Each schistosomulum then makes its way to the blood vessels, stimulating an immune response but avoiding its consequences by acquiring red cell antigens that render it immunologically invisible. However, subsequent schistosomula that have not acquired host antigens are fully susceptible to attack from the immune system and thus superinfection is effectively prevented. The schistosomulum is the obvious weak link against which any immunological attack must be directed and the development of a vaccine against schistosomiasis will depend to a large extent on our understanding of what happens to the schistosomulum during the first few hours in its host.

The immune responses against schistosomula in previously sensitised hosts are extremely rapid and effective and several effector mechanisms have been postulated including antibody and complement and complement activated through the alternative pathway. Recently, various cells have also been found to be involved, in particular macrophages in the presence of IgE (A. Capron *et al.* *Nature* **253**, 474; 1975), eosinophils in the presence of IgG (A. Capron *et al.* *Amer. J. trop. Med. Hyg.* **26**, 39; 1977; Mackenzie *et al.* *Clin. exp. Immun.* **30**, 97; 1977) and eosinophils in the presence of complement (Ramalho-Pinto, McLaren & Smithers *J. exp. Med.* **147**, 147; 1978). It is known that eosinophils adhere to a schistosomulum through either Fc receptors or complement receptors and, in *in vitro* systems, empty their contents on to the surface of the worm (McLaren, Mackenzie & Ramalho-Pinto *Clin. exp. Immun.* **30**, 105; 1977). Until now, however, the evidence that eosinophils actually kill the schistosomulum has been circumstantial: labelled chromium release, for example, is increased but no ultrastructural damage has been detected. It has now been conclusively shown by the group of workers at Mill Hill that the schistosomulum is damaged and killed (McLaren, Ramalho-Pinto & Smithers *Parasitology* **77**, 313; 1978).

The death of the schistosomulum begins when eosinophils attach to its surface, flatten out and release their contents causing a small lesion in the tegument through which the cells migrate into the interstitial tissue of the worm. The tegument is prised off and the worm dies. The actual method of attachment of the eosinophils to the surface of the worm is important as is the ratio of cells to schistosomula. 3,000–5,000 eosinophils per schistosomulum are necessary (3,000 were used

in the previous studies) and attachment through complement receptors results in 100% killing in 20 h whereas attachment through Fc receptors results in little damage within 20 h and, in some worms, no damage at all.

The eosinophil product that actually causes damage to the schistosomulum seems to be the major basic protein (MBP) from the eosinophil crystalloid core (Butterworth *et al.* *J. Immun.* **122**, 221; 1979). The destructive effects of MBP are nonspecific but schistosomulum killing is effectively specific because of the tight binding of eosinophils to antibody-coated worms and the release of MBP into a small space between the eosinophil and the schistosome surface. The action is therefore localised and no bystander host cell damage occurs.

There is now abundant evidence that eosinophils in conjunction with either IgG or complement can kill schistosomula but the whole story may be more complicated than this. Monique Capron and her colleagues at Lille have observed that both eosinophils and mast cells adhere to schistosomula and have found that, in the presence of IgG and with 5,000–10,000 effector cells per worm, the proportion of mast cells to eosinophils is a very important factor in killing (M. Capron *et al.* *Europ. J. Immun.* **8**, 127; 1978). The cytotoxic effects decrease as mast cells are depleted and increase with mast cell enrichment. Ultrastructural studies show that little damage is done to the tegument in the first 3 h but that it is very vacuolated after 20 h, findings comparable with those of the Mill Hill group. In a further series of experiments (M. Capron *et al.* *J. Immun.* **121**, 2518; 1978) it has been shown that eosinophil-dependent cytotoxicity proceeds in three steps, the activation of the mast cells, the release by these cells of a soluble mediator that acts as a signal to the eosinophils and the reception of this signal by the eosinophils. Activation of the mast cells depends on IgG binding to the schistosomulum, the IgG involved being IgG2a, the second anaphylactic antibody in rats. This antibody also seems to be involved directly in eosinophil-mediated cytotoxicity. As both mast cells and eosinophils possess receptors for IgE and complement there may be several patterns of eosinophil activation in schistosomiasis and these may act separately or together. Whether the eosinophil-complement mechanism also involves mast cells remains to be determined, for mast cells also adhere to schistosomula through complement receptors (Sher & McIntyre *J. Immun.* **119**, 722; 1977).

An understanding of the role of eosinophils in immunity to helminth infections is likely to become

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creasingly important as the attachment of these cells and the release of their contents have also been seen with the adult nematodes, *Nippostrongylus brasiliensis* and *Trichinella spiralis* (McLaren, Mackenzie & Ramalho-Pinto *Clin. exp. Immun.* **30**, 105; 1977) and larval *T. spiralis* (Kazura & Grove *Nature* **274**, 588; 1978). The finding that the nematode *Ascaris suum* contains a substance that is chemotactic for eosinophils and neutrophils (Tanaka, Baba & Torisu *J. Immun.* **122**, 302; 1979) may mean that certain worms are actually instrumental in their own destruction. Immunity to helminths is complex and there are unlikely to be any simple explanations of all the facts observed so the vast amount of fundamental research being carried out is easy to justify in terms of its possible importance to our understanding of human and animal diseases.

Growing embryos *in vitro*

from Gillian Morriss

THE once inaccessible postimplantation mammalian embryo can today be grown in transparent vessels in the laboratory for various periods between implantation and an advanced stage of organogenesis. This is largely due to the work of New and his collaborators in Cambridge, and (the earliest stages) Hsu and colleagues in Baltimore (reviewed by New, *Biol. Rev.* **53**, 81; 1978). Mammalian embryologists have been slow to exploit the opportunities of whole embryo culture, but recent progress in the culture technique represents a significant increase in its potential, since the *in vitro* requirements of postimplantation mouse embryos have now been defined.

Pre and peri-implantation culture of mouse embryos are now routine procedures, whereas for postimplantation stages the techniques were previously well worked out only for the rat. Rat embryo culture is an important technique for the investigation of environmental factors such as energy supply, gas phase constituents, and chemical teratogens. For specific chemical interference with intra or extracellular components such as basement membrane collagen, glycosaminoglycans and microfilaments, it is immaterial which mammalian species is used. However, for the investigation of abnormal development which is genetic in origin,

mouse mutants provide a range of abnormalities unrivalled by any other experimental species. These mutants represent an important potential source of information on normal and abnormal mechanisms of mammalian morphogenesis. The onset of abnormal development of several of them occurs during the early postimplantation period (primitive streak stage to mid-organogenesis), and *in vitro* studies will make an important contribution to elucidation of the mechanisms involved.

Y.-C. Hsu (*Devl Biol.* **68**, 453; 1979) has achieved a significant improvement in the proportion of preimplantation stage mouse embryos which attach *in vitro*, develop into 'egg cylinders' and subsequently reach the 5–10-somite stage. The technical alterations were simple: blastocysts were cultured singly in 2 ml medium in 35 mm culture dishes, and 2 days after attachment (early 'egg cylinder' stage) the serum component in the medium was changed from 20% fetal calf serum to 20% human cord serum (the synthetic component was CRML 1066 throughout). Twelve out of twenty embryos developed to the 5 to 10 somite stage, after which they began to degenerate. It is important to note that the photographs of these 'successful' embryos closely resemble rat embryos grown at this stage in dish cultures: the spinal neural tube closes, but the cranial neural folds fail to change from their early convex shape to the concave shape which precedes closure of the brain tube.

From the primitive streak stage onwards, rat embryos undergo normal morphogenesis only when the culture vessel and/or the culture medium is continuously moving. T. W. Sadler (*J. Embryol. exp. Morph.* **49**, 17; 1979) has modified this technique for the culture of mouse embryos from the 2–4-somite stage for up to 48 h. During this period mouse embryos require a greater volume of gas phase than rat embryos; however, their liquid requirements are apparently identical, with rat serum supporting normal development. Successful culture of younger mouse embryos, from the primitive streak stage for 48 h, has been achieved by M. Snow and P. P. L. Tam (personal communication), using 30% newborn calf serum in Dulbecco's modified Eagle's medium supplemented with glucose (4.5 g l^{-1}).

Meanwhile, the better-established techniques of rat embryo culture are yielding information on the metabolic requirements of the early embryo, and on some aspects of the relationship between these and morphogenesis. Klein *et al.* (*J. exp. Zool.* **203**, 313; 1978) have improved development of 10-day embryos in small quantities of serum (four embryos per 2 ml) by changing the serum after 24 h. The im-

provement was related to depletion of a protein (or proteins) of approximately 125,000 molecular weight; this protein may be identical to the α_2 -globulin of 126,000 molecular weight, identified by Hoch and McVey (*J. biol. Chem.* **252**, 1881; 1977) as one of the two major human serum DNA-binding proteins.

Energy production of 10-d embryos is primarily through anaerobic glycolysis (Tanimura & Shepard *Proc. Soc. exp. Med. Biol.* **135**, 51; 1970); subsequently (day 10 to day 12) oxygen dependence progressively increases, as the yolk sac and then the chorioallantoic placental circulations develop. Not surprisingly, these observations are matched by the oxygen requirements of cultured embryos (reviewed by New *op. cit.*). Robkin and Cockcroft (*Teratology* **18**, 337; 1978) have compared the effects of oxygen deprivation and carbon monoxide elevation on cultured 11–12-d embryos. Decreased availability of oxygen produced a greater response (depression of metabolism and growth rate) than elevated carbon monoxide, and the authors suggest that at this stage of development oxygen transport is primarily through solution rather than in combination with haemoglobin.

Our understanding of the mechanisms involved in controlling most aspects of normal and abnormal organogenesis has progressed very little compared with progress in other areas of developmental biology during the past decade or so. It is to be hoped that the recent improvements in techniques of mammalian embryo culture will soon be fully utilised in pursuit of such studies. □



A hundred years ago

ON Tuesday morning, in the presence of a small number of his sorrowing friends, the remains of the late Prof. W. K. Clifford were placed in their last resting-place in Highgate Cemetery.

THE following grants have lately been made from the Research Fund of the Chemical Society: —10*l.* to Dr. C. A. Burghardt for an investigation into the constitution of topaz; 20*l.* to Mr. Francis Jones for the investigation of boron hydride; 15*l.* to Mr. F. D. Brown for the study of the theory of fractional distillation; 30*l.* to Dr. Dupré for the estimation of organic carbon in air; and 15*l.* to Prof. T. E. Thorpe for the investigation of albitene, the hydrocarbon of nut-pine.
From *Nature* **19**, 27 March, 491; 1879.

review article

The new frontier of particle physics

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Quarks and leptons have emerged as elementary constituents of matter. To these may perhaps be added the field particles, or mediating agents, of a unified theory of the weak and electromagnetic forces and also those of a new theory of the strong (nuclear) force. This article briefly outlines some of the main items of evidence for this new picture and indicates a few of the many crucial questions to be answered.

THE first lepton to be discovered was the electron, by J. J. Thompson in 1897. The leptons are a family of particles with spin 1/2 (in units of $\hbar$) and a membership summarised in Table 1 which includes two recent additions.

The charged leptons participate in both the electromagnetic and the weak interaction (responsible for processes such as radioactive β -decay) while the neutrinos, with zero electric charge, are influenced only by the weak force.

Since its discovery 40 years ago, the muon has remained one of the great enigmas of particle physics; it differs from the electron only in mass, has equal strength couplings to the electromagnetic and weak forces and shows no sign of additional modes of interaction which might account for this mass difference. Moreover, in 1961 the electron and muon were found to be associated with different neutrinos, ν_e and ν_μ . Another lepton pair has now been added to the list; with the matching expansion in the numbers of types of quarks this multiplicity of forms is one of the most tantalising aspects of particle physics at present.

The annihilation of high energy electrons (e^-) and positrons (e^+) brought together in the head-on collision of oppositely circulating beams of e^- and e^+ can lead to the creation of charged lepton pairs or numbers of hadrons (particles which participate in the strong interaction as well as in the weak and electromagnetic interactions). The τ -lepton was discovered in 1975 by Perl *et al.*¹ using the e^+e^- machine SPEAR built at the SLAC laboratory. Its existence has been confirmed by experiments² at the similar machine, DORIS, at the DESY Laboratory, Hamburg. It is almost certainly associated with a new neutrino, ν_τ , and although the mass limits on ν_τ are still high it is for the moment assumed, like ν_e and ν_μ , to have zero mass.

The magnetic moments of the electron and muon have been measured to remarkable precision in two experiments of great

technical ingenuity and scientific elegance. The work on the electron, by Dehmelt *et al.*^{3,4} at Seattle has reached an accuracy of 1 part in 10^{10} and at CERN the group led by Picasso⁵ has achieved 1 part in 10^8 for this static property of the unstable muon. These experiments test the validity of quantum electrodynamics (QED), the quantised electromagnetic theory; they also probe the structure of the particles and the possibility of couplings to additional fields of force. Dirac's theory for point-like particles of spin 1/2 predicts a magnetic moment of $\mu_D = eh/2mc$ (m is the particle mass), however, the measured values are very slightly higher (by about 1 part in 10^3) and the difference is called the anomalous magnetic moment. In QED the anomalous moment is due to corrections to μ_D , called radiative corrections, which arise from interactions of the charge with the electromagnetic field; these can be illustrated by the

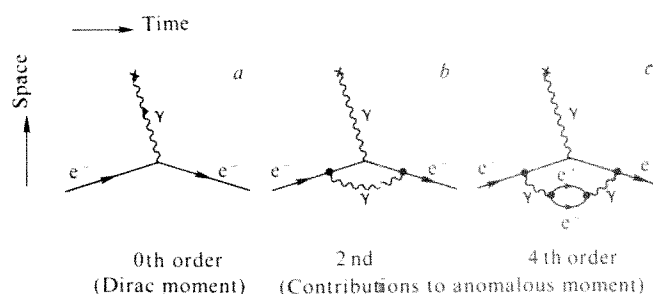


Fig. 1 Feynman diagrams showing contributions to the magnetic moment of the electron.

Feynman diagrams of Fig. 1. (Feynman diagrams illustrate details of the interactions between particles and fields by showing their evolution in space and time; with a set of rules given by Feynman they provide a prescription for calculating transition rates. One space dimension is shown and time 'flows' from left to right.)

The lowest order diagram corresponds to interaction with a virtual photon, mediator of the external electromagnetic field, and gives the Dirac moment, μ_D ; the radiative corrections involve emission and re-absorption of virtual photons, including in higher orders the transient presence of virtual charged lepton pairs. This 'structure' associated with the field is the source of the anomalous moment. The calculations have been carried out to sixth order (96 diagrams contribute) and the excellent agreement between theory and experiment^{6,7} represents a remarkable success for QED. In addition the experiments led to the following conclusions about the electron and muon

Table 1 The leptons (and anti-leptons)

Name		Charge*	Mass (MeV)†	Lifetime
Electron	$e^-(e^+)$	$-1(+1)$	0.51	Stable
Electron-neutrino	$\nu_e(\bar{\nu}_e)$	0	$0(<4 \times 10^{-5})$	Stable
Muon	$\mu^-(\mu^+)$	$-1(+1)$	105.7	2.2×10^{-6} s
Muon-neutrino	$\nu_\mu(\bar{\nu}_\mu)$	0	$0(<0.5)$	Stable
τ -lepton	$\tau^-(\tau^+)$	$-1(+1)$	1807	$<3 \times 10^{-12}$ s
τ -neutrino	$\nu_\tau(\bar{\nu}_\tau)$	0	$0(<300)$	Stable(?)

*In units of the electron charge, e .

†The proton mass in these units is 938.3. The neutrinos are assumed to have zero mass; the present experimental limits are given in parentheses.

there is no evidence of a new coupling sufficient to account for the heavier mass of the muon; (2) the electron and muon behave like point-like, structureless particles at least down to distances approaching 10^{-15} cm.

The quarks

Leptons can be created and their properties measured to very high precision in the laboratory. In contrast all we know about quarks has to be inferred from indirect information like the patterns of hadron spectroscopy, the products of e^-e^+ annihilation, the scattering of leptons probing the nucleon and similar processes. This is a severe technical difficulty, and the failure to establish the existence of free quarks has cast doubt on this concept. These difficulties make it worth summarising some of the evidence which has led to the present common acceptance of the quarks as constituents of the hadrons.

The quark proposal^{8,9}, made by Gell-Mann and independently by Zweig in 1964, was the culmination of a successful attempt to explain the numerous spectrum of hadronic states (more than 200 are known). There are two main classes of hadrons: the baryons have half-integer spin and include the proton and neutron (nucleon); the mesons have integer spin. (All particles of half-integer spin are called fermions and those of integer spin are called bosons.)

When the quark model was proposed only three components were required to 'build' all the known hadronic states; these were the u, d and s quarks listed in Table 2. The u and d quarks form an isospin doublet (like the proton and neutron), while the s quark carries the attribute of strangeness, a hadronic property discovered in the late 1940s. The different quark types have been given the collective name 'flavours'. Quarks not only have the exotic property of fractional electric charge, which should make them easily recognisable if freed from hadronic confinement, but also baryon-number (B) of $1/3$ ($-1/3$ for $\bar{q}$). Baryon-number $+1$ is assigned to all baryons (-1 to anti-baryons) and 0 to all mesons; it is a quantity believed to be strictly conserved by all interactions, leading to the apparently absolute stability of the lightest baryon, the proton.

The baryons are formed from three quarks ($3q$) and the mesons from quark-antiquark pairs ($q\bar{q}$); examples are: proton (uud), neutron (udd), π^+ -meson ($u\bar{d}$) and K^+ -meson ($u\bar{s}$). The ($3q$) and ($q\bar{q}$) recipe assembles multiplets of states, all with the same spin and parity; the multiplets formed from the u, d and s quarks correspond to those representations of the unitary symmetry group $SU(3)$ which are just the ones needed to accommodate the states observed (at least up to a mass of 3 GeV). The quark triplet corresponds to the simplest (non-trivial) representation of $SU(3)$. Baryons ($3q$) form multiplets of dimension (number of members) (1), (8) and (10) while the mesons ($q\bar{q}$) occur in (1) and (8).

Invariance to transformations of the $SU(3)$ group would imply equal mass for all the members of a multiplet. However, this symmetry is broken to the extent that mass differences occur; one may accommodate these by making the s-quark ~ 150 MeV heavier than the u and d quarks. The masses and magnetic moments of the low mass hadrons can be understood if the quarks have spin $1/2$, have Dirac-like moments and if the effective masses of the u and d quarks are ~ 350 MeV. (But these should not be taken as the masses free quarks might have—if they exist.)

Table 2 Quarks and anti-quarks

Name (Flavour)	Charges			
	q		$\bar{q}$	
	$+2/3$	$-1/3$	$-2/3$	$+1/3$
Up	u		$\bar{u}$	
Down		d		$\bar{d}$
Charm	c		$\bar{c}$	
Strange		s		$\bar{s}$
[Top?]	[t]		[$\bar{t}$]	
Bottom		b		$\bar{b}$

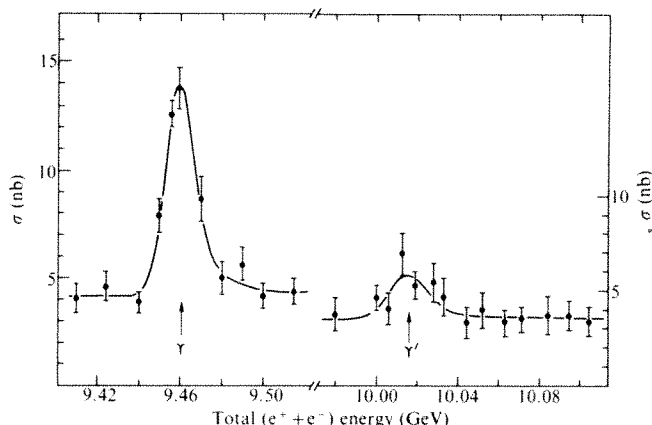


Fig. 2 Production of Y and Y' in e^+e^- annihilation at DORIS¹⁹.

Giving the quarks spin $1/2$ permits a description of hadron states embracing $SU(3)$ multiplets of different spin, within representations of the higher symmetry $SU(6)$. The quark model has been extended by including orbital (angular momentum) and radial excitations to account for higher mass and spin regions of the spectrum.

The original triplet of quarks had to be extended to a quartet (and thus $SU(3) \rightarrow SU(4)$) by the discovery of a new hadronic flavour, charm (c), in 1974. This was first signalled by the observation of a heavy, surprisingly stable meson state of mass 3.1 GeV called the J/ψ found simultaneously by the groups led by Ting¹⁰ at Brookhaven and Richter¹¹ at SPEAR. The J/ψ is formed of ($\bar{c}c$) and is accompanied by a number of other 'charmonium'^{12,13} states in a spectrum typical of that expected for a two fermion system (analogous to positronium, e^+e^-). The existence of a fourth type of quark with charge $+2/3$ had been suggested earlier by Glashow (who chose the name) and his collaborators; at first this was to achieve a quark-lepton symmetry (two pairs of each) and then¹⁴ to explain why a strangeness changing neutral-current decay mode of the K^0 -meson ($K^0 \rightarrow \mu^+ + \mu^-$) was strongly suppressed.

A fifth quark, named 'bottom' (b), (or 'beauty') is required by the experiments¹⁵ of Lederman *et al.*; this almost certainly heralds the existence of a sixth quark with a new flavour, to be called 'top' (t) (or 'truth'). Lederman's experiment¹⁶ detected a new meson state, called the Y (upsilon), as a peak at 9.5 GeV in the mass spectrum of ($\mu^+\mu^-$) pairs produced by 400 GeV protons at Fermilab. The same state has since been found^{17,18} at the DORIS e^+e^- colliding beam machine, running close to its upper limit of energy, and in pp collisions at the CERN ISR. The upsilon is partnered by at least one higher level, Y' (see Fig. 2)¹⁹ and probably a second, making a heavy analogue of the charmonium spectrum; this time the states are of hidden bottom ($b\bar{b}$) rather than hidden charm ($c\bar{c}$). The evidence points to charge $-1/3$ for this massive, ~ 5 GeV, quark and the new higher energy e^+e^- machine, PETRA, just starting to operate at DESY will be able to make states containing a single b-quark (such as, ($b\bar{d}$), and ($u\bar{b}$)).

The pattern of quark flavours, pairs of charge $-1/3$ and $+2/3$, demonstrated by the first four, is related to a quark-lepton symmetry embedded in the new unified theory of the weak and electromagnetic interactions. This is the reason for anticipating the sixth flavour, top, to partner bottom. It is not possible to predict the mass of hadrons carrying the t-quark but 'toponium' and 'exposed top' hadrons probably lie within reach of PETRA and another new e^+e^- machine called PEP, due to start up at SLAC in a year or so, both with maximum energies in the 40 GeV region.

Another important property of quark states, called 'colour', has emerged from the study of hadron spectroscopy. Certain baryons (such as the Δ^{++} state of mass 1238 MeV and spin $3/2$) contain three identical quarks with parallel spins in a state which is symmetric (the wave function does not change sign) to the

interchange of a pair of quarks. This breaks a cardinal rule, the spin-statistics theorem of Pauli, which requires the overall wave-function describing a system of identical fermions to be anti-symmetric on interchange of a pair. The difficulty has been resolved by introducing another degree of freedom²⁰, a means to distinguish the otherwise identical quarks. This requires a set of three labels say, red, blue and green. Then the forces between quarks are required to be such that at any instant each quark is a different colour (for example, the $\Delta^{++}(1238)$ state is $(u_R u_B u_G)$). The requirement that the colour part of the wave function be anti-symmetric to interchange of a pair of colour indices (to satisfy the spin-statistics rule) limits the low-lying hadron states to colour singlets—that is, states with no net colour; thus this new factor need not lead to a further great expansion of the hadron spectrum through the existence of coloured states.

Alternatively, by postulating that only colour singlet states may exist the possible quark combinations are reduced to just $(3q)$ and $(q\bar{q})$ so explaining the absence of free quarks (q), or such systems as (qq) , $(q\bar{q}q)$, and (qqq) . (But quark 'molecules' such as $(q\bar{q}-q\bar{q})$ or $(qqq-q\bar{q})$ would be allowed and there is some tentative evidence for them).

The lifetime of the π^0 -meson provides one direct piece of evidence for colour: without it the prediction of the lifetime is wrong by a factor of 9. Colour is evidently closely related to the nature of the quark binding force, a point we shall discuss with the new theory of the strong interaction. But we now look at three different types of experiment giving evidence for the constituent quark model and further support for colour.

Lepton-nucleon scattering

Although experiments at the highest possible energies have failed to detect quarks knocked out of the nucleon, they have, nevertheless, played an essential part in creating the presently accepted picture of hadron structure. This is particularly true of the use of leptons as high energy probes of the nucleon; they are, apparently, structureless themselves and the nature of the interaction, electromagnetic for electron and muon, weak for the neutrino, is well understood.

The electron scattering experiments at SLAC late in the 1960s, and those soon afterwards in the Gargamelle bubble chamber at CERN, Geneva, using beams of neutrinos, repeated for the nucleon what Rutherford's α -particle scattering experiments did for the atom early this century (for a review see ref. 21). They found evidence that high momentum transfer collisions occurred with point-like constituents within the nucleon; moreover, the two experiments together showed that these constituents (or partons as Feynman named them) possessed just the properties expected of quarks: fractional electric charge and spin $1/2$. Furthermore, although subject to rather large errors, the neutrino experiment can 'count' the number of 'valence quarks' in the nucleon (those determining its physical attributes) and the result is close to three.

There was a significant additional observation, the quarks (plus a small component of $(q\bar{q})$ pairs forming a 'sea') only carry ~50% of the nucleon momentum. This forced the conclusion that the remaining 50% is carried by other constituents which display no electromagnetic or weak interaction; the obvious

speculation was that these other constituents must be associated with the strong force binding the quarks and so they were called 'gluons'. In the quark-parton mode, of lepton-nucleon scattering the elementary interaction with the quark can be represented by Fig. 3a and b for the electron (or muon) and neutrino cases respectively. The struck quarks do not escape as nucleon debris so it must be supposed that in some subsequent strong interaction between the quarks and gluons the quark is 'dressed' and what emerges is a jet of conventional hadrons. Nevertheless, in some average sense this jet is expected to carry information about its parent quark.

This simple point-like behaviour which implies no dependence on a fundamental length or scale and in which the quarks are treated as free, independent entities is often referred to as scaling; it leads to a linear rise with energy of the weak interaction total cross-sections. However, further experiments with electrons at SLAC, muons and neutrinos at Fermilab and recently with large detectors exposed to the high energy neutrino beams of the CERN-SPS, have revealed departures from this simple picture. This is no surprise. The linear rise must be halted before the cross-section reaches an upper bound set by reaction theory, the unitarity limit; a damping of the rise sets in when momentum transfers become comparable with the mass of the mediators of the weak interaction, but these effects are not significant in the present experiments. There is also the apparent paradox in a picture of quarks so strongly bound they cannot escape yet act quasi-free under the impulse of a high momentum transfer collision. In fact these 'scale-violating' effects, occurring at the 10–20% level, may prove to be important clues to an understanding of the interaction between quarks and gluons, now seen as the basic form of the strong interaction.

Electron-positron annihilation

The creation of lepton pairs or hadrons in the annihilation of high-energy electrons and positrons takes place through an intermediate virtual photon and there is good evidence that in hadron production the second step is creation of a $(q\bar{q})$ pair, (see Fig. 4b). There are three kinds of evidence for the quark mechanism.

First, the strength of the electromagnetic coupling to the virtual photon is proportional to electric charge and this leads to a prediction of the ratio of hadron production (via quarks) to that of muon pairs:

$$R = \frac{\text{rate to hadrons}}{\text{rate to } \mu^+ \mu^-} = 3 \sum_j e_j^2$$

where e_j is the charge of the quark of flavour j , and the 3 is due to the three quark colours. At total annihilation energies ~2.5 GeV, where the u , d and s quarks should contribute, the value of R is ~2.5, quite close to $3[(1/3)^2 + (1/3)^2 + (2/3)^2] = 2$ which is the simple quark model prediction. Note that, for fractional charges, agreement requires the colour factor of 3, otherwise the prediction would be $2/3$. At energies ~5 GeV, above the threshold for production of charmed hadrons and the τ -lepton, R has risen to 4.7 ± 0.05^{22} again in reasonable agreement with the new expected value of 4.33 (1 is added by the τ -lepton and 1.33 by charm.)

A second observation^{23,24} is that the hadrons tend to emerge in two 'back-to-back' jets, presumably the progeny of the intermediate $(q\bar{q})$ pair; this effect is particularly clear at high total energies, above 5 GeV, where the angular spread in each jet is less broad. A remarkable confirmation of this comes from the angular distribution in the angle made by the axis of these two jets, with respect to the line of the e^+e^- collision; this is exactly as predicted for quarks of spin $1/2$.

Muon-pair production by hadrons

The quark-parton model is also successful in describing the creation of pairs of muons in high energy collisions between hadrons.

The mechanism proposed by Drell and Yan²⁵ is shown in Fig. 5: it is assumed that in the collision an anti-quark of the $(q\bar{q})$ sea

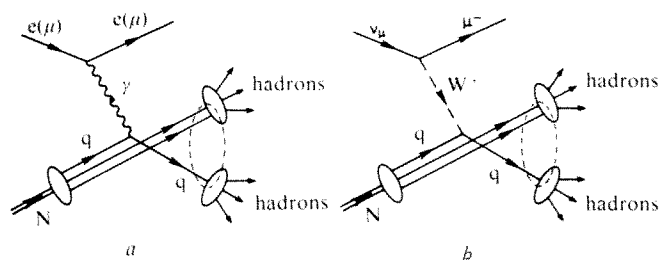


Fig. 3 a, Electromagnetic—photon exchange. b, Weak—vector boson (W^+) exchange.

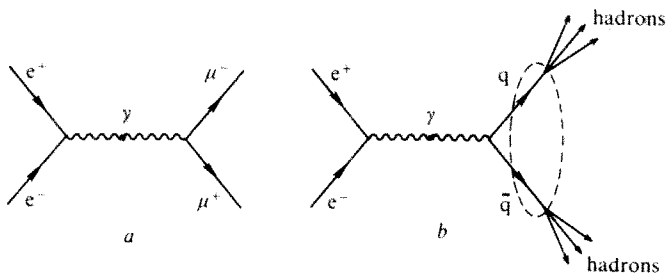


Fig. 4 a, Lepton pair production. b, Hadron production.

in one hadron annihilates with a quark in the other hadron resulting in the creation of a lepton pair. This is like a reversal of the e^+e^- annihilation to hadrons (Fig. 4b) and the probability of making muon pairs of a certain mass can be written as a product of the rate for the $(q\bar{q})$ annihilation to leptons and the probability of finding a q and $\bar{q}$ carrying given fractions of the hadron momenta. The first quantity is calculable, and already tested by the e^+e^- annihilation experiments, while the second can be related to the high energy lepton scattering experiments.

The distribution in mass of muon pairs created by 400 GeV protons at Fermilab in the experiment of Lederman^{26,27} is shown in Fig. 6. The continuum (away from the Υ region) is well described by the Drell-Yan model using q and $\bar{q}$ momentum fraction distributions for the nucleon which are quite compatible with those deduced from lepton scattering; the comparison also favours the colour factor of 3. Muon pair production provides a means of sampling the $\bar{q}$ of the sea and also the valence q and $\bar{q}$ of mesons when these are used as incident hadrons.

Thus three quite different types of experiment reflect the same internal structure and taken together with hadron spectroscopy (and a number of other phenomena not mentioned) the total body of evidence in favour of quark constituents is now overwhelming. (For recent reviews see refs 28,29.) But there are significant discrepancies, quantitative and qualitative, which show that the naïve quark-parton model is not the whole story. The next stage must be to see if these discrepancies also have a common origin, in particular, whether they are reflections of the quark-gluon interaction.

Weak and electromagnetic interactions

Like the electromagnetic force the weak interaction is believed to be mediated by spin 1 particles, called intermediate vector bosons (Table 3) which must be massive (>20 GeV) because at present energies there is no significant damping of the energy dependence of the cross section. The weak interaction responsible for β -decay, and many other processes, is well described by the V-A theory in which two forms of vector interaction (V and A) participate. This mixing of V and A causes the parity violation, or lack of invariance with respect to transformations between mirror-image situations, which is characteristic of the weak interaction.

The law of conservation of electric charge can be linked to the fact that the electromagnetic field potential is not unique or,

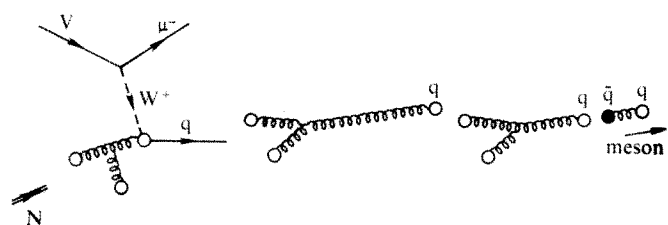


Fig. 5 Drell-Yan mechanism for $\mu^+\mu^-$ pair production in nucleon-nucleon collision.

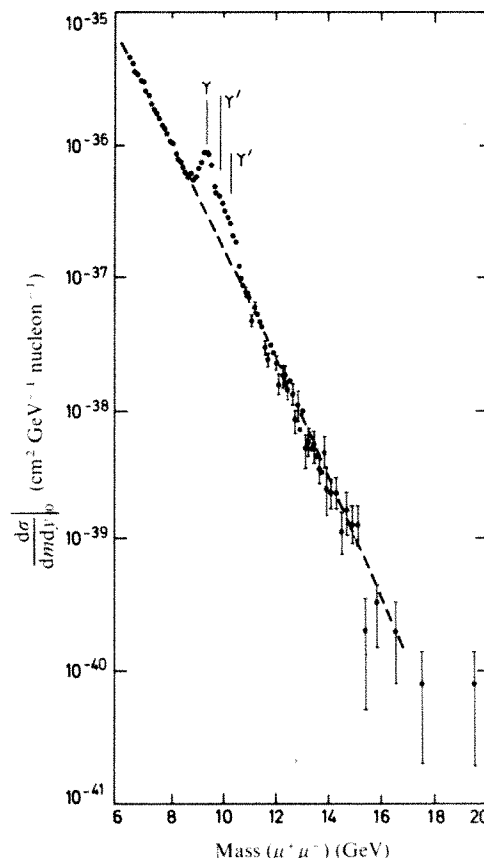


Fig. 6 The mass distribution of $\mu^+\mu^-$ pairs produced by 400 GeV protons at Fermilab^{26,27}.

equivalently, that the theory, QED, is invariant to transformations, called gauge transformations, of the unitary group U(1). The electromagnetic interaction does not violate parity. Nevertheless, the vector nature of both forces made it natural to seek a theoretical framework, a gauge theory³⁰, able to describe both phenomena. However, there seemed to be at least one fundamental obstacle: the only known form of the weak interaction involved a change of charge by the participants, requiring the exchanged field particle to carry electric charge. In contrast the electromagnetic exchange current is neutral and seemed to have no counterpart in the weak interaction. Most unification schemes called for neutral as well as the familiar charged weak current.

This crucial question was settled at CERN in 1973 by the discovery of weak neutral current interactions in an experiment made by Lagarrigue and his collaborators^{31,32} using the Gargamelle bubble chamber exposed to the CERN neutrino beam. The most successful unified gauge theory is also the simplest; it is due, independently, to Salam and Weinberg³³⁻³⁵ and owes its firm theoretical foundation to the work of 't Hooft^{36,37}. Experimental support has now been assembled for this theory in various neutrino experiments and also, very recently, by an elegant experiment³⁸ carried out by Taylor *et al.* at SLAC in which minute parity violating effects due to the weak neutral current have been observed in the scattering of polarised electrons by deuterium. This provides the first test of the Salam-Weinberg theory outside neutrino induced processes and the result is in excellent agreement with those from the neutrino experiments. Attempts have been made to detect parity violation due to weak neutral current effects in certain atomic transitions and after some early null results parity violating effects have now been observed; however, the experiments are difficult and the situation remains unclear.

The Salam-Weinberg theory is a gauge theory in which the intermediate vector bosons couple to left-handed doublets (Table 4) of the quarks or leptons (or to right-handed doublets of anti-quarks or anti-leptons). (Left-handed and right-handed

describe the correlation between spin and momentum directions. A right-handed particle moves in the direction which also corresponds to a clockwise twist—like a right-handed corkscrew. The asymmetry in couplings is clearly parity-violating.) The charged current interaction, mediated by a W^+ , transforms one member of a doublet into the other, like a rotation in a 'weak isospin' space. These operations are those of an $SU(2)$ gauge transformation group; thus the basic gauge symmetry structure of the theory is $SU(2)_W \times U(1)_E$.

Actually the charge $-1/3$ members of the weak $SU(2)$ doublets are not identical with the flavour states d and s of hadron spectroscopy. The weak interaction couples to a mixture of s and d ;

$$d' = d \cos \theta_c + s \sin \theta_c \quad s' = -d \sin \theta_c + s \cos \theta_c$$

where θ_c is the mixing angle, introduced by Cabibbo³⁹. (Any mixing with b is small.) This structure explains the depressed rates of decay of strange particles ($s \rightarrow u$ is proportional to $\sin^2 \theta_c \sim 0.06$) and also provides the mechanism involving the charm quark proposed by Glashow, Illiopolous and Maiani⁴⁰ to explain the extremely low rate of $K^0 \rightarrow \mu^+ \mu^-$ decay.

Table 3 The mediators

Force	Name	Mediators Mass (GeV)	Spin	State of theory
Gravity	Graviton	0	2	Quantised theory not established.
Weak +	Intermediate Vector Bosons	$W^\pm$ (~ 78) Z^0 (~ 89)	1 (V-A) —	Unified gauge theory $SU(2)_W \times U(1)$
Electromagnetic	Photon	γ 0	1	QED Salam-Weinberg almost established.
Strong	Gluon	g 0	1	QCD Attractive new gauge theory; some support but not established.

A condition which must be satisfied to ensure that unmanageable infinities do not prevent calculations with the theory takes the form, in the Salam-Weinberg case, of the requirement that the sum over the charges of the quark and lepton doublets must be zero. Table 4 shows this condition is satisfied provided there are three colours and the numbers of lepton and quark pairs are equal.

The success of the Salam-Weinberg theory is especially remarkable as it contains only one free parameter, the Weinberg angle, θ_w , which measures the relative strengths of the electromagnetic (e) and weak (g) interactions:

$$\sin^2 \theta_w = e^2 / g^2$$

$\sin^2 \theta_w$ has been determined in various experiments, including the one on electron-deuteron scattering at SLAC, and all the values obtained are close to the average, 0.23 ± 0.02 . One prediction of the theory gives the masses of the weak vector bosons; these are:

$$M(W^\pm) = \frac{37.3}{\sin \theta_w} \text{ GeV} \sim 80 \text{ GeV}$$

and

$$M(Z^0) = \frac{M(W)}{\cos \theta_w} \sim 90 \text{ GeV}$$

No existing particle accelerator or colliding beam machine can reach the energies needed to create these states which thus present a major challenge for the next generation of super high energy devices. The first such opportunity will come from the proton-anti-proton collider⁴⁰ now under construction at

CERN. The aim is to store both protons and anti-protons in the CERN SPS; the two beams will circulate in opposite directions at an energy of 270 GeV so that when they are made to collide in the centre of the detecting apparatus 540 GeV will be available for the creation of W s and Z^0 s.

Table 4 Weak $SU(2)$ doublets

Charge	Quarks	Leptons	Charge
+2/3	$\begin{pmatrix} u \\ d' \end{pmatrix}_L$	$\begin{pmatrix} \nu_e \\ e^- \end{pmatrix}_L$	0
-1/3	$\begin{pmatrix} c \\ s' \end{pmatrix}_L$	$\begin{pmatrix} \nu_\mu \\ \mu^- \end{pmatrix}_L$	-1

A theory for the strong interaction

A gauge theory of the strong interaction in its most elementary form, the binding of quarks in their hadrons, has been developed based on the idea that colour is the 'strong charge' and the force between quarks is mediated by the exchange of coloured, zero mass gluons. The gluons form a colour octet, (8), of spin 1 and the $SU(3)_c$ symmetry of colour is supposed to be exact. This theory, known as quantum chromodynamics or QCD^{41,42}, generates a force with two remarkable properties which provide a plausible basis for understanding the paradoxical behaviour of quarks and the theory is now receiving more quantitative support from experiment.

In contrast to QED, where the exchange particle carries no charge and so photons do not interact with each other (at least in lowest order), in QCD the gluons carry colour-charge and can interact strongly. This leads to a profound difference in the forces: whereas the Coulomb potential between electric charges falls at large distances ($\propto 1/r$), in QCD the force between quarks may increase; conversely the force becomes weaker at small distances, such as are probed in high momentum transfer collisions. The latter property, known as asymptotic freedom, provides a qualitative basis for understanding the success of the simple quark-parton model. On the other hand, the increase at large quark separations may be sufficient to prevent free quarks escaping from their confinement within the hadrons; instead the increase in field energy may be released in the form of created ($q\bar{q}$) pairs, or mesons. This can be visualised as shown in Fig. 7. The high momentum W imparts an impulse to the 'almost free' quark q ; as q moves away the binding force increases, eventually the stored energy is more than sufficient to create a new ($q\bar{q}$) pair. In effect, the 'spring' breaks, a new hadron is created, the quarks remain confined. Like the quarks the coloured gluons are also permanently imprisoned.

The large distance behaviour, where the quark-gluon coupling is strong, is not, so far, amenable to calculation except in a strongly model-dependent way. However, considerable success has been achieved in constructing models including quark confinement and describing the spectroscopy of hadron states^{21,30}. On the other hand, a perturbation method of calculation can be applied in the small distance, high energy regime where the coupling strength is weaker. This permits, for example, the calculation of 'strong radiative corrections' to the quark-parton model. As already described, the naïve version assumes the quarks are free but the first order effects of gluon coupling lead to departures from exact scaling which are calculable and so can be compared with those found by experiment.

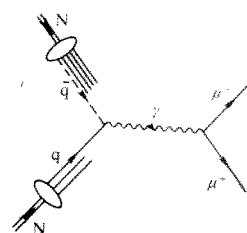


Fig. 7 Illustration of quark confinement mechanism.

These corrections are shown by examples of first order QCD processes in Fig. 8. Recent analyses of experimental data on scaling violations show good agreement on the whole with the QCD predictions. One example is the case of neutrino scattering studied by Perkins and collaborators using the BEBC and Gargamelle bubble chambers in the CERN neutrino beams. Scaling violation is demonstrated^{44,45} in this experiment by the dependence of moments of the observed quark momentum-fraction distribution in the nucleon on the momentum transfer in the collision. The results obtained are shown in Fig. 9 as plots of three different pairs of moments; the lines in this figure have slopes predicted by QCD which are in good agreement with the data. This result demonstrates that the form of the scaling violation is consistent with that predicted by QCD but more particularly the magnitude of the slopes is evidence for the vector nature of the gluon.

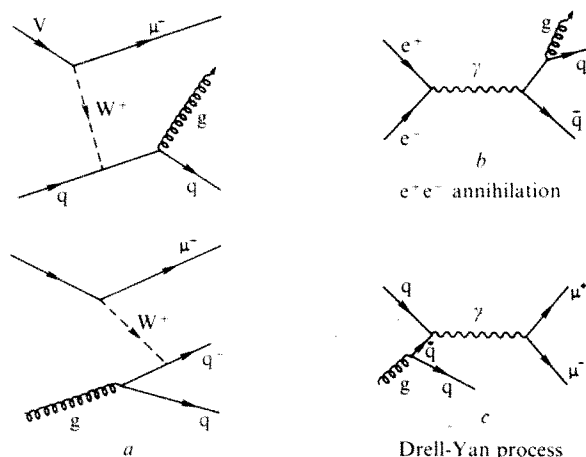


Fig. 8 Examples of 1st order QCD processes.

The first order QCD correction (see Fig. 8b) may also account for the measured values of R being rather larger than the naïve prediction. More direct evidence for gluons is being sought among the showers of hadrons created in high energy collisions, some of which should be born in gluon jets. The heavy-onium states, like charmonium or bottomonium, formed by e^+e^- annihilation are constrained to decay to hadrons through three gluons ($Y \rightarrow 3g \rightarrow \text{hadrons}$) so providing another 'laboratory' for gluon studies. So far a clear difference has been established between hadron production through Y formation and direct e^+e^- annihilation, but a proof of the intermediate role of three gluons may have to wait for the higher mass toponium.

Many other experiments are now bringing QCD under scrutiny and the present indications are that we may have, for the first time, a serious contender for a theory of the strong interaction.

Questions for tomorrow

On the experimental front further results from the polarised electron scattering experiment at SLAC may soon close (or open) one of the few remaining loop-holes left for an alternative to the Salam-Weinberg unified theory of the electro-weak interaction, a synthesis of significance equal to Maxwell's unification of the electric and magnetic forces. But the crucial tests must await the observation of the intermediate vector bosons, W^+ and Z^0 . These may be identified at the high energy colliders under construction like the CERN $\bar{p}p$ facility due to operate in 1981 using the SPS ring or the 400 GeV proton-proton collider, ISABELLE, at Brookhaven which is planned to be ready in 1986. Another contender will be the $\bar{p}p$ collider planned at Fermilab using the superconducting magnet ring being installed to double the present accelerator's energy to 1,000 GeV; this may eventually allow 2,000 GeV to be reached in the collision. However, a full study of their properties and decisive tests of the unified gauge theory through study of the

process $e^+e^- \rightarrow W^+W^-$ will need a super high energy e^+e^- collider such as the large electron positron (LEP) machine⁴⁶ now under discussion in Europe which could have an energy of up to 100 GeV in each beam.

The almost conclusive experimental support for the Salam-Weinberg theory brings another question to the fore. It is based on a gauge theory, due to Yang and Mills⁴⁷, which only applies to massless particles. The introduction of mass depends on a device called the Higgs mechanism and a consequence is the existence of at least one neutral, spin 0, particle called a Higgs meson. Its general properties are known but the mass is not predicted, it must be greater than ~ 12 GeV but may be as heavy as 1,000 GeV. The search for the Higgs meson, or at least an indirect hint of its existence, must be one of the prime objectives for the future and especially for a LEP.

The programme seems well defined, but suppose that there are no W^+ or Z^0 to be found? At the high energies of a LEP the strength of the weak interaction will overtake that of the electromagnetic force and a new view of their character is bound to be revealed.

In the field of strong interactions QCD has brought new hope of reaching and understanding although daunting problems remain, especially in the 'large distance, low energy and strong coupling' regime where the problem of quark confinement remains to be understood. Great efforts are being applied to making tests in the 'short distance' regime probed in high energy processes and for which calculations using perturbation methods can be attempted. QCD has great theoretical attraction, being a gauge theory with vector mediators it carries the promise of eventual unification with the electro-weak theory, but decisive experimental tests are going to be very difficult. The principal actors, quarks and gluons, move on a stage hidden from direct observation.

Remembering the sequence atom, nucleus, nucleon, the frontier has now moved inside the nucleon; but the old hadron spectroscopy seems to have been replaced by a new spectroscopy of quarks and leptons. How many flavours of quarks and leptons are there and is there an even deeper layer of structure to be unravelled? Only experiments can show; still more flavours may be discovered at PETRA, PEP and LEP. However, one significant sign may be the growing connection between the elementary particle states (quarks and leptons) and the character of the forces. The Salam-Weinberg theory links the quarks and the leptons; QCD loses its property of asymptotic freedom if there are more than 16 quark flavours; the

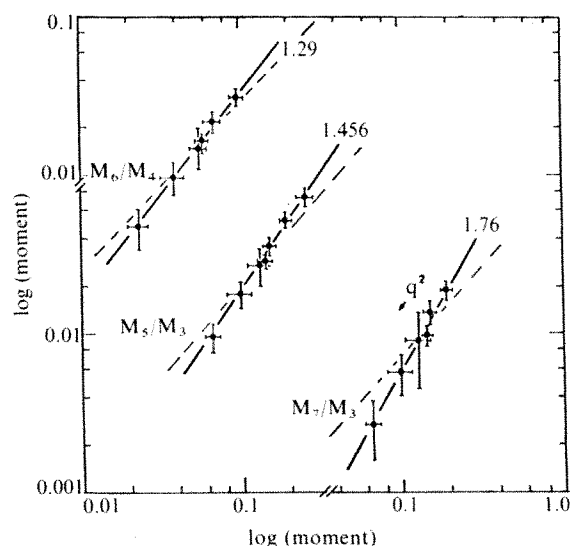


Fig. 9 Data from neutrino scattering experiment at CERN^{44,45}. The scale violating dependence on momentum transfer squared (q^2) spreads the points; the lines shown have the slopes predicted for spin 1 gluons (full-line) or spin 0 gluons (dashed line).

increase from 4 to 6 quarks is just what is wanted⁴⁸ to provide a mechanism for the violation of time-reversal invariance observed in rare decays of the K^0 -meson.

A fundamental challenge is the search for a theoretical structure which would unify the three forces within one strong-electro-weak theory. This requires a larger gauge symmetry including $SU(3)_c \times SU(2)_w \times U(1)_E$. Such a grand unification may provide answers to many outstanding questions including the relative masses of the quarks and leptons, the values of $\sin^2 \theta_w$ and the Cabibbo angle, θ_c . In these schemes the proton usually loses its absolute stability, decaying with a lifetime which may be as short as 10^{34} yr; the present limit is $>10^{30}$ yr. Beyond this lies the still greater task, left by Einstein, of achieving a unification

with gravity, the force which moulds the Universe but which is too weak for its effects to be studied at the particle level.

Most of the quarks and leptons seem superfluous; the enigma of the muon has only deepened as its new companions have arrived. To construct our world, outside the high energy physics laboratories, Nature needs only one quark pair (u,d) and one pair of leptons (ν_e, e^-). The other flavours of quarks and leptons seem to play no essential role (except perhaps the extra neutrinos which (if massless) may have helped to determine the course of the first few moments⁵⁰ following the Big Bang); they are clues to the overall scheme of Nature, which, in Salam's view, may be more sparing of principles than of particles.

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articles

Radiocarbon measurements on submerged forest floating chronologies

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The submerged forests along the west coast of England and Wales provide a unique source of wood for radiocarbon/dendrochronological studies. We report ^{14}C age determinations on sequential growth increments from three 'floating' chronologies. A sampling frequency of ~ 10 samples per century was used. Fluctuations in atmospheric ^{14}C levels of 2–3% over several decades can occur, these variations being superimposed on a smoothly changing trend.

THERE is increasing interest and controversy surrounding the issue of calibration of the radiocarbon time scale^{1–5}. The new data and interpretation presented here justify a view of moderation between the opposing extremes of opinion demonstrated by Pearson *et al.*^{1,3} and Suess² respectively.

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We report the first results of a study of past radiocarbon concentrations as reflected in ancient wood. The source of our material was the 'submerged forests' exposed at low tide around the coast of the UK. These submerged forests are the consequence of drowning, by post-glacial sea-level rise, of coastal woodlands. The trees grew at about mean high water mark, usually behind a coastal barrier of some kind, and were usually killed by the rising water table or by the ingress of salt water rather than by catastrophic overwhelming on a large scale. Preliminary radiocarbon measurements (J.A.C. and M.S.B., in preparation) have shown that there is a wide age range of wood available. As rates of isostasy and eustasy are fairly well understood, it is therefore possible, by reference to established sea-level curves⁶, to collect samples for analysis systematically, thus accelerating compilation of a tree-ring chronology. At any one site, there are large numbers of trees, mainly oak with pine and, to a lesser extent, alder, yew and birch. Thus a master chronology can be constructed, this record then being overlapped with chronologies at different locations. The trees are ideal

for dendrochronological studies; individual growth rings are wide and the growth pattern is extremely sensitive and therefore well characterised. (The dendrochronology of the submerged forests is by Heyworth at Aberystwyth.)

The submerged forests also provide excellent material for radiocarbon calibration studies. Trees of different ages all grew at the same altitude, that is, contemporary sea level and should not experience such local effects as higher ambient ^{14}C concentration and *in situ* production which may limit the previously derived bristlecone pine calibration. Plentiful supplies permit collection of large samples and a high sampling frequency, that is, 10 samples per century, with the benefit of duplication of samples and collaborative analyses to maintain the essential high level of objectivity.

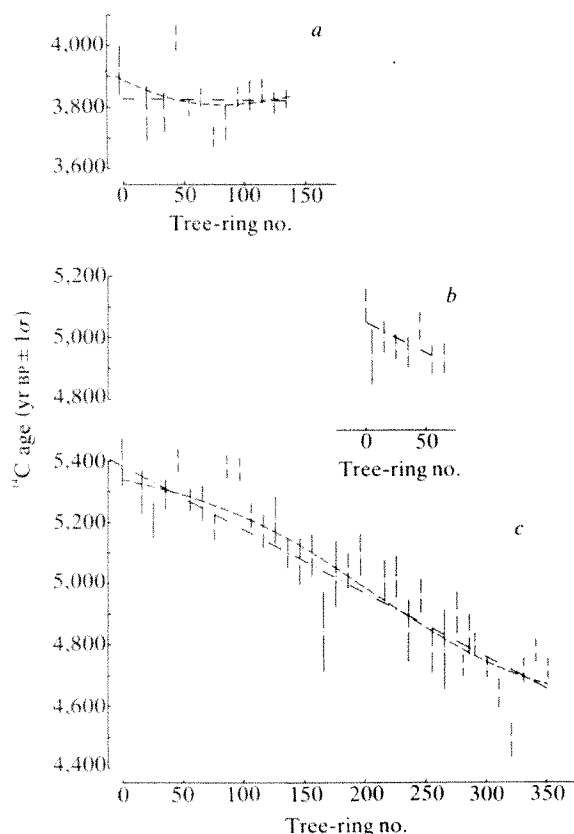


Fig. 1 Radiocarbon age (yr BP) versus tree-ring number for a, Borth 4; b, Ynyslas 1; and c, Stolford 4-5 floating chronologies. The vertical error bars indicate the 1σ counting statistics uncertainty associated with individual measurements. On the axis of abscissa age increases to the left, - - - - represents the linear least-squares fit, - - - - represents a least-squares polynomial approximation.

Experimental methods

Ten-year growth increments were separated by hand from the bulk sections which had previously undergone dendrochronological analysis. Individual wood samples were finely chipped in preparation for chemical pretreatment and subsequent conversion to benzene for liquid scintillation counting. Fairhall and Young⁷ have demonstrated that there can be significant differences in the ^{14}C levels of different components of wood, namely cellulose, lignin and resin. Therefore the ^{14}C assay of wood samples was performed on the cellulose fraction only, as the cellulose in each ring is not in dynamic equilibrium with the metabolism of the tree but, once synthesised, remains fixed and thereby satisfies the closed system assumption of the radiocarbon dating method.

Cellulose was extracted by digestion of the finely divided wood in aqueous 2M KOH followed by bleaching in hot $\text{HCl}/\text{NaClO}_2$ for 48 h. The conversion of cellulose to benzene for liquid scintillation counting was performed using standard procedures^{8,9}. Following sample combustion, an aliquot of carbon dioxide was removed for mass spectrometric analysis of

the stable carbon isotope enrichment to allow correction for natural fractionation. Subsequent radiometric measurements were normalised to account for this effect.

The possibility of artificially inducing isotope fractionation through non-quantitative chemical conversion yields was investigated (J.A.C. and M.S.B., in preparation). Aliquots of synthesised benzene were converted to CO_2 and the stable carbon isotope enrichment was measured. Only small differences in the isotopic composition between CO_2 from sample combustion and CO_2 from synthesised benzene were recorded. These differences ($\sim 0.5\%$), uncorrelated with conversion yields, do not contribute significantly to the overall precision.

^{14}C measurement was by liquid scintillation counting on a Packard series 3330 Tricarb spectrometer. Three channels monitored the ^{14}C pulse height spectrum, the positioning of discriminators minimising contributions from ^3H (to $<0.01\%$) and background pulses. The principal channel monitored the ^3H -free portion of the ^{14}C pulse-height spectrum, and the other two channels subdivided the spectrum to monitor small changes in counting efficiency induced by quenching agents. A quench correction calibration was evaluated and all count rates corrected accordingly.

Standard low potassium glass vials were used for ^{14}C counting. A sample volume of 10 ml was adopted and scintillation vials were precoated with matt black paint to this liquid level to reduce spurious cross-talk pulses. Where necessary, samples of synthesised benzene were diluted with spectroscopic grade 'dead' benzene to bring the volume up to the standard amount. The scintillation mixture contained 2 ml of a PPO/POPOP solution and vials were sealed with the supplied, high density, screw-on caps. Close monitoring of spiked samples did not reveal evaporative losses during time periods considerably longer than a typical counting cycle.

The background count rate was determined by repeated measurements on samples of benzene synthesised from materials containing no ^{14}C and samples of spectroscopic grade benzene. A rigorous study of sources of background pulses was conducted. Variability between vials induced by differences in vial weight and by small variations, ± 1 mm, in that part of the vial not masked, was insignificant. In contrast to the work of Pearson *et al.*¹ no correlation with barometric pressure was found. Over several months the variability in background count rate, for a particular vial, expressed as a standard deviation on the mean value of individual counting cycles, was comparable to the uncertainty associated with a single measurement. Hence only statistical variability in the number of counts has a role in background determinations and thus a high degree of long-term instrument stability clearly exists.

Similarly, data on individual NBS oxalic acid samples, compiled over many months, also indicated excellent long-term counter stability, the standard deviation of individual counts about the mean (2.2%) being comparable with the standard deviation of a single measurement (2.4%). Only random variability in the count rates is again found with no long-term evaporative losses. A slow increase in the degree of quenching was found but the sample channels ratio calibration corrected observed count rates. Replicate NBS oxalic acid determinations show a scatter comparable with the uncertainty on an individual count (2-3%). Thus the dominant factor in the precision of our ^{14}C age determination procedure is the counting statistics uncertainty, and errors associated with experimental procedures contribute only a small amount to the overall analytical error.

To ensure the validity of data, several intercalibration studies were carried out. These data will be published elsewhere (J.A.C., M.S.B., L.A. *et al.* in preparation) but agreement between laboratories implies satisfactory analytical accuracy.

Based on the long-term counter stability reflected in the reproducibility of background and modern standard data, and on the findings of the replicate analysis and intercalibration studies, any observed variations in the radiocarbon/tree-ring data presented here can be attributed to true changes in atmos-

pheric ^{14}C concentrations rather than to experimental variability.

Results and discussion

The results of sequential growth-increment analysis of three floating chronologies are presented in Table 1 and Fig. 1. The shortest chronology, spanning 80 yr, was pine wood from the Ynylas exposure (YS-1, $52^{\circ}29'\text{N}$, $4^{\circ}4'\text{W}$), and the two longer oak chronologies from Borth, Cardigan Bay (BH-4, $52^{\circ}29'\text{N}$, $4^{\circ}4'\text{W}$) and Stolford, Bridgewater Bay, Somerset (SD-4-5, $51^{\circ}12'\text{N}$, $3^{\circ}6'\text{W}$) comprised respectively 155 yr and 360 yr. Figure 2 shows results for an additional oak timber recovered from a late prehistoric site in north-east Scotland. Analysis of these samples was shared with the radiocarbon laboratory of the Scottish Universities Research and Reactor Centre, East Kilbride. Sample preparation was performed at East Kilbride with ^{14}C counting being carried out at the Glasgow laboratory. (Details of the floating chronology are published elsewhere¹⁰.) The tree-ring numbering sequence was from the oldest growth ring to the youngest. On each radiocarbon analysis, one sigma counting errors are given based on accumulated sample counts and on established modern standard and background uncertainties. This error also includes a contribution from the imprecision of stable carbon isotope enrichment measurement required for correction for natural fractionation.

Analysis of tree-ring/radiocarbon data for possible variations in the natural ^{14}C content of the atmosphere requires subjectivity in the selection of a relationship between the two time scales and there is therefore little to choose between statistical fitting and free-hand drawing. As relatively short time periods are presented here only a simple least-squares model is considered and non-random variability about this trend line is discussed below.

Linear least-squares analysis was applied to the data. If the linear model is to be a good approximation to the results, then the mean square deviation about the regression line (MSDR)

Table 1 Radiocarbon/tree-ring data for the Borth 4, Ynylas 1, Stolford 4-5 sequences

Laboratory code	Tree-ring no	Radiocarbon age (yr BP $\pm 1\sigma$)	Laboratory code	Tree-ring no	Radiocarbon age (yr BP $\pm 1\sigma$)
Borth 4 series—oak wood					
GU-742	-15-+10	3,917 $\pm$ 84	GU-749	81-90	3,751 $\pm$ 61
GU-743	11-30	3,781 $\pm$ 95	GU-750	91-100	3,835 $\pm$ 29
GU-744	31-40	3,783 $\pm$ 58	GU-751	101-110	3,838 $\pm$ 46
GU-745	41-50	4,027 $\pm$ 35	GU-752	111-120	3,856 $\pm$ 33
GU-746	51-60	3,798 $\pm$ 30	GU-753	121-130	3,812 $\pm$ 31
GU-747	61-70	3,832 $\pm$ 31	GU-754	131-140	3,828 $\pm$ 32
GU-748	71-80	3,705 $\pm$ 37			
Ynylas 1 series—pine wood					
GU-711	-10-+10	5,106 $\pm$ 48	GU-715	31-40	4,946 $\pm$ 46
GU-712	0-10	4,935 $\pm$ 89	GU-716	41-50	5,035 $\pm$ 46
GU-713	11-20	5,009 $\pm$ 52	GU-717	51-60	4,924 $\pm$ 40
GU-714	21-30	4,974 $\pm$ 39	GU-718	61-70	4,930 $\pm$ 48
Stolford 4-5 series—oak wood					
GU-763	-12-+10	5,398 $\pm$ 79	GU-781	181-190	5,039 $\pm$ 61
GU-764	11-20	5,298 $\pm$ 68	GU-782	191-200	5,092 $\pm$ 67
GU-765	21-30	5,201 $\pm$ 55	GU-784	211-220	5,007 $\pm$ 61
GU-766	31-40	5,285 $\pm$ 45	GU-785	221-230	5,015 $\pm$ 68
GU-767	41-50	5,399 $\pm$ 40	GU-786	231-240	4,844 $\pm$ 97
GU-768	51-60	5,269 $\pm$ 40	GU-787	241-250	4,958 $\pm$ 54
GU-769	61-70	5,258 $\pm$ 60	GU-788	251-260	4,783 $\pm$ 67
GU-770	71-80	5,186 $\pm$ 45	GU-789	261-270	4,775 $\pm$ 124
GU-771	81-90	5,382 $\pm$ 43	GU-790	271-280	4,908 $\pm$ 68
GU-772	91-100	5,377 $\pm$ 41	GU-755	275-284	4,733 $\pm$ 34
GU-773	101-110	5,220 $\pm$ 39	GU-791	281-290	4,831 $\pm$ 67
GU-774	111-120	5,169 $\pm$ 53	GU-756	285-294	4,803 $\pm$ 36
GU-775	121-130	5,204 $\pm$ 72	GU-757	295-304	4,718 $\pm$ 31
GU-776	131-140	5,094 $\pm$ 51	GU-758	305-314	4,645 $\pm$ 41
GU-777	141-150	5,067 $\pm$ 81	GU-759	315-324	4,490 $\pm$ 47
GU-778	151-160	5,092 $\pm$ 70	GU-760	325-334	4,715 $\pm$ 31
GU-779	161-170	4,840 $\pm$ 127	GU-761	335-344	4,778 $\pm$ 42
GU-780	171-180	5,020 $\pm$ 104	GU-762	345-355	4,707 $\pm$ 32

Growth-ring numbers increase from the old to young extremes of the sequences

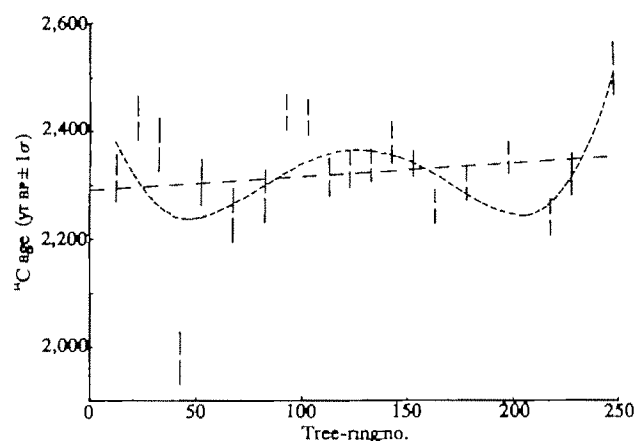


Fig. 2 Radiocarbon age (yr BP) versus tree-ring number for Cullykhan 1 floating chronology¹⁰. Vertical error bars indicate 1σ counting statistics uncertainty associated with individual measurements. — — — And — — — represent respectively linear and polynomial approximations to the data.

should reflect only the analytical contribution which, for the Glasgow laboratory, is dominated by the counting statistics uncertainty ($\bar{\epsilon}_c^2$). Table 2 compares the observed MSDR and $\bar{\epsilon}_c^2$ values. In the case of the short pine chronology, YS-1, the linear model is a reasonable fit to the trend line (Fig. 1), indicating that, during the time period represented by this tree-ring section, atmospheric ^{14}C concentrations were decreasing at approximately 0.5% per century parallel to growth. The linear approximation is not good in the case of oak sections BH-4, SD-4-5 and CK-1, the mean square deviation terms considerably exceeding the expected values based solely on analytical uncertainty. The trend lines for these floating sequences indicate that during the BH-4 chronology atmospheric ^{14}C concentration was decreasing at 0.8% per century whereas for the Stolford chronology the trend was one of increasing ^{14}C at 1.5% per century. Over the 250 yr Cullykhan growth period a general decrease of 1.5% per century is evident. Polynomial fitting applied to the three oak chronologies reduces the discrepancy between observed and expected values of MSDR (see Figs 1 and 2). Nevertheless, the fit remains unsatisfactory.

Visual inspection of the tree-ring chronologies indicates non-linear trends in the ^{14}C age profile. For example, the rapid increases in atmospheric ^{14}C displayed at the old and young extremes of the Stolford oak chronology are clearly non-random effects. Fluctuations such as these, amounting to 2-3% in 50 yr, have not generally been observed in previously reported bristlecone pine-radiocarbon data. This may be due to a damping effect arising from the lower sampling frequency of the American work¹¹. Reduction of the sampling frequency to 5 per century, whether as contiguous 20-yr samples or as five discrete samples, seems to smooth the Glasgow data. For example, if the Stolford data are subjected to averaging of adjacent sections to provide five samples of 20 rings per century, the MSDR decreases from 8,455 to 4,797.

Thus, for the time periods measured, the atmospheric ^{14}C concentration seems to have changed smoothly with occasional more rapid fluctuations superimposed on the gradual trends. This evidence is therefore between the traditional views either of a rapidly wriggling or almost linearly changing ^{14}C record. Periods of stability and of rapid fluctuation apparently occur in succession. While these findings apparently contradict, in particular, the results obtained from the Irish bog oak measurements, the real variability in the submerged forest data is believed to be a consequence of the increased sampling frequency used in the latter work. For the same reason, short-term variability of 2-3% over several decades is generally more rapid than those proposed in the bristlecone pine chronology.

A possible explanation for the perturbations may be that ^{14}C in wood, but not necessarily ^{14}C in the atmosphere as integrated over each year, may experience local effects. Lerman *et al.*¹² have reported small differences in the ^{14}C specific activity of tree rings from different locations. Furthermore, the work of Young

Table 2 Comparison of mean square deviation residuals

Floating sequence	Observed residual MSDR	Expected residual ($\bar{\epsilon}^2$)
Borth 4	5,577	2,016
Ynyslas 1	3,424	2,601
Stolford 4-5	8,455	3,448
Cullykhan 1	12,030	1,399

Expected value is based on counting statistics uncertainty, observed value is calculated by linear least-squares analysis of the floating sequences (Cullykhan 1 is also included).

and Fairhall¹³ on the distribution of fallout ^{14}C , has shown the existence of significant seasonal, latitudinal and meridional variations in the input of radiocarbon from the stratosphere, the source of natural ^{14}C , to the troposphere. Thus the temporal relationship between the critical periods of tree-ring growth and maximum tropopause instability is likely to be fundamentally important in the incorporation of local effects. Very rapid growth during this time of maximal stratosphere-troposphere incursion should lead to high sensitivity to short-term fluctuations in cosmic-ray-produced ^{14}C . Thus as both the tree-ring growth and stratospheric input periods are brief and tend to coincide (in spring/summer) the ^{14}C record in wood may not reflect the mean annual ^{14}C level throughout the troposphere, the latter being smoothed by atmospheric mixing. Hence, at locations at which stratospheric influx is extensive, tree rings will tend to record higher amplitude variations in ^{14}C content. Transitions from a smooth to irregular ^{14}C trend could therefore reflect either a change in solar, or, on Earth, global parameters such as geomagnetism, or could simply imply modification of meteorological conditions at the sampling site. The exceptionally slow growth of bristlecone pine wood may then give rise to a much slower response through a longer storage of nutrients. Clearly ^{14}C measurements on single tree rings from the same growth year should show some correlation with ring widths if this is a true source of local effects. Also Tans *et al.*¹⁴ have suggested that differences in ^{14}C in the cellulose fraction of tree rings from the same year but at different locations may reflect a shift in the major growth season of individual trees.

Reiter¹⁵ has shown that influxes of stratospheric air into the troposphere can be triggered by solar flares. As present-day transport of natural ^{14}C cannot be observed directly because of the effect of fallout radiocarbon, evidence must be based on the distribution of those sister cosmogenic radionuclides which were not produced in nuclear weapon tests. Thus measurements of ground-level concentrations of ^7Be and ^{32}P following such events on the Sun have shown increases of 50–100%, which are clearly not related to production rate changes. Moreover the elevated levels of these radionuclides persist for several days. If similar local and short-term enrichments of tropospheric ^{14}C occur during the tree-ring growth period, variability in the ^{14}C record in wood is quite feasible.

The full value of the floating tree-ring sequence data can only be assessed when they become fixed on an absolute scale. Statistical comparison of the submerged forest floating chronologies with a master chronology such as the bristlecone pine tree-ring record, using the method of Clark and Renfrew¹⁶, is unsatisfactory in this case because of the disparate sampling frequency. Comparisons have, however, been made for the UK data. Differences in the growth pattern of submerged forest oak and pine wood sections preclude dendrochronological overlapping of the tree-ring records. Statistical comparison of Ynyslas 1 (pine) to Stolford 4-5 (oak) shows the excellent agreement of the ^{14}C data. In Fig. 1, the tree-ring axes for these chronologies have been aligned to show this concordance. Matching of the Borth 4 (oak) chronology with a section of the Irish bog oak chronology is shown in Fig. 3. The agreement between the two floating chronologies is good. Although for the time period studied both laboratories agree about the overall trend in atmospheric ^{14}C concentrations, the higher sampling frequency of the Glasgow work suggests that there are short-term variations superimposed on this trend.

The manipulation of floating and master chronologies to produce a statistical fit requires linear least-squares analysis of the separate chronologies as well as combined least-squares analysis yielding the placement of the floating series on the master time-scale. With the Irish tree-ring sequence taken as master chronology, analysis of variance indicated that the average uncertainty associated with a Belfast radiocarbon assay, shown by the mean square deviation about the regression, is ~ 35 yr. Such a value is greater than the 25-yr analytical error reported by Pearson *et al.*¹. This ± 25 -yr uncertainty was calculated by combining error contributions assessed by laboratory standardisation (Baillie, personal communication). However, the magnitude of the uncertainty indicated by the regression analysis suggests either that the laboratory standardisation has failed to evaluate all of the uncertainty associated with the ^{14}C assay procedures, or that there is an additional, non-experimental contribution to the total variability.

The statistical fit produced by the treatment of Clark and Renfrew is limited by the precision of the radiocarbon data comprising the floating and master chronologies. Thus, the comparison of Borth 4 with the Irish chronology is limited mainly by the higher average variability of the submerged forest data. The Belfast tree-ring/radiocarbon data have also been aligned against an absolute chronology, presumably the bristlecone pine dendrochronology. This analysis, using an interactive data analysis technique (Baillie, personal communication), places the Belfast data to within 10 yr on the absolute time scale. Bearing in mind the ± 25 yr uncertainty for the floating chronology radiocarbon data and ~ 40 yr for the bristlecone pine ^{14}C analyses, the apparent precision of the fit seems optimistic. Pearson *et al.*¹ predict an ultimate precision for their ^{14}C analyses of ± 12 yr resulting solely from an increase in counting time. At present, quoted counting errors are $< \pm 17$ yr, implying an uncertainty arising from non-counting sources of at least ± 18 yr. Hence it would not be possible to decrease the total uncertainty associated with ^{14}C assay by increasing the counting time.

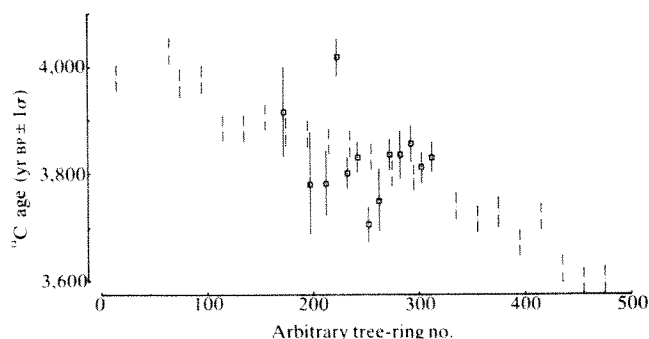


Fig. 3 Statistical comparison of Borth 4 chronology against a section of the Irish bog oak chronology. ● Represents the Irish data with associated ± 25 yr uncertainty, □ represents the submerged forest data with appropriate 1σ counting error.

In conclusion, ^{14}C measurements on floating chronologies from the UK submerged forests indicate that short-term variations in natural radiocarbon concentrations can occur. Fluctuations of 2–3% over several decades superimposed on smoothly changing trend have been detected with a sampling frequency of ~ 10 samples per century. Periods of relative stability of ^{14}C also seem to occur. Until the past ^{14}C record is precisely defined, therefore, calibration of the radiocarbon timescale must continue to involve a significant uncertainty and the accuracy of existing radiocarbon dating techniques will remain correspondingly limited.

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Crystal structure and molecular interactions of tropomyosin

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The three-dimensional structure of tropomyosin filaments has been determined by X-ray crystallography. The ends of the molecules were located by reference to the single pair of cysteine residues. Departures from the α -helical-coiled coil conformation may occur in localised domains along the molecule as well as at the overlapping ends.

TROPOMYOSIN is a key protein in the regulation of muscle contraction. The molecule is a two-chain α -helical-coiled coil that forms filaments wound in the two grooves of the actin helix. In many muscles, a calcium-sensitive protein complex, troponin, which binds to both tropomyosin and actin, controls contraction^{1,2}. Tropomyosin is present in all muscles, and most species have two populations with slightly different sequences termed α and β . Tropomyosin from rabbit cardiac muscle contains only the α subunit^{3,4}. The complete sequence of the α -chain of rabbit tropomyosin, comprising 284 residues, has been determined^{5,6}. There is a short-range periodicity of nonpolar residues, as envisaged in Crick's model for the coiled coil⁷ so that a regular pattern of interactions stabilises the structure⁸. The two identical chains in the molecule run in the same sense⁹; and have little or no relative stagger, as the single cysteine residue at position 190 can form an intramolecular disulphide crosslink (refs 10–13 and P.C. and C.C., unpublished observations). Analyses of the tropomyosin sequence^{14–17} have led to a model where the two chains are in exact register and bond through head-to-tail linkages to form filaments. A 14-fold repeat of acidic and nonpolar residues has also been demonstrated and related to muscle regulation^{14,17}.

X-ray diffraction studies of whole muscle have shown that the location of tropomyosin on the actin helix depends on the states of troponin: at low levels of Ca^{2+} , tropomyosin prevents the interaction of nucleotide-containing myosin heads with actin; when contraction is turned 'on', tropomyosin moves deeper into the actin groove, permitting the myosin heads to bind to actin^{18–20}. This work has been extended by three-dimensional reconstruction of thin filaments from electron micrographs²¹. One troponin complex binds to each tropomyosin molecule, probably about one-third of the distance from its C terminus^{22–24}. McLachlan and Stewart¹⁷ have interpreted the 14 quasi-equivalent zones on tropomyosin as two sets of alternative binding sites for actin. When tropomyosin is bound to the actin filament it would make seven half-twists in one molecular length, so that quasi-equivalent regions of the coiled coil would

link to seven identical actin monomers in the 'off' position. When the muscle is switched 'on', the tropomyosin molecule would make a quarter-turn, and the seven quasi-equivalent sites on the molecule would interact with a second set of seven sites on actin. These arguments, necessarily speculative, assume a uniformly α -helical tropomyosin molecule that makes highly regular symmetrical interactions.

Tropomyosin crystals²⁵ have a uniquely high water content of 95% and are made up of an open meshwork of cross-connected filaments. One projection of the crystal has been solved⁹, and shows that the molecules are bonded head-to-tail to form filaments that are bent into gentle sinusoids, implying a supercoiling of the coiled-coil structure. Further studies have shown that the filaments run parallel to the body diagonals of the unit cell with an effective length of a molecule in the filament of 410 ± 4 Å (refs 23, 26). The 385-Å axial repeat of troponin in muscle²⁷ thus indicates that there is a regular bending or 'kinking' of the filaments as they wind in the actin grooves²⁶. Small amounts of troponin can be incorporated into the crystal lattice and produce changes in unit cell parameters and molecular bending; these motions may be related to those occurring in the thin filaments of muscle^{23,26,27}.

We have determined the low-resolution structure of tropomyosin by single crystal X-ray diffraction analysis. The Bragg reflections used in this study extend to 20 Å, near the limit for this crystal form, and from these data, based on a solution derived from the three centric projections of the crystal, the three-dimensional electron density map has been calculated. We describe the winding of the tropomyosin filaments in three dimensions, the precise molecular length, locations of cross connections, molecular motions in the crystal lattice, and a preliminary report on the relationship of the amino acid sequence to the observed structure, including the location and possible conformation of the molecular ends.

Preparation and X-ray photography of tropomyosin

Tropomyosin was prepared as described by P.C. and Perry⁴ from frozen rabbit cardiac muscle. The procedure is based on the method of Bailey²⁵ with the following modifications. Muscle was homogenised with an equal volume of 50 mM KCl, 10 mM Tris-HCl, 0.5 mM EDTA, pH 7.5, and allowed to stand for 30 min. After centrifugation at 1,000g for 15 min, the supernatant was discarded and the residue washed several times more to remove as much blood as possible. Dried muscle fibre was extracted with 1 M KCl, 25 mM Tris-HCl, 1 mM dithiothreitol (DDT) and 0.5 mM EDTA, pH 8.0, and ammonium sulphate fractionation cycles were carried out in 0.2 M KCl, 5 mM Tris-

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HCl, 1 mM DTT and 0.5 mM EDTA, pH 7.9. After fractionation, tropomyosin solutions were dialysed exhaustively against 1 mM DTT, and freeze dried.

Tropomyosin crystals were grown in quartz capillary tubes as previously described⁹. The final pH of the crystallising solution was 5.8. To grow mercury-labelled crystals, the cysteines of tropomyosin were reacted with approximately stoichiometric amounts of the organomercurial 2-chloromercuri-4, 6-dinitrophenol²⁸. After dialysis to remove unreacted mercurial the protein was set up to crystallise in the usual way, with the addition of 0.1–1.0 μ M mercurial for stability. Assays for free sulphhydryl groups indicated essentially complete reaction when at least 1.0 Hg per SH group was present initially.

X-ray photographs of crystals in mother liquor were taken at 2–4°C using an air-stream cooling device²⁹. Three-dimensional data were collected by screened precession photography from crystals that had been reacted with 0.6 mercurial per SH group. The unit cell parameters for these crystals were: $a = 120.0 \pm 0.5$ Å; $b = 241.7 \pm 1$ Å; $c = 296.0 \pm 1$ Å. Over 95% of the reflections with Bragg spacings greater than 20 Å were examined. Although the maximum Bragg angle for our data is only about 3°, precession angles up to 21° were required to reduce the radius of the blind region in upper-level photographs to an acceptable value. (Within one film pack R values were typically 0.08.) Least-squares scaling of all film packs produced a data set of 352 reflections with an overall R of 0.077, where

$$R = \frac{\sum_i |I_{\text{ave}} - I_i|}{\sum_i I_i}$$

Space group determination

The space group of Bailey tropomyosin crystals has been difficult to determine because of the lack of strong diffraction along the reciprocal lattice axes. The pmg symmetry of the [100] was unambiguously determined from X-ray patterns in conjunction with electron micrographs of negatively stained crystals (Fig. 1). Moreover, early X-ray photographs showed $h = \text{odd}$ reflections along $h00$ to be absent, so that the symmetry element along a was taken to be a twofold screw axis. The space group was therefore originally reported to be $P2_12_12$ (ref. 9). Because of the nature of the transform, however, the data along $h00$ are generally very weak. A few photographs obtained later showed observable 100 reflections, indicating that the space group was $P22_12$ (refs 23, 26). Of the many crystals recently examined, only one had observable $h = \text{odd}$ reflections.

There are two strong arguments in favour of the space group being $P2_12_12$, despite the occasionally observed weak 100. The first uses the minimum wavelength principle. The large unit cell

and localisation of the protein within 5% of the cell volume produce a reciprocal lattice sampling that is fine relative to the molecular transform, especially at very low resolution. This means that along reciprocal lattice lines perpendicular to the filament paths there can be no abrupt intensity changes except those caused by space group extinctions. The 210 and 201 reflections are always very strong whereas the 100 reflection is always either absent or weak (Fig. 2). The difference in intensity between the 100 and its neighbouring reflections is too great to be caused by changes in the molecular transform, and there must therefore be an extinction arising from the symmetry of the structure. Second, in a case where a weak 100 reflection was observed, inspection by polarised light revealed within the crystal a small crystal of a different orientation. We would attribute atypical photographs to anomalies in crystal growth. In all subsequent discussions, therefore, the space group is taken to be $P2_12_12$.

In the case of tropomyosin, the existence of screw axes instead of diads parallel to the a axis has implications about the contacts that can be made between filaments. There can be no contact in regions near the screw axes, because the filaments, which have a maximum diameter of 25 Å, are separated by one-half the unit cell, or 60 Å. This means that the bonding regions are confined to a relatively limited area of the molecule (see below).

Solution of the structure

The approximate path of the molecule in three dimensions can be deduced from the intensity distributions in the three centric projections. In special cases it is possible to solve a structure from these projections; the continuity of the tropomyosin filaments and the large solvent content make this one such a case. The projections, in fact, contain some redundancy: two projections are sufficient to determine x , y and z ; the third provides an independent check.

A net of strands has been visualised in the electron microscope and established as the view down the a axis of the crystal (Fig. 1). The X-ray pattern could then be interpreted as arising from a pair of gently curved filaments running along the cell diagonals⁹. The cross connections between filaments produce a kite-shaped mesh for the net, so that the filaments have a long and a short arm. Note that all three views show similar intensity distributions, despite the change in scaling due to differences in lattice spacings (Fig. 2). As in the [100] projection, these patterns are indicative of sinusoids with small separations of filaments in projection. Hence, the problem of determining the three-dimensional filament path becomes one of correlating the origins of the projections. Because of the fine reciprocal lattice sampling, preliminary electron density maps can be calculated for each of the projections in a straightforward manner. For the [010] and [001] projections only three phases are required to calculate maps incorporating the ~ 12 strong reflections in each projection. The phases along the reciprocal space direction perpendicular to the filament will be positive (relative to some origin) and the phases of reflections on rows next to the diagonal will be positive on one side of the diagonal and negative on the other, the two possible choices being related by origin shifts. One has only to choose origins for the projections that produce a three-dimensional filament path consistent with the space group and the continuity of the filament. Only one solution (and its enantiomorph) satisfies these criteria. This approximate filament path was used to model the structure as discussed below.

Refinement

Tropomyosin, with its high solvent content, offers a unique method of correcting initial X-ray diffraction phases, and hence, improving the quality of the electron density map. Phases were first calculated from the Fourier transform of point scatterers placed at small enough intervals along the filament to approximate a uniform rod at 20 Å resolution. About 95% of the unit cell is solvent which must, at this resolution, have a uniform electron density, placing a powerful constraint on the phases.

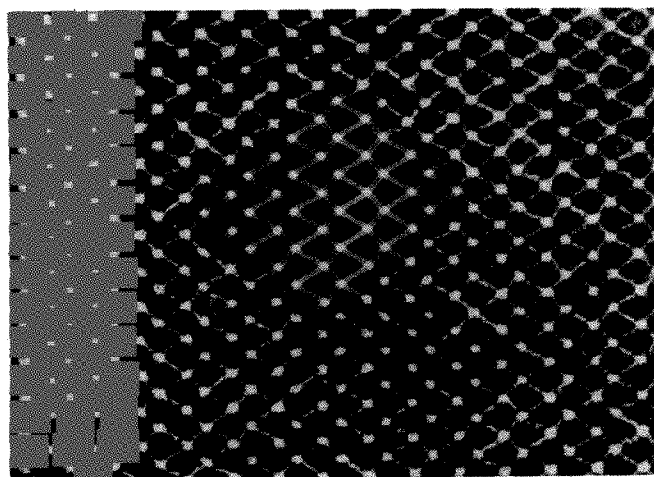


Fig. 1 Electron micrograph of a tropomyosin crystal corresponding to the view in the [100] direction. The strands form a kite-shaped net with long and short 'arms' defined by cross connections. The insert shows the pairs of tropomyosin filaments making up the strands. Negatively stained with 1% uranyl acetate ($\times 190,000$).

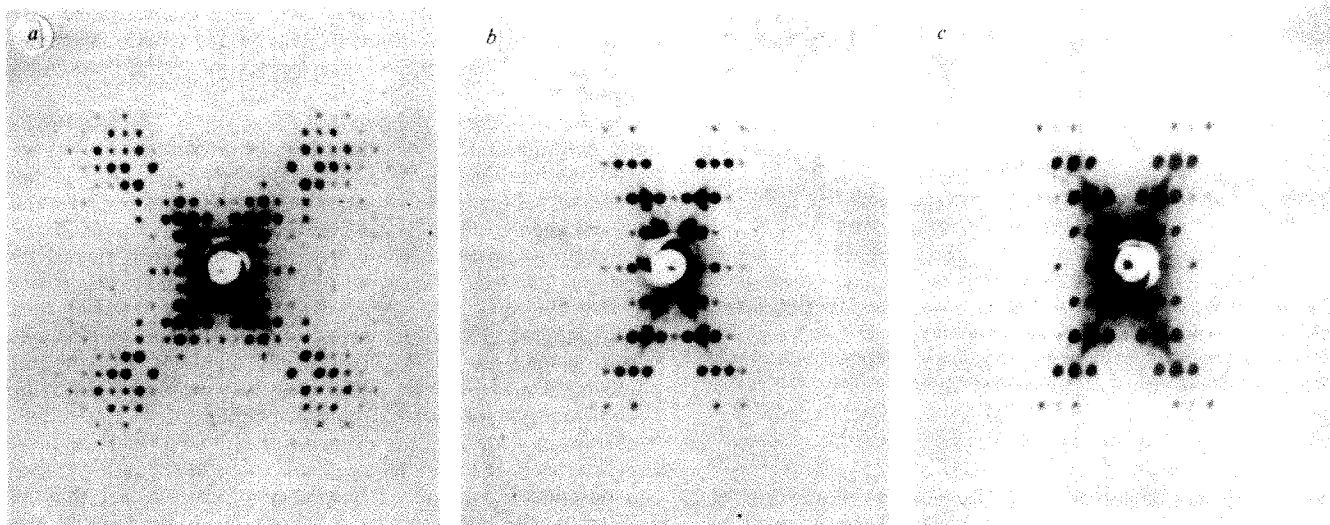


Fig. 2 Precession photographs ($\mu = 3\frac{1}{2}^\circ$) of the three principal projections of tropomyosin taken on a 10-cm camera with a double mirror focusing monochromator. Except for the lattice spacings, note the similarities of all three views. Each projection shows strong spikes arising from closely spaced pairs of filaments. *a*, [100]; *b*, [010]; *c*, [001]. In the [100] projection, nodes arising from the separation of the filaments and their curvature can be seen.

This information was included by seven cycles of setting the solvent region to a constant and calculating a Fourier transform to obtain new phases for an improved electron density map (G.N.P., in preparation). (The final *R* factor between the observed and calculated structure factors was 0.16 compared with 0.35 for the initial model.) Several views of the refined electron density map are given. Figure 3 shows the three-dimensional arrangement of filaments and their cross-connecting regions. Figure 4 shows a single filament sectioned parallel to its axis. It is relatively easy to trace the filament path through the unit cell including the area of cross connections.

Molecular length and three-dimensional winding

The tropomyosin filament winds along a line parallel to the body diagonal of the unit cell. The effective length of the molecule, that is, the repeat along the filament, can be determined to the limit of accuracy of the unit cell parameters. This length was previously estimated from projections as 410 ± 4 Å. As the path can now be traced in three dimensions, we can establish this length more precisely as 410.3 ± 1.4 Å. This information can be used to deduce the number of residues involved in the end-to-end bonding region. Assuming an average axial translation of 1.49 Å per amino acid residue in the α -helix of a perfect coiled coil, the molecular overlap is predicted to be $284 - 410.3/1.49 = 8.6 \pm 1.0$ residues. The three-dimensional winding of the filament is not, in fact, helical, but has the form of an ellipse when projected down the axis of the molecule. The sense of this supercoiling cannot yet be established. The tropomyosin filament is wound in the grooves of the actin helix, with a right-handed sense; and, for illustrative purposes we have chosen right-handed supercoiling of the filaments in the crystal.

Location of the Cys 190 residue

Rabbit cardiac tropomyosin consists of two identical chains of known sequence; there is one cysteine per chain^{5,6}. These SH groups have now been located within the unit cell. X-ray diffraction data to 20 Å have been collected from both Hg-labelled and reduced forms of tropomyosin for two projections, corresponding to views down the [100] and [001] axes. Difference Fourier maps were calculated using the phases obtained by the model building and refinement procedures. A single site is observed in each projection (Fig. 5). (Previous problems with difference Fourier maps of Hg derivatives have been accounted for by the presence of small amounts of

contaminating proteins within the lattice.) The redundancy of measurement of the value of y provides a check on the solution; the values obtained for y agree to within 6 Å. Difference Patterson maps were also calculated to test the validity of the solution. Major peaks were found corresponding to heavy atom positions, although other strong peaks were also present. Efforts are underway to determine the usefulness of including isomorphous replacement data in the structure analysis. The fact that there is a single peak for the two SH groups of the molecule supports a structure where the two chains are in register, although the resolution of the difference Fourier maps does not rule out a small stagger ($< \sim 10$ Å) (see below).

The Hg serves as a marker for the location of Cys 190 along the filament, but leaves a twofold ambiguity, however, in the direction of the polypeptide chain in the filament. If the N to C direction is chosen, as shown in Fig. 4, the intermolecular overlap would occur in the short arm of the filament near a crossover region. The map shows a relatively dense peak at precisely that point. This feature presumably corresponds to the end-to-end molecular overlap. The alternative choice of direction for the polypeptide chain would place the molecular overlap

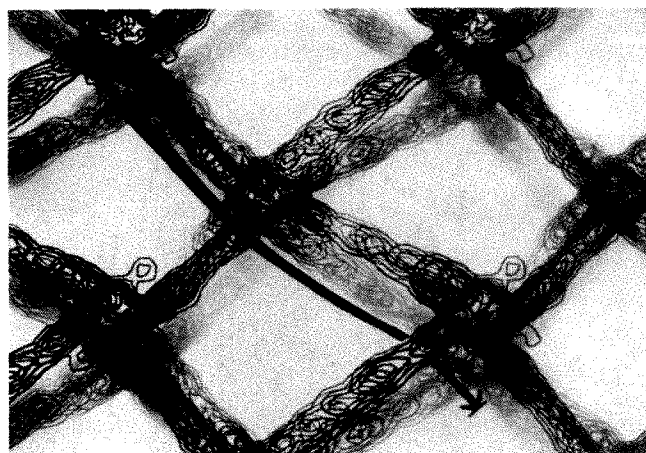


Fig. 3 The three-dimensional electron density map of tropomyosin. Several unit cells are shown at a slight angle to the [100] direction to illustrate the packing of filaments in the lattice and the location of a single molecule in the structure. (The head and tail of the arrow represent the C and N termini, respectively.) Contour values are at equal increments, the lowest of which is about 0.34 electrons Å⁻³.

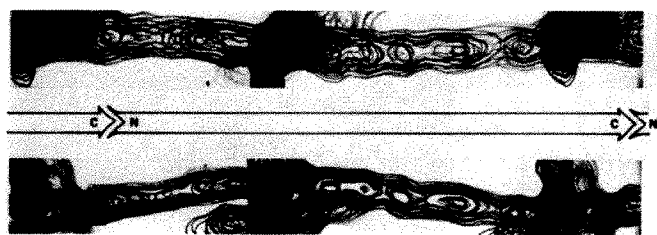


Fig. 4 A filament sectioned along its length. The top and bottom view are rotated by 90° about the filament axis. Contour values are the same as in Fig. 3 and sections are at intervals of $4 \text{ \AA}$. Slightly more than one effective molecular repeat is displayed. The arrow shows the direction of the polypeptide chain and the location of the molecular ends.

within a crossover region and does not readily account for the electron-dense region in the short arm. Sequence information can also be used to distinguish these possibilities. A line projection of the electron density was calculated by integrating cross sections of the filament in regions where no crossovers were present. The corresponding 'theoretical' distribution was estimated based on the amino acid sequence and a 9-residue overlap. Relative weights for each amino acid were derived from Hulmes *et al.*³⁰, with the exception of cysteine, which was corrected to include its labelling with Hg. Figure 6 shows the theoretical and observed density profiles for both chain directions. The better fit of the two curves is clearly represented by Fig. 6a and confirms the solution shown in Figs 3 and 4. Introducing a stagger of seven residues did not produce a significant effect, and hence cannot be ruled out. The Hg labelling of β -tropomyosin (which contains an additional pair of SH groups) or a molecule containing an α and a β chain should allow us to confirm this solution of the chain direction.

Molecular structure

We can interpret certain additional features observed in the electron density map on the assumption that the preceding analysis gives the correct direction of the polypeptide chain. We should note initially that one might expect to establish certain parameters of the coiled coil at this resolution. The theoretical molecular cross section is, however, approximately $20 \times 15 \text{ \AA}$. This small difference is not discernible in the structure that we have so far achieved, and we have not, therefore, been able to determine the pitch of the coiled coil, its sense of twist or its phase in the unit cell. (As α -helices are right-handed and the coiled coil is left-handed, when we are able to visualise the twist,

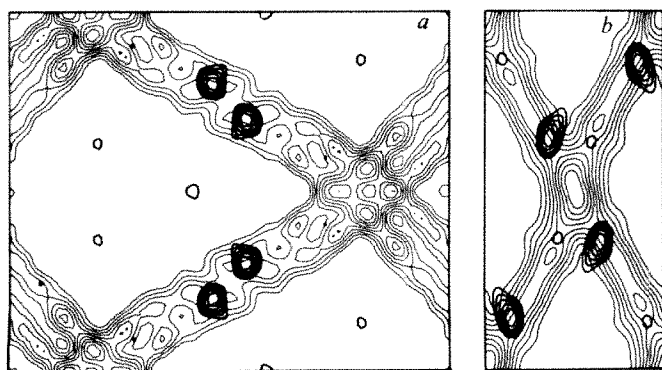


Fig. 5 Difference Fourier projections (dark lines) superimposed on the electron density map (light lines) showing the location of the Hg and, hence, Cys 190. a, [100] projection; b, [001] projection. The difference maps are contoured at values corresponding to the range 2σ – 4σ , where σ is the r.m.s. deviation in the map.

the absolute hand of the supercoiling can be determined.) By comparing the limited intensity data to $15 \text{ \AA}$ with calculated molecular transforms it may soon be possible to visualise these features.

Inspection of the map shows that the run of the filament seems quite continuous (Fig. 4), including the dense region that we have identified as the molecular ends, where a head-to-tail overlap of about nine residues must take place. Here the filament shows only a slight swelling in all directions. If the molecular ends were simple α -helical-coiled coils linked by overlapping of the 'broad faces' (ref. 17), we would expect to see a discontinuity in this region of the map corresponding to a lateral stagger. However, an alternative explanation of this feature is that the molecule might not maintain the α -helical conformation at the ends so that the chains would be joined by intermeshing to form a small globular domain. This kind of link is consistent with both the electron density map and chemical characteristics of the molecule. Predictions of secondary structure based on the amino acid sequence indicate, in fact, that the ends of the chain may not be α -helical; this is particularly true for the sequence at the C terminus, whereas the N terminus does not allow a strong prediction (Argos, personal communication). Tropomyosin has been seen as the archetype of α -helical-coiled coil, but its functional design to form filaments by end-to-end bonding may have produced a specialised structure for the molecular ends.

The general smoothness of curvature of the molecular filaments—apart from the ends—implies that there are no major deviations from coiled coil structures at 4°C . However, this observation does not mean that there are no local regions of 'flexibility'. The filament seems to be slightly kinked in several places in the map. These deviations from a uniform structure may be more pronounced at physiological temperatures.

The molecular filaments can be traced relatively easily through the cross-connecting 'knots' in the lattice. There are two regions of the molecule where intermolecular contacts are made; one is near the C terminus, and the other is about $140 \text{ \AA}$ from the N terminus (Fig. 4). (These 'sticky' regions on the molecule may also be used for cross connections in other polymorphic forms of tropomyosin²³.) Although we can now identify some of the residues involved in the intermolecular bonding, we wish to establish the direction of the molecule in the filament unambiguously (by the use of β -tropomyosin) before reporting the detailed relationship between the amino acid sequence and the electron density map.

Molecular dynamics

The tropomyosin crystal is unique not only because of its high water content but because it is made up of filaments rather than discrete molecules. Moreover, there is only limited contact between filaments in the lattice. Correspondingly, these crystals display some unusual structural features. There are considerable variations in the b and c parameters of the unit cell that have been shown to be related to the presence of other proteins in the lattice²³. We have confirmed and extended these findings. Despite these unit cell differences, however, the length of the body diagonal remains $402 \text{ \AA}$. This figure is the projected molecular repeat along the tropomyosin filament, and its constancy implies that the molecular structure and precise end-to-end overlap are maintained despite small variations in molecular bending that occur in unit cells of different sizes.

A striking aspect of the diffraction patterns is the intense diffuse scattering seen in unscreened photographs. This is due to various types of disorder in the crystal (see ref. 31). The most plausible explanation is that this feature arises from vibrations of the filaments in the crystal lattice. In three dimensions, the diffuse scatter consists of 'disks' normal to the direction of the long arms; there is also a weaker streak associated with the short arm of the filament. This finding implies that the conformation of the long arm of the filament is relatively variable, but that there is also some vibration in the short arm (Fig. 4). This

interpretation is consistent with the following experiments. X-ray photographs have been taken of crystals at 30 °C rather than 4 °C. In these conditions, reflections arising from the long arms but not those from the short arms are essentially eliminated. Moreover, this effect is reversible. This result confirms the difference in stability of the two arms of the filament, and shows that the magnitude of the effect is considerable at physiological temperatures.

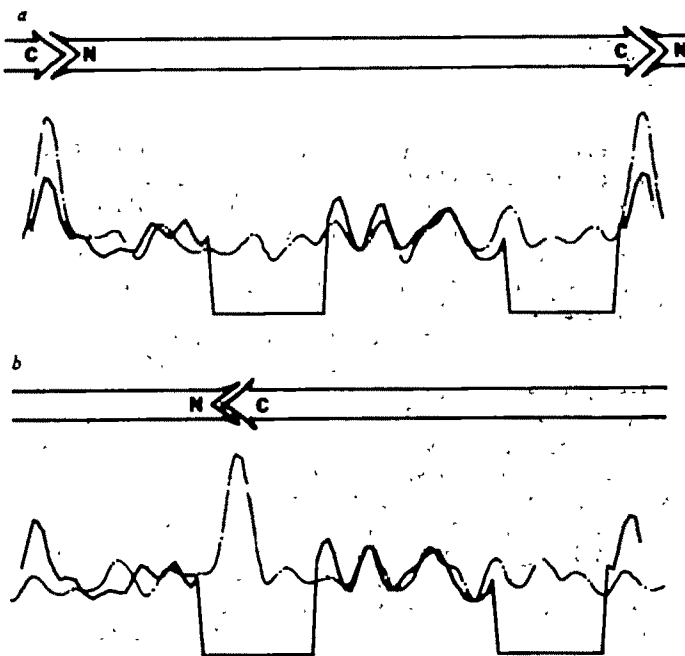


Fig. 6 Comparison of the observed (solid lines) and calculated (broken lines) electron density profiles of the tropomyosin filament for the two possible directions of the polypeptide chain. The observed electron density is taken as zero where cross-connecting filaments prevent integration of the molecular cross section. The calculated profiles were obtained by assigning weights to each amino acid in the sequence and convoluting with a gaussian of width appropriate to match the resolution of the observed curve. Curves in *a* correspond more closely than curves in *b* and the chain direction has, therefore, been taken as in *a*.

Relevance to muscle

Our results provide insight into the role of tropomyosin in muscle regulation. The molecular length we have established allows an estimate of the filament's radius of bending when it is bound on the actin helix. Taking the projected axial repeat of tropomyosin as 385 Å, this average radius can be calculated to be 45.2 Å ($(410.3^2 - 385^2)^{1/2} / \pi$). Assuming the same sense of twist, the radius of supercoiling of the molecular filament in the crystal lattice is about one quarter as large as that of tropomyosin on the actin helix. Interactions with actin, therefore, play an important part in determining the precise conformation(s) of tropomyosin in muscle. There must be some local flexibility of the tropomyosin structure to accommodate this large difference in radius of bending. The slight irregularities of the filament seen in the electron density map and the motions of the filament deduced from the diffuse scattering analysis imply localised flexible regions of the molecule³²⁻³⁴ and also indicate that there may be regions of the molecule other than the ends that are not in stable α -helical conformations.

The motions of tropomyosin during regulation depend on the precise structure(s) of the molecule and its specific interactions with actin. Attempts to determine the design of tropomyosin

have led to the recognition of some rather subtle structural features. The sequence results are consistent with an almost fully α -helical-coiled coil molecule; however, certain small regions may depart from this conformation, either in a conserved fashion (at the molecular ends) or only transiently, depending on varying interactions. The effective length of tropomyosin spans seven identical monomers on the thin filament, but there is no sevenfold repeat in the sequence; rather, 14 quasi-equivalent regions have been detected, which differ, however, rather markedly. (A single region of the molecule, for example, binds troponin.)

The presence of the high proportion of α -helical-coiled coil, and the quasi-equivalent bonding regions have led to models for tropomyosin that tend to stress its regular symmetrical features. This is, perhaps, a deceptively simple view. The kind of picture that is now beginning to emerge from our crystallographic analysis, and other studies, is that of a somewhat less regular and rigid structure. In its regulatory role in muscle, the tropomyosin supercoil moves on the actin helix. The molecular motions displayed by tropomyosin supercoils in the crystal lattice may reflect to some extent the real nature of the dynamic elements in the structure (see refs 23, 26). It is likely that at some stage of the contractile cycle, the tropomyosin molecule may be rather loosely wound about the actin helix, perhaps held strongly at only a few sites, and locally kinked at other regions. In view of the unusual characteristics of the molecule that have been deduced thus far, we suggest that the motions of tropomyosin in muscle depend on two aspects of its design: the regular coiled coil structure and quasi-equivalent regions that interact with actin; and the aperiodic features. These uncommon domains may play a critical part in the function of this highly specialised molecule.

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Endogenous mammary tumour virus DNA varies among wild mice and segregates during inbreeding

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Proviruses of the mouse mammary tumour virus (MMTV) endogenous to normal mice can be identified by molecular hybridisation and distinguished using restriction endonucleases. Feral mice display marked heterogeneity with respect to the number of copies and the sites of insertion of endogenous MMTV-specific DNA, with occasional mice apparently free of MMTV DNA. Several different MMTV proviruses present in laboratory mice have segregated like stable, independent genetic elements during the inbreeding which followed a cross between Bagg albino and DBA mice 60 years ago. The results favour the hypothesis that endogenous proviruses have been established by multiple, independent infections of germ cells rather than by somatic mutation of ancestral proviruses or of cellular genes.

DNA related to the genomes of retroviruses and capable of directing synthesis of viral gene products has been found endogenous to many animal species^{1,2}, but the origin and function of endogenous proviruses remain relatively obscure. Since retroviruses are extremely widespread in nature and have a very similar genetic organisation in different hosts, they are likely to have a common origin. It has generally been argued that endogenous proviruses have evolved either by alteration of cellular genes⁴ or by rare infection of the germ line(s) of early vertebrates⁵. To account for the retention of functional proviral DNA during speciation, it is attractive to propose that virus-specific DNA serves a function which confers some selective advantage to the host. It is also possible, however, that contemporary retroviruses evolved as viruses from a single progenitor virus and that endogenous proviruses were occasionally established by infection of the germ lines of individuals from diverse species. This proposal does not imply that endogenous proviruses have any utility for the host; their wide distribution might be simply a consequence of the ability of retroviruses to infect germinal cells, thereby introducing stably inherited proviral DNA.

Several features of the mouse mammary tumour virus (MMTV) make it suitable for investigating some of these issues. Pathogenic strains of the virus are transmitted in laboratory mice either as infectious agents in the milk of certain animals or as endogenous proviruses integrated into germ line DNA (refs 6, 7). Molecular hybridisation tests indicate that laboratory and feral mice may harbour approximately 3–15 copies of MMTV DNA per diploid genome^{9–12}; MMTV DNA has also been detected in Asian mice⁹, but little if any viral DNA has been found in rodents outside the genus *Mus*¹³.

Endogenous MMTV DNA differs markedly among feral mice

To characterise virus-specific DNA endogenous to normal cells, most investigators have relied on assays which measure the rate

of formation and stability of hybrids between labelled viral nucleic acids and cellular DNA, often using cellular DNA pooled from several individuals. Restriction endonucleases which recognise sequences within and adjacent to virus-specific DNA allow important refinements in the analysis of viral DNA: fragments generated from internal regions can be used to differentiate closely-related proviruses, and fragments which include adjacent host DNA can be used to identify the location of the proviruses in host DNA¹⁴. Since the recognition sites for these enzymes are short sequences (4–6 base pairs in length), they are likely to be conserved among members of a single species; the single copy DNA of individual mice, for example, appears to manifest less than 1% overall difference in base sequence¹⁵. We have used restriction endonucleases to compare endogenous viral DNA among individual animals, as a means of determining the origin of the DNA. If, for example, MMTV DNA had evolved from element(s) present in a progenitor of *Mus musculus* at a rate similar to that of other non-reiterated DNA, individual mice would be expected to appear similar when analysed for restriction fragments bearing virus-specific sequences.

To perform such tests, we prepared DNA from the livers of 12 wild mice trapped in four geographically distinct areas in California. (The liver is relatively resistant to horizontal infection by MMTV. Using tests for new proviruses in tissues of susceptible animals receiving virus in milk, no new viral DNA could be measured in livers in conditions adequate to detect infection of 1% of the cells¹⁶. Hence we have assumed here that proviruses in hepatic DNA represent only genetically transmitted DNA.) Following digestion with the restriction endonucleases *Pst*I and *Eco*RI, the DNA samples were assayed for fragments containing virus-specific sequences using agarose gel electrophoresis and the DNA transfer method¹⁷. These two enzymes were chosen because mapping studies with both viral DNA introduced into cells by acute infection¹⁸ and virus-specific DNA endogenous to inbred mice (see below; also ref. 16 and our unpublished results) indicated that *Pst*I recognises several sites within proviral DNA, whereas *Eco*RI cleaves at a single site (Fig. 1). Hence *Pst*I would be expected to generate several virus-specific fragments whose size would depend solely on sites within viral DNA, while cleavage of each provirus with *Eco*RI would normally produce two fragments whose size depends on sites in flanking cellular DNA. This approach has been used to show that MMTV proviruses introduced by experimental infection are located at many sites in cellular DNA (as manifested by variations in the size of *Eco*RI fragments), but that the same small region of viral DNA is always joined to cellular DNA^{16,19} (see legend to Fig. 1).

Analysis of the *Pst*I digests of wild mouse DNA (Fig. 2) showed all of the tested mice to be unique with respect to their MMTV proviruses, although fragments of certain sizes (for example, molecular weights of 3.3, 3.1, 2.5, 1.1, 0.9, 0.6, and 0.5×10^6) could be found in several of the digests. It is presumably significant that these fragments are observed in digests of DNA from inbred strains (Fig. 1; also Fig. 4, Table 1); we infer that units of viral DNA with very similar or identical internal sequences are variably distributed throughout the feral (and inbred) mouse population. We noted with particular interest the

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widespread occurrence (Fig. 2, lanes D, E, F, G, J, L, and M) of the constellation of *Pst*I fragments of 2.5, 1.1, 0.9 and 0.6×10^6 Mr also associated with the provirus acquired by infection with MMTV commonly isolated from milk (Fig. 1); in at least one case (lane F), these fragments appear to be present in the absence of any others.

The most striking finding, however, was the virtual absence of MMTV-related DNA in two of the 12 specimens examined with *Pst*I (Fig. 2, lanes H and I); annealing with a labelled MMTV-specific DNA revealed only a faint band, corresponding to a fragment of 9.0×10^6 MW common to all DNAs. Annealing of DNA immobilised on the same filter with labelled cDNA specific for rRNA demonstrated fragments common to all digests, including those in lanes H and I (bottom panel, Fig. 2), thereby excluding the possibility that the poverty of MMTV-specific bands in lanes H and I was due to some mechanical impediment to annealing. Moreover, several additional wild mice from the same location (Lake Casitas, California) have been found free of MMTV DNA (unpublished results of J.C.C. and M. Gardner). The common 9.0×10^6 MW fragment in annealings with both MMTV- and rRNA-specific cDNAs indicates a minor contamination of our viral cDNA with ribosomal sequences.

Examination of MMTV proviral DNA in feral mice with *Eco*RI produced grossly heterogeneous patterns (Fig. 3). Again, all tested mice were different; but in the *Eco*RI digests few, if any, fragments were common to two or more individuals, and the fragment sizes were not identical to those encountered in digests of DNA from inbred mice (see below, Fig. 5). One sample found to be free of MMTV-specific *Pst*I fragments (Fig. 2, lane I) also proved to lack *Eco*RI fragments which react with MMTV-specific cDNA (Fig. 3, lane I). The other negative

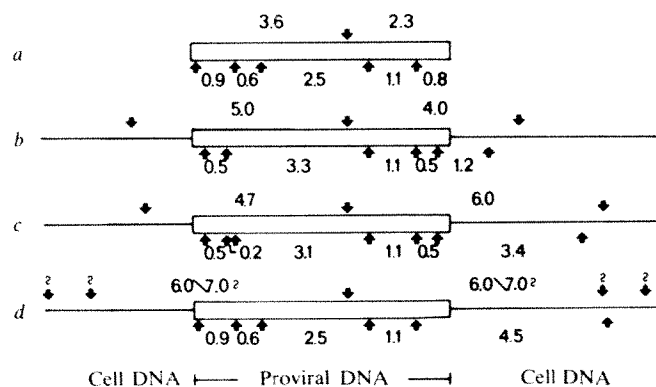


Fig. 1 Restriction endonuclease maps of MMTV DNA. The sites of cleavage of MMTV DNA and adjacent cellular DNA by the two enzymes used in this report are indicated by arrows (pointing down for *Eco*RI sites, up for *Pst*I sites). The numbers denote the molecular weights $\times 10^{-6}$ of consequent fragments. *a*, Linear form of unintegrated MMTV DNA in cultured cells infected by milk-borne virus from mouse strains GR or C₃H (refs 16 and 18). The linear form is approximately colinear with a subunit of viral RNA, with the right-hand half of viral DNA representing the 3' half of viral RNA¹⁸. Integrated MMTV DNA in cells infected by these viruses is colinear with the unintegrated linear species and has been found inserted in many different positions in host genomes^{16,19}. (We have not attempted to illustrate the many possible restriction endonuclease sites in cellular DNA adjacent to viral DNA introduced by experimental infection.) *b*, *c*, and *d*, Units of MMTV DNA found endogenous to certain inbred mice of the Bagg albino $\times$ DBA lineage (Fig. 6, Tables 1 and 2). The maps for Unit II (*b*) and Unit III (*c*) were determined by secondary cleavages of isolated *Eco*RI fragments of BALB/c mouse DNA; details will be presented elsewhere (unpublished data of J.C.C., J. Majors, and H.E.V.). The *Pst*I fragments of 1.2 (Unit II), and 0.2 and 3.4×10^6 Mr (Unit III) could be visualised well only after annealing with cDNA specific for the 3' terminus of viral RNA and were not therefore used routinely to identify these proviruses. (cDNAs specific for either end of viral RNA anneal to both ends of viral DNA^{18,19}, indicating that terminally redundant sequences are present in MMTV DNA in a manner similar to that described for avian sarcoma virus DNA³⁷⁻⁴⁰.) The *Pst*I map of Unit IV of endogenous MMTV DNA (*d*) is identical to the *Pst*I map of proviruses acquired by infection with milk-borne viruses (*a*). The question marks indicate uncertainty about the assignment of *Eco*RI sites in flanking cellular DNA to the right or left side of the provirus.

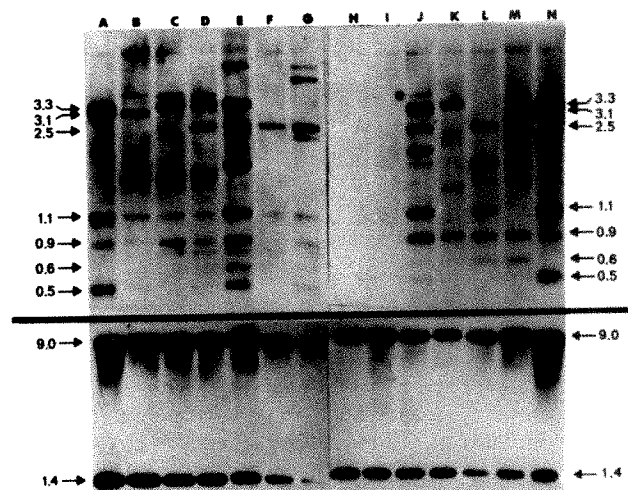


Fig. 2 Products of *Pst*I digestion of MMTV DNA endogenous to feral mice. DNA was extracted from the livers of individual wild mice using established procedures^{9,16}. Samples of 10 μ g were digested to completion with *Pst*I (prepared as described by Greene *et al.*⁴¹) and subjected to electrophoresis (1–2 V cm^{-1}) in horizontal 3–6 mm slab gels of 0.8% agarose (Seakem) in buffer containing 0.04 M Tris-acetate, pH 8.15, 0.02 M Na acetate, 0.018 M NaCl, 0.002 M Na₂EDTA. After staining with 5 $\mu\text{g ml}^{-1}$ ethidium bromide and subsequent inspection under UV illumination, the DNA was transferred to sheets of nitrocellulose (Schleicher and Schull) using the procedure of Southern¹⁷ as previously described^{16,18}. Filters were baked *in vacuo* for 2 h at 80 °C, incubated at 41 °C in a pre-annealing mixture containing 50% formamide, Denhardt's solution⁴², 20 $\mu\text{g ml}^{-1}$ alkali sheared and denatured salmon sperm DNA, 0.05 M HEPES buffer, pH 7.0, and $3 \times \text{SSC}$ ($\text{SSC} = 0.15 \text{ M NaCl}$, 0.015 M Na citrate), then incubated 60–72 h at 41 °C in a minimal volume of a similar mixture initially containing $1-2 \times 10^6$ c.p.m. ml^{-1} of MMTV ³²P-cDNA (upper panel). Following the washing and autographic procedures (see below), the filter subsequently was incubated with $1-2 \times 10^6$ c.p.m. ml^{-1} ribosomal ³²P-cDNA (lower panel) first at 68 °C for 1 h to denature hybrids with MMTV-specific cDNA and then at 41 °C for 60–72 h. These cDNAs were prepared as described by Shank *et al.*¹⁸ using oligomers of calf thymus DNA to prime synthesis randomly on either MMTV 20–35S RNA or 18S and 28S rRNA²⁷. After incubation with specific cDNAs the filters were rinsed briefly at 23 °C with $2 \times \text{SSC}$, incubated at 50 °C for 1–2 h with two changes of $0.1 \times \text{SSC}$, 0.1% SDS, and washed frequently at 23 °C with $0.1 \times \text{SSC}$. Kodak Royal X-omat film was exposed to the filter using Cronex 'Lightning Plus' intensifying screens (Dupont) for up to 10 d at -70°C ⁴³. *Hind*III fragments of ³²P-DNA were included as MW standards (not shown). The MW $\times 10^{-6}$ of major viral-specific fragments are shown on the left for lanes A–G and on the right for H–M. *Pst*I fragments of MMTV DNA endogenous to BALB/c mice, lanes A and M, and from Californian wild mice⁴⁴ obtained from Bouquet Canyon, lanes B, C, and D; San Francisco, lanes E, F, and G; Lake Casitas, lanes H, I, and J, and Norwalk, lanes K, L, and M.

sample was not tested with *Eco*RI. Although the MMTV proviruses in feral mice have internal similarities, as judged by the shared, relatively small *Pst*I fragments (Fig. 2), they are presumably located at different positions in cellular DNA, as judged by the variability of the size of the *Eco*RI fragments (Fig. 3). (The large sizes of these fragments supports our prediction that at least one of the determining sites is in flanking host DNA.) The results are most readily interpreted as evidence for variable numbers of independent integrations of MMTV proviruses at various sites in host DNA. The situation appears analogous to that in MMTV-infected tissues and cells, with viral DNA inserted at different sites in host DNA in each independently infected cell^{16,19}.

We have considered the possibility that some of our results could reflect a high degree of mutability of DNA surrounding endogenous proviral DNA, with relative conservation of the internal regions (as reflected in results of digestion with *Pst*I (Fig. 2) and by previous thermal denaturation studies⁹). However, this interpretation cannot account for the absence of proviral

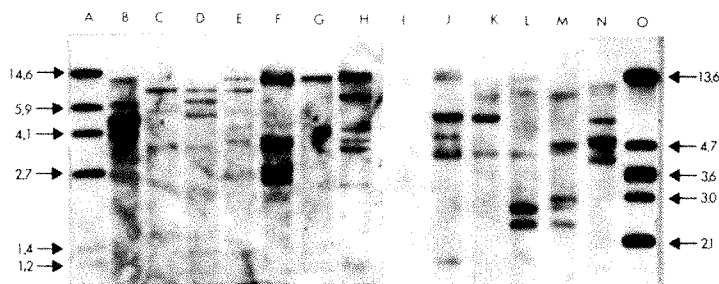


Fig. 3 Products of *EcoRI* digestion of MMTV DNA endogenous to feral mice. DNAs from the feral mice analysed in the experiment shown in Fig. 2 were digested with *EcoRI* (a gift from P. Greene) and analysed as described in the legend to Fig. 2. *HindIII* fragments (lane A) and *EcoRI* fragments (lane O) of phage λ ^{32} P-DNA served as MW standards for lanes B-H and I-N, respectively. *EcoRI* fragments of MMTV proviral DNA from BALB/c mice, lanes B and N; Bouquet Canyon, lanes C, D, and E (lanes B, C, and D, respectively in Fig. 1); San Francisco, lanes F, G, and H (lanes E, F, and G, respectively in Fig. 1); Lake Casitas, lanes I and J (lanes I and J, respectively in Fig. 1); and Norwalk, lanes K, L, and M (lanes K, L and M, respectively in Fig. 2).

DNA in some wild mice or for what seem to be major differences in the number of units of viral DNA among the wild mice (for example, compare the numbers of virus-specific *EcoRI* fragments in lanes F, H, J, and M with those in lanes C, E, G, and K in Fig. 3). In addition, the required frequency of change in the hexanucleotide recognition sequences for *EcoRI* in *Mus musculus* DNA would greatly exceed the base sequence divergence expected among individuals within a single species of *Mus*^{15,20}.

Units of endogenous MMTV DNA segregate like stable genetic elements during inbreeding

Our results could also be seen as evidence of a marked propensity of retroviral DNA to undergo genetic gymnastics, including deletion, translocation, rearrangement, and amplification, in manner similar to certain elements in bacteria²¹ or predicted for retroviral DNA in the provirus hypothesis⁴. (The latter hypothesis cannot, by itself, account for the absence of viral DNA from wild mice, but we consider the possibility that it might be operative in conjunction with other mechanisms for radical genetic change.) We have been able to perform a provisional test of this explanation by examining whether multiple units of endogenous MMTV DNA have segregated like stable genetic elements, retaining their characteristic restriction endonuclease recognition sites, during the almost 60 yr of inbreeding which followed the mating of a Bagg albino female with a DBA male in 1920 (ref. 22).

Figures 4 and 5 illustrate the patterns of virus-specific fragments in *PstI* and *EcoRI* digests of DNA obtained from the livers of mice from several contemporary colonies descended from the progeny of the initial Bagg albino $\times$ DNA mating. Despite several obvious differences among these digests (to be considered below), the similarities are very striking, especially when they are viewed in comparison with the digests of DNA from feral mice (Figs 2 and 3). As the tested mice were obtained from branches of the genealogical tree that had been separated for nearly 60 yr (see Fig. 6, ref. 22), the apparent stability of restriction sites suggested that neither viral DNA nor flanking cellular DNA was highly mutable and that the elements of endogenous DNA might be amenable to segregation analysis.

To simplify this analysis, we correlated the presence of *EcoRI* and *PstI* fragments in digests of each sample, as summarised in Table 1. In making these correlations we took advantage of mapping data from related studies. (1) We knew from studies of cells and tissues infected by milk-borne MMTV¹⁶ that *PstI* fragments of 2.5 and 0.6×10^6 MW could be used to distinguish provirus acquired by horizontal infection from proviruses found endogenous to certain inbred mice (for example BALB/c). As shown here, several other strains derived from the Bagg albino $\times$ DBA mating (C₃H/Bi, CBA/J, and CBA/St; Fig. 4) carry a provirus indistinguishable with this enzyme from the provirus acquired by infection with milk-borne MMTV (Fig. 1a, d). This provirus (Unit IV in Table 1) is also associated in these mice with the appearance of *EcoRI* fragments of 7.0 and 6.0×10^6 MW; Unit IV can be found in contemporary DBA/2 mice and presumably was carried by the DBA male participating in the original cross. (2) We have generated physical maps of the MMTV DNA endogenous to contemporary BALB/c (Fig. 1b, c; Table 1) by performing secondary digestions with several endonucleases of isolated *EcoRI* fragments of BALB/c liver DNA bearing viral sequences (data not shown). These studies validated several of the associations we were able to make on genetic grounds between *PstI* and *EcoRI* fragments, and they revealed the structure of three units of endogenous viral DNA (I-III) which differ from the provirus (Unit IV) resembling DNA acquired by infection with milk-borne MMTV. These three units include two (Units II and III) very similar in complexity and organisation to the proviruses acquired by infection with milk-borne virus (that is 5×10^6 – 6×10^6 Mr); the third (Unit I) bears viral sequences with about one-half the complexity of a unit length provirus (Table 1).

These approaches led to the definition of six units of endogenous MMTV DNA which seem to be segregating in the progeny of Bagg albino $\times$ DBA; the restriction fragments associated with each unit are listed in Table 1 to facilitate inspection of the digests shown in Figs 4 and 5. When each of the contemporary colonies was scored for carriage of each of the six units, we were able to define six genotypes (A-F, Table 2). No strain carried less than two units of endogenous DNA, but only one unit (Unit II) was present in the DNA of all the laboratory mice

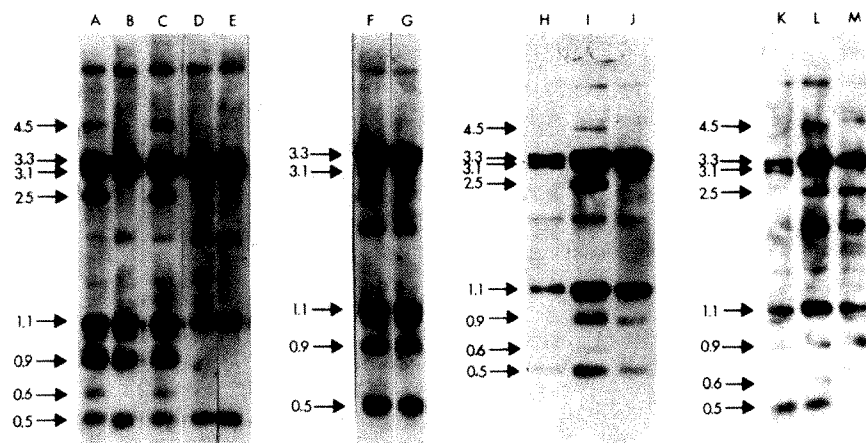


Fig. 4 Products of *PstI* digestion of MMTV DNA endogenous to progeny of Bagg albino $\times$ DBA. 10 μ g of DNA from the livers of individual mice from the strains indicated were analysed as described in Fig. 2. *HindIII* fragments of λ DNA were used as MW markers and the MW $\times 10^{-6}$ of major MMTV-specific fragments are indicated to the left of each gel. Lane A, C₃H/Bi; B, C₃H/St; C, CBA/J; D, CBA/CaJ; E, CBA/Ht-6; F, C₃H/HeN; G, C₃H/HeJ; H, CBA/N; I, C₃H/Bi; J, C₃H/An; K, BALB/c; L, DBA/2; M, CBA/St.

Table 1 Restriction fragments derived from six defined units of endogenous MMTV DNA

Units of endogenous MMTV DNA	<i>Pst</i> I fragments	<i>Eco</i> RI fragments
I	0.9*	10.0
II	3.3, 1.1, 0.5	5.0, 4.0
III	3.1†, 1.1, 0.5	6.0, 4.7
IV	4.5, 2.5, 1.1, 0.9, 0.6	7.0, 6.0‡
V§	3.1†	3.9¶, 3.5, 2.7
VI§	—	5.4, 9.0, 1.0**

The *Pst*I and *Eco*RI fragments useful for the identification of each of the six units of endogenous MMTV DNA observed in the progeny of Bagg albino × DBA are listed according to molecular weight ($MW \times 10^{-6}$). For discussion of the definition of the units, see text. The restriction endonuclease maps of Units II, III, and IV are illustrated in Fig. 1.

* The 0.9 *Pst*I fragment is diagnostic of Unit I when it appears in the absence of Unit IV.

† *Pst*I fragments of 3.1 are derived from Units III and V, but the corresponding band is equal in intensity to the 3.3 *Pst*I band in the first case, but is less intense in the second (compare lanes G and H in Fig. 4).

‡ Two *Eco*RI fragments, derived from Units III and IV, migrate at 6.0×10^6 Mr as shown by analysis of CBA/St (Table 2; Fig. 5, lane L) in which Unit IV but not III is present and a 6.0×10^6 MW fragment is still observed.

§ These units have not been characterised fully by biochemical techniques.

¶ The 3.0 *Eco*RI fragment is inferred from the broadening of the 4.0 band (see lane G compared with lane I in Fig. 5).

** This small molecular weight fragment is not included on the gel shown in Fig. 5.

examined here; results with feral mice (see above) indicate that this unit is not, however, ubiquitous in mice. When the genotypes were correlated with the genealogical tree for the mouse colonies used (Fig. 6), three units (I, III, and IV) were found in both major branches of the tree but not in all the members. Unit V was identified in colonies on one branch and in contemporary DBA/2 mice. Unit VI was also found in DBA/2 mice but not in any of the progeny from the original Bagg albino × DBA cross; we cannot say whether it is likely to have been present in the DBA male used in the initial cross. These results indicate that the parental Bagg albino and DBA animals were not both homozygous for any of the defined units of endogenous MMTV DNA, with the probable exception of Unit II; Units I, III, IV, and V (and perhaps VI) have consequently segregated during inbreeding.

MMTV genotypes can be used to monitor breeding programmes

To ensure that the observed results were not the result of errors in breeding protocols, we examined several colonies of some strains maintained at widely separated locations. Colonies of C₃H/He mice maintained at Jackson Laboratories (Bar Harbor), the National Institutes of Health (Bethesda); and the University of California (Berkeley) yielded identical results, as did colonies of C₃H/Bi (and C₃Hf/Bi) from Simonson Laboratories (Gilroy, California), the Sloan-Kettering Institute (New York), and the Imperial Cancer Research Fund (London) (data not shown).

An interesting exception to the general rule of identity among separately maintained colonies was provided by the differences in genotype between the C₃H/St mice maintained at the L.C. Strong Foundation (San Diego, California) and a subline, C₃H/StWi, derived in 1947 by Wilson from the original Strong colony²³ and maintained at the National Institutes of Health (Bethesda); Unit IV is present in the colony derived by Wilson but not in that kept by Strong (data not shown). Since these colonies were separated in the 1940s it is probable that the animals were not fully inbred at that stage and were heterozygous with respect to Unit IV. It is, of course, also possible that Unit IV either was lost from the Strong colony as a consequence of deletion or introduced into the Wilson subline by an inadvertent interruption in the pedigree.

In view of the result with C₃H/St, we looked for evidence of incomplete inbreeding or genetic instability in the contemporary colony of C₃H/St at San Diego by testing the DNA from several individuals for endogenous MMTV DNA. So far no differences

have been observed among members of this colony, in tests that have included 20 individuals. In addition, analysis of endogenous MMTV sequences among numerous BALB/c colonies has not revealed any differences. These results suggest that contemporary mouse strains are truly homozygous at most or all viral loci, as predicted from other kinds of analysis²², and that endogenous MMTV DNA seems stable by the criteria which revealed marked heterogeneity among feral mice. Such tests for defined patterns of endogenous viral DNA may be a particularly sensitive assay for the success of an inbreeding programme; for example, we would have been likely to have measured the heterogeneity in C₃H/St at the time of separation of its colonies (see above).

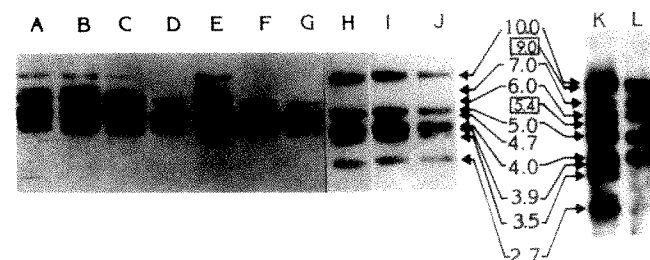


Fig. 5 Products of *Eco*RI digestion of MMTV DNA endogenous to progeny of Bagg albino × DBA. 10 µg of DNA from the livers of individual mice from the strains indicated were digested to completion with *Eco*RI and then analysed as described in Fig. 2 legend. ³²P-labelled *Eco*RI fragments of λ DNA included in the gel were used as MW standards in obtaining $MW \times 10^{-6}$ listed between lanes J and K for major viral fragments. Viral fragments found only in the DBA/2 (lane K) parent strain are presented in the squares while fragments shared by more than one strain are indicated by the larger numbering. Lane A, C₃Hf/Bi; B, C₃H/Bi; C, C₃H/St; D, CBA/N; E, CBA/J; F, CBA/CaJ; G, CBA/Ht-6; H, C₃H/HeJ; I, C₃H/HeN; J, C₃H/An; K, DBA/2; L, CBA/St.

Implications for origins of endogenous DNA

Our results are most readily interpreted as evidence favouring the hypothesis that endogenous proviruses result from multiple, independent, but presumably infrequent infections of germ line tissues with retroviruses. According to this view, the similarities among retroviruses from many species would reflect their probable evolution from a single progenitor virus; the rationale for their ubiquity would be no more obvious than the rationale for the ubiquity of any other of the several far-flung classes of virus; and their appearance as endogenous viruses would be a consequence of their capacity to establish their genomes in infected cells, presumably including germ cells, as proviruses covalently integrated into host cell DNA. Several ancillary observations are in accord with this proposal. (1) The four MMTV proviruses endogenous to the A strain mouse are located on more than two chromosomes²⁴, consistent with their acquisition by multiple, independent infections. (2) Evidence for trans-species transmission of viruses now recovered as endogenous viruses argues that they were established by infection of germinal tissues^{5,25,26}. (3) Avian leukosis virus-specific DNA cannot be detected in two types of wild jungle fowl, despite its presence in a third type of jungle fowl, in closely related domestic chickens, and in more distantly related pheasants (D.

Table 2 Genotypes observed in progeny of Bagg albino × DBA

Genotype designation	Units of MMTV DNA	Example
	I II III IV V VI	
A	+ + - + + +	DBA/2 (Figs 4L, 5K)
B	+ + - + - -	CBA/St (Figs 4M, 5L)
C	+ + + - - -	BALB/c (Figs 2A, 3B, 4K)
D	+ + + + - -	C ₃ H/Bi (Figs 4A, 5B)
E	- + + - - -	CBA/CaJ (Figs 4D, 5F)
F	+ + - - + -	C ₃ H/HeN (Figs 4F, 5I)

The units of endogenous MMTV DNA defined in Table 1 were sought by examination of *Pst*I and *Eco*RI digests of liver DNA from mice in colonies derived from Bagg albino × DBA (see Figs 4, 5). The presence of a unit is indicated by +, its absence by -. Six genotypes, designated, A, B, C, D, E, and F, were observed in the tested samples. An example for which complete data are provided is listed for each genotype.

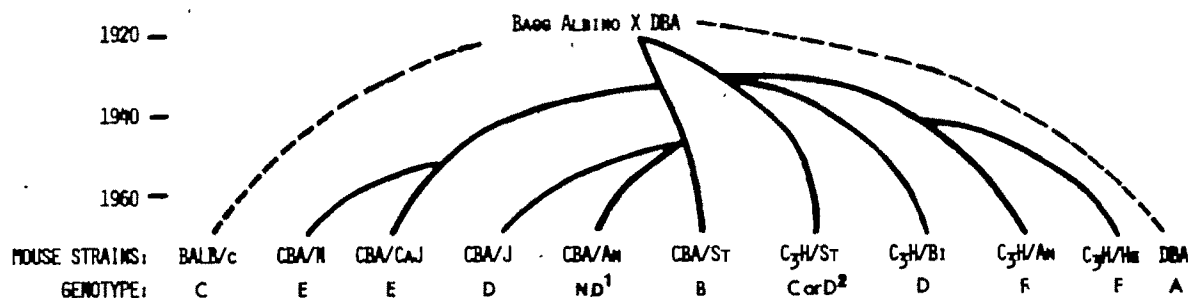


Fig. 6 Segregation of endogenous MMTV proviruses during inbreeding of progeny of Bagg albino $\times$ DBA. The genealogy of mouse strains was derived from the summary prepared by Staats²¹ and plotted against the recorded dates for mating and segregation. Solid lines are used for the progeny of the Bagg albino $\times$ DBA mating; dashed lines indicate the ancestors of contemporary BALB/c and DBA colonies, but are not meant to imply that the animals involved in the indicated cross were the direct predecessors of BALB/c and DBA/2 mice used here. The genotype (see Table 2) for each strain examined was determined from analyses of the sort shown in Figs 4 and 5 and is noted below the name of the strain.

* Not done because the strain was not available.

† C3H/St is assigned 'C or D' because two colonies gave different results (see text).

Frisby, R. Weiss, M. Roussel and D. Stehelin, personal communication). The lack of concordance of viral DNA with established phylogenetic relationships again argues for infection of germ lines after speciation. (4) Biochemical studies of normal chickens^{27,28} and mice^{29,30} indicate considerable heterogeneity from animal to animal^{27,28} or from strain to strain^{29,30} with respect to the amount and/or structure of endogenous leukaemia virus-related DNA. There is, however, one notable difference between results reported here and the results of studies of endogenous avian leukosis virus-specific proviruses in domestic chickens: all of the tested chickens shared one common proviral element, despite the variable distributions of other elements^{27,28}. This observation might mean that domestic chickens have been derived from a relatively small genetic pool, or it might imply that a least one provirus was introduced into the germ line of chickens at some relatively early time. (5) In the studies of avian viruses, the variability in number, location, and structure of endogenous proviruses contrasted with uniform or nearly uniform results obtained with reagents specific for cellular genes, such as globin, ovalbumin, ovomucoid, and transferrin²⁷; these findings further support the thesis that endogenous proviruses either originate or evolve in a manner fundamentally different from cellular genes.

Our results obviously run counter to the widely held view that endogenous viral DNA has evolved either from some ancient cellular sequences⁴ or from an ancient provirus present in early vertebrates⁵. This point of view has encouraged examination of the relatedness of endogenous proviruses in attempts to assess the evolutionary relationships among many animals³¹, including man³². The proposal favoured by our data, if supported by similar studies of other kinds of proviral DNA, would challenge the assumptions that underlie such studies. Certainly it is possible that some kinds of proviral DNA other than MMTV DNA appeared in germ lines, by infection or other mechanisms, sufficiently long ago to serve as useful markers for vertebrate evolution. In addition, interpretation of our data would be difficult if retroviral DNA exhibits a propensity to undergo deletion, rearrangement, or translocation. Although our results with the progeny of Bagg albino $\times$ DBA mice argue for stability of germinal proviral elements over a 60-yr period, we cannot discount rare events of these kinds over a much longer period as explanations for some or all of our observations with feral mice. Future experiments with other endogenous proviruses and with proviruses acquired by infection of cultured cells may be helpful in judging such possibilities.

Implications for mammary tumorigenesis

The hypothesis that endogenous proviruses arise by infection rather than by conservative evolution from sequences common to primitive vertebrates does not favour any functional role for viral DNA in the growth and development of normal organisms. Nevertheless endogenous viral DNA may significantly alter

major biological processes in ways that have yet to be recognised; certainly its presence may have pathological consequences for the host animal. This idea has been dramatically documented in the laboratory by the markedly increased frequency of leukaemia in BALB/c mice following the introduction of the provirus of the Moloney strains of murine leukaemia virus as an endogenous provirus³³. In the case of MMTV, the GR strain is apparently prone to early mammary tumours⁷ as a consequence of its carriage of endogenous provirus(es) indistinguishable in tests described here from that acquired by infection with milk-borne virus of known oncogenic potential (our unpublished data). The GR mouse may be a special case, in that the provirus in question seems to be present in multiple, probably closely-linked copies^{9,10,34,35}. The biological effects of a similar endogenous provirus (Unit IV) present in some of the mice studied here remains to be determined. It is known, however, that several strains of mice develop mammary tumours late in life under the influence of genetically transmitted viruses⁶⁻⁸; examination of the DNA from such tumours by methods described here indicates that the tumour cells bear additional copies of proviruses similar to that endogenous provirus termed Unit II (our unpublished data). Presumably some alteration in the control of expression of such proviruses results in virus spread and subsequent tumorigenesis, with the pathogenic potential of the element augmented when it is integrated into a new site after secondary infection. A similar mechanism has been proposed to account for the regulation of expression of an endogenous virus of chickens³⁶. This suggests that the site at which an endogenous provirus is integrated in cellular DNA may have a considerable influence upon its expression; depending upon the consequences of viral gene expression for the host, the entry of new proviruses into the germ line at certain sites could therefore provide a selective advantage or disadvantage to the host.

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Nucleotide sequence of cloned cDNA for bovine corticotropin- β -lipotropin precursor

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The nucleotide sequence of a 1,091-base pair cloned cDNA insert encoding bovine corticotropin- β -lipotropin precursor mRNA is reported. The corresponding amino acid sequence indicates that the precursor protein consists of repetitive units and includes a third melanotropin sequence in its cryptic portion. Pairs of lysine and arginine residues separate the component peptides of the precursor.

THE pituitary hormones corticotropin (ACTH) and β -lipotropin (β -LPH) and their gene provide a useful system for studying the structure and regulation of hormonally regulated genes. These two hormones, which are formed from a large common precursor protein¹⁻⁸, contain small component peptides with biological activity; α -melanotropin (α -MSH) and corticotropin-like intermediate-lobe peptide (CLIP) are derived from ACTH⁹, whereas γ -lipotropin (γ -LPH), β -melanotropin (β -MSH), endorphins and methionine-enkephalin are elaborated from β -LPH^{10,11}. The intracellular level of the mRNA coding for the common precursor protein is depressed by glucocorticoids, which probably act at the transcriptional level by means of the glucocorticoid receptor^{12,13}. As ACTH and β -LPH together account for only one-third to one-half of the molecular weight of the precursor protein, there has been considerable interest in and speculation about the primary structure and possible biological functions of the remaining portion.

Recently developed techniques of DNA cloning¹⁴ and nucleotide sequence analysis^{15,16} together provide a useful approach to an understanding of the primary structure of eukaryotic genes and the regulation of their expression. Previously¹⁷, we described the construction of bacterial plasmids that contain the nucleotide sequence for the bovine ACTH- β -LPH precursor and presented the DNA sequence coding for ACTH and a portion of β -LPH. Here, we report the complete nucleotide sequence of a 1,091-base pair cloned cDNA insert that contains a duplex copy of the ACTH- β -LPH precursor mRNA. Our results define the precise locations of ACTH and β -LPH in the precursor protein and predict the amino acid

sequence of its remaining portion. They further indicate that the precursor protein consists of several repetitive units separated by pairs of basic amino acid residues, lysine and arginine, and that a third melanotropin sequence is located within the cryptic portion of the molecule.

Sequencing strategy

The bacterial plasmid pSNAC20 was constructed by inserting *in vitro*-synthesised double-stranded cDNA for the bovine ACTH- β -LPH precursor into the *Pst*I endonuclease cleavage site of the plasmid pBR322 with the use of poly(dG)·poly(dC) homopolymeric extensions¹⁷. Nucleotide sequence analysis of the inserted cDNA was carried out by the procedure of Maxam and Gilbert¹⁵; the sequence map is shown in Fig. 1. The restriction fragments used were obtained either from intact pSNAC20 or from the cDNA insert (including poly(dG)·poly(dC) tails), which was isolated by cleavage of the plasmid at the regenerated *Pst*I sites¹⁷.

For accurate prediction of the amino acid sequence of the cryptic portion of the ACTH- β -LPH precursor protein, the nucleotide sequence was determined on both strands of the cDNA for all but 33 residues corresponding to the 5' end and 47 residues corresponding to the 3' end of the mRNA; for these regions this was technically difficult, but the sequence data were reliable. In addition, care was taken to overlap the sequence data derived from different endonuclease-generated fragments. These procedures provided a useful verification of the sequence. Furthermore, some of the endonuclease-generated fragments were analysed for nucleotide sequence in duplicate or in triplicate. Thus, at least three sets of sequence data were obtained for all segments of the cloned cDNA, thus eliminating any ambiguity resulting from closely lying bands of chemically degraded DNA, which were occasionally observed on electrophoretic analysis.

Another potential problem in the use of cloned cDNA for sequence analysis of mRNA is a possible error caused by deletion, addition or rearrangement of nucleotides during *in vitro* cDNA synthesis or DNA cloning itself¹⁸⁻²⁰. Thus, an independent method, such as analysis of protein sequence or mRNA sequence, should preferably be used to verify that the

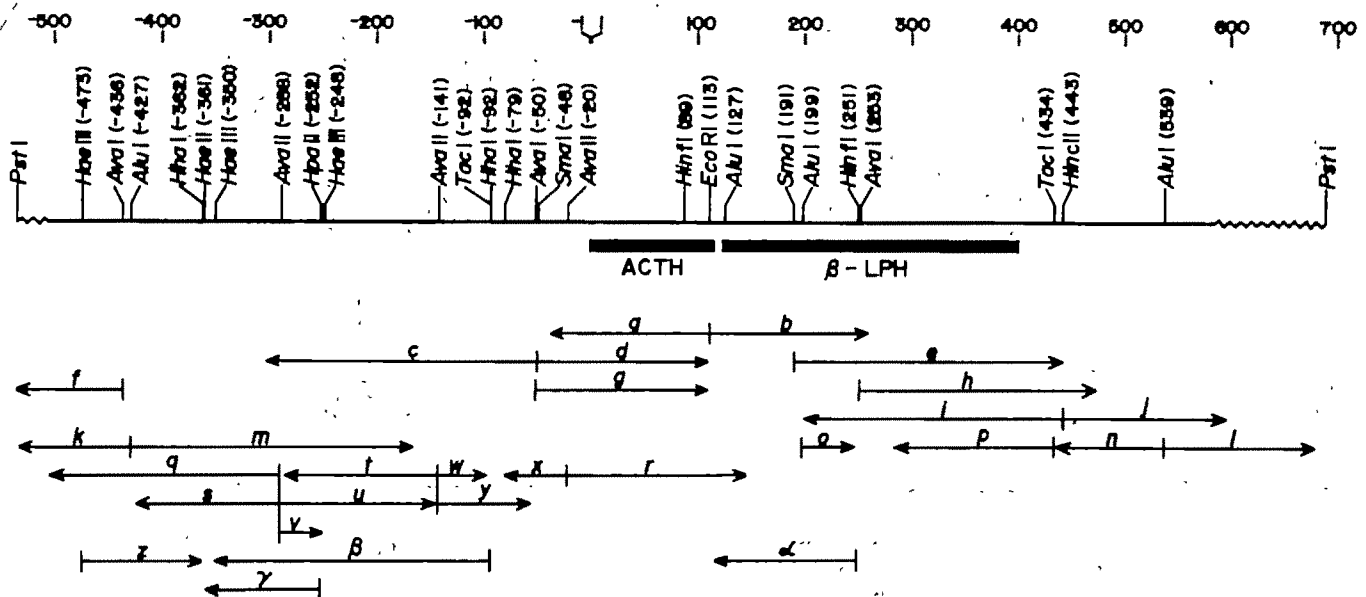


Fig. 1 Sequence map for the cDNA insert in the pSNAC20 plasmid. Nucleotide residues are numbered in the direction from 5' to 3' in the message strand, beginning with the first residue in the coding region for ACTH, and the nucleotides on the 5' side of residue 1 are indicated by negative numbers. The wavy lines represent the poly(dG)·poly(dC) tail of the cDNA insert (27 and ~100 base pairs long on the left and right sides, respectively). The coding regions for ACTH and β -LPH are indicated by closed boxes. Only the restriction sites used for sequencing are shown and are identified by numbers indicating the 5'-terminal nucleotide generated by cleavage. DNA sequence analysis was carried out by the procedure of Maxam and Gilbert¹². The direction and extent of the sequence determinations are indicated by horizontal arrows α -z and α -y. The 5'-end-labelled restriction fragments used for sequencing, which were derived either directly from intact pSNAC20 or from the cDNA insert obtained by cleavage of the plasmid with PstI followed by electrophoretic isolation, were prepared as follows (the letters in parentheses refer to the horizontal arrows, the labelled site is designated by an asterisk, and the number indicates the fragment chain length in base pairs): α , Cleavage of pSNAC20 with EcoRI, labelling, cleavage with PstI, isolation of *EcoRI-PstI-645 (a) and *EcoRI-PstI-570 (b). β -c, Cleavage of pSNAC20 with SmaI, labelling, cleavage with EcoRI, isolation of *SmaI-EcoRI-1,241 (c), *SmaI-EcoRI-160 (d) and *SmaI-EcoRI-4,100 (e). β -f-h, Cleavage of pSNAC20 with AyoI, labelling, cleavage with PstI, EcoRI or PstI, isolation of *AyoI-PstI-223 (f), *AyoI-EcoRI-162 (g) and *AyoI-PstI-430 (h). β -i, Cleavage of pSNAC20 with HaeIII, labelling, cleavage with PstI, isolation of *HaeIII-PstI-975 (i) and *HaeIII-PstI-240 (j). β -k-p, Cleavage of the cDNA insert with AyoI, labelling, isolation of *AyoI-PstI-106 (k), *AyoI-PstI-150 (l), *AyoI-PstI-553 and *AyoI-PstI-340 (the PstI site was not accurately labelled), cleavage of *AyoI-PstI-553 with SmaI, isolation of *AyoI-SmaI-579 (m), cleavage of *AyoI-PstI-340 with TceI, isolation of *AyoI-TceI-105 (n) and *AyoI-TceI-235, labelling of *AyoI-TceI-235, cleavage with HaeIII, isolation of *AyoI-HaeIII-52 (o) and *TceI-HaeIII-183 (p). β -q-y, Cleavage of the cDNA insert with AyoI, labelling, isolation of *AyoI-PstI-245 (q), *AyoI-PstI-710 (r), *AyoI-PstI-147 (s) and *AyoI-PstI-121 (t) (in one experiment, *AyoI-PstI-245 was cleaved with AyoI, and *AyoI-PstI-139 (z) was isolated, alkali treatment²³ of *AyoI-PstI-147, isolation of single-stranded *AyoI-PstI-147 (u) (in one experiment, *AyoI-PstI-147 was cleaved with HaeIII, and *AyoI-HaeIII-40 (v) was isolated), cleavage of *AyoI-PstI-121 either with HaeIII or with SmaI, isolation of *AyoI-HaeIII-49 (w), *AyoI-HaeIII-59 (x) and *AyoI-SmaI-93 (y). β -z, Cleavage of the cDNA insert with AyoI, isolation followed by cleavage with HaeIII of AyoI-PstI-245, isolation followed by labelling of HaeIII-HaeIII-123, cleavage with HaeIII, isolation of *HaeIII-HaeIII-112 (z). α , Cleavage of the cDNA insert with AyoI, isolation followed by cleavage with HaeIII of AyoI-PstI-710, isolation followed by labelling of HaeIII-HaeIII-162, cleavage with EcoRI, isolation of *HaeIII-EcoRI-138 (a). β , Cleavage of the cDNA insert with EcoRI, isolation followed by cleavage with TceI of EcoRI-PstI-645, isolation followed by labelling of TceI-PstI-441, cleavage with HaeIII, isolation of *TceI-HaeIII-270 (b). γ , Cleavage of the cDNA insert with TceI, isolation followed by cleavage with HaeIII of TceI-PstI-441, labelling, cleavage with HaeIII, isolation of *HaeIII-HaeIII-110. Cleavage with restriction endonucleases was carried out as the conditions described by the vendors with optimal amounts of the enzymes. Separation of the restriction fragments was carried out by electrophoresis on either 5% or 8.3% polyacrylamide slab gels in 50 mM Tris-borate containing 1 mM EDTA (pH 8.3), except that alkali-treated *AyoI-PstI-147 was electrophoresed on 15% gel for strand separation. Fractionated DNA was extracted from the gel by maceration. Treatment of DNA with 7 μ g of bacterial alkaline phosphatase (Worthington) was carried out at 55 °C for 1 h in 20 mM Tris-HCl (pH 8.3). Labelling of DNA was carried out at 37 °C for 1 h with 200–250 μ Ci [γ -³²P]ATP (~7,000 Ci mmol⁻¹, New England Nuclear) and 0.5 μ g of T4 polynucleotide kinase (Boehringer-Mannheim Corporation) in a reaction mixture containing 50 mM Tris-HCl (pH 9.5), 10 mM MgCl₂, 5 mM dithiothreitol and 0.75 mM spermidine.

cloned cDNA we have studied represents an exact copy of the mRNA. However, the fidelity of cDNA cloning in bacterial plasmids has been amply established for several mRNA species^{21–26}. We have also obtained evidence indicating that the coding regions for the known segments of the precursor protein (that is, the segments containing ACTH and β -LPH) are faithfully represented in the cloned cDNA (see below). In any case, the conclusions reported here result from study of the cDNA copy of a single mRNA molecule and heterogeneity of the ACTH- β -LPH precursor mRNA cannot be excluded.

Sequence of ACTH- β -LPH precursor mRNA and assignment of protein sequence

Figure 2 gives the sequence of 1,091 nucleotides comprising the cDNA inserted in the bacterial plasmid pSNAC20 (excluding poly(dG)·poly(dC) tails). Our previous work has demonstrated that the sequence of nucleotide residues 1–207 corresponds precisely to the amino acid sequences determined for bovine ACTH and the first portion of bovine β -LPH (28 amino acid residues) plus the two amino acid residues Lys-Arg connecting the two peptides¹⁷. The nucleotide sequence we have determined for the remaining portion of β -LPH indicates the presence of two additional amino acid residues, Ala-Glu, between positions 35 and 36 of the generally accepted structure of bovine β -LPH²⁷. Thus, our results are consistent with a structure of bovine β -LPH that contains 93 amino acid residues, as reported by Pankov²⁸; however, our finding that the residue at position 16 is Gln agrees with the assignment at this position made by Li *et al.*²⁷.

The nucleotide residues encoding the glutamine at the carboxyl terminus of β -LPH are followed by the translational termination codon UGA. This observation indicates that there is no peptide extension beyond the carboxyl terminus of β -LPH and that the 3' noncoding region of the mRNA is composed of an untranslated region of 175 nucleotides plus a poly(A) terminal sequence. Another significant finding is that the amino terminus of ACTH, like the carboxyl terminus, is connected to the adjacent peptide by a pair of basic amino acid residues, Lys-Arg. Thus, the formation of ACTH and β -LPH from their precursor seems to involve proteolytic processing similar to that observed in the conversion of proinsulin to insulin²⁹ and parathyroid hormone to parathyroid hormone^{30,31}.

In assigning the amino acid sequence of the cryptic portion of the ACTH- β -LPH precursor, the reading frame of amino acid codons was determined from the frame corresponding to the amino acid sequences of ACTH and β -LPH. However, the translational initiation site on this mRNA cannot be located definitively without knowing the amino acid sequence of the amino-terminal region of the precursor protein. A possible location for the translational initiation site is the methionine codon AUG at positions –393 to –391. On the basis of this assignment, the molecular weight of the ACTH- β -LPH precursor protein is calculated to be 29,259, a smaller value than the previously estimated molecular weight of the cell-free translation product of bovine ACTH- β -LPH precursor mRNA (35,000 (ref. 1) or 41,000 (ref. 2) depending on the electrophoresis system used). However, the relatively large number of basic amino acids (lysine and arginine) encoded by this mRNA (Fig. 2) or possible glycosylation of the cell-free trans-

lation product may result in overestimation of the molecular weight of the protein by electrophoretic analysis³². The assignment of the translational initiation site at the methionine codon mentioned above seems plausible because the first 20 amino acid residues starting with this putative initiative methionine (residue -131), which are followed by the characteristic sequence Ser-Met-Glu--- (see below), include a large number of hydrophobic amino acids (13 nonpolar residues including 7 leucine residues). A high content of hydrophobic amino acids is known to be present in the signal peptides of other proteins destined for secretion³³⁻³⁶. On the other hand, if the previous estimation of the molecular weight of the precursor protein is correct, the translational initiation site would be located 1,125-1,295 nucleotides (950-1,120 nucleotides for the coding region plus the 175 noncoding nucleotides we have identified at the 3' end) away from the first residue of the poly(A) sequence of the mRNA and therefore would be absent from the cloned cDNA segment we have analysed. In Fig. 2, we have assigned amino acid residues up to the putative initiative methionine, although it remains possible that the region of the mRNA proximal to this site is translated.

Computer analysis of the amino acid sequence determined from the pSNAC20 clone indicates that the sequence of amino acid residues -55 to -44 is strikingly similar to the sequences of α -MSH and β -MSH, which are known to have common structural and biological features³⁷ (Fig. 3). This segment of the precursor protein shares with the known MSHs tyrosine and methionine residues as well as the characteristic tetrapeptide sequence His-Phe-Arg-Trp, which is required for melanotropic activity, at equivalent positions³⁸. Moreover, the peptide fragment discovered by our DNA sequence analysis is flanked on both sides by a pair of basic amino acid residues. This suggests that the new peptide, like α -MSH or β -MSH, can be formed by proteolytic processing of the precursor; by analogy with α -MSH, it is possible that phenylalanine amide is formed at the carboxyl terminus of this peptide during processing. In view of the remarkable structural homology between the newly found peptide fragment and α -MSH or β -MSH, we propose to name the peptide γ -melanotropin (γ -MSH). Another peptide fragment within the precursor protein that exhibits some structural similarity to the MSHs is that containing amino acid residues -111 to -105 (Fig. 3). Note that this fragment contains

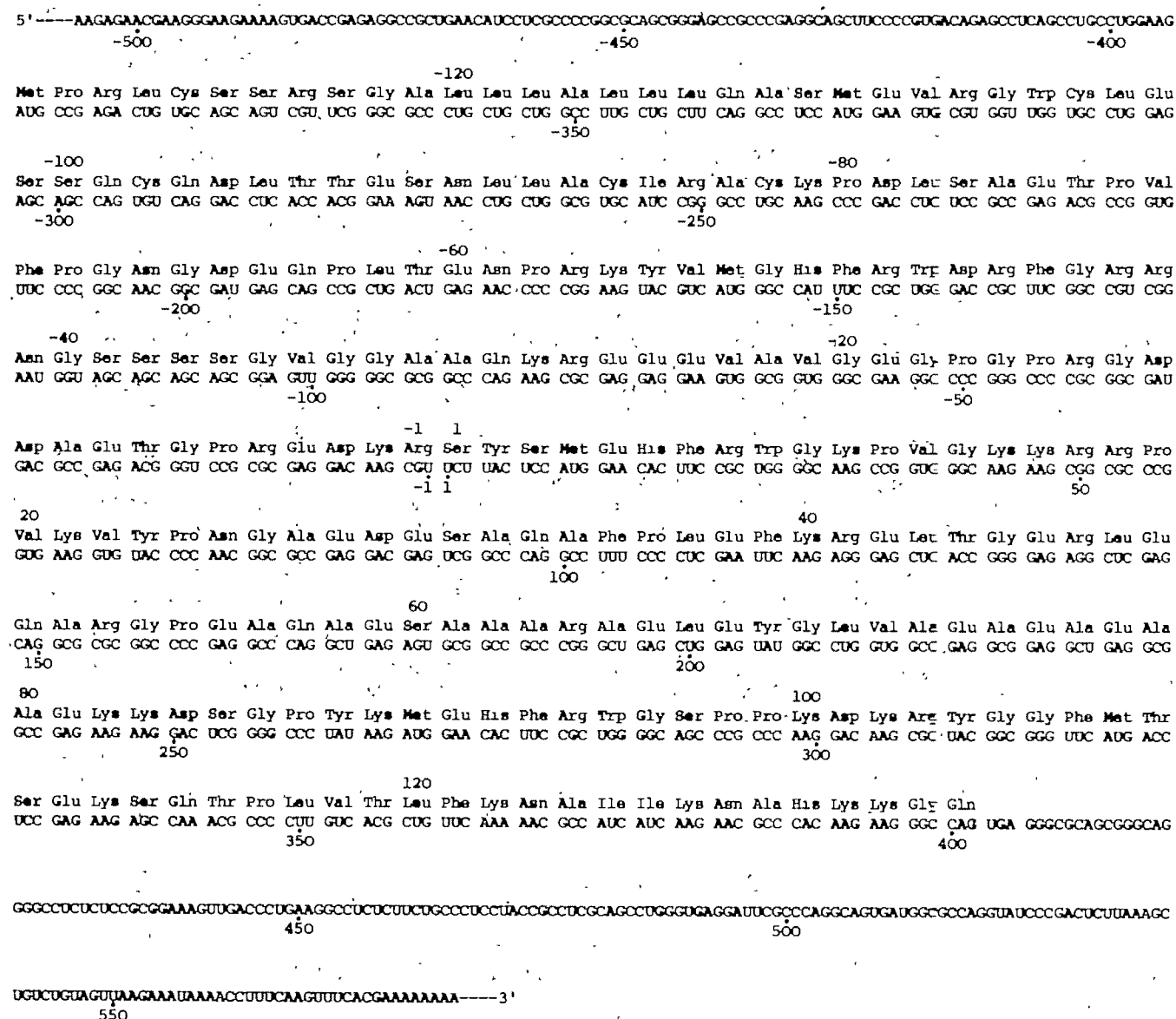


Fig. 2 Primary structure of bovine ACTH- β -LPH precursor mRNA. The nucleotide sequence of the ACTH- β -LPH precursor mRNA was deduced from that of the cDNA insert in pSNAC20. The nucleotide numbers (see legend to Fig. 1) are indicated below the sequence. The predicted amino acid sequence up to the methionine residue encoded by nucleotide residues -393 to -391 is shown above the nucleotide sequence. Amino acid residues are numbered in the direction from amino terminus to carboxyl terminus, beginning with the first residue of ACTH, and the residues on the amino-terminal side of ACTH are indicated by negative numbers, the amino acid numbers are given above the sequence. The sequence of nucleotide residues 1-207 was reported previously¹⁷ and was confirmed by sequencing additional restriction fragments as shown in Fig. 1. The cDNA insert includes a poly(A) tract of eight nucleotides at positions 578-585, but the average length of the poly(A) sequence in the mRNA was previously estimated to be 68 nucleotides.

methionine and tryptophan residues at the positions characteristic of the MSH structure and shares with α -MSH serine and glutamic acid residues at equivalent positions. Computer analysis indicates no structural correspondence of other amino acid sequences in the cryptic portion of the precursor with hormones of known sequence³⁹.

From the findings described above, we conclude that the ACTH- β -LPH precursor protein is probably composed of four repetitive structures as shown in Fig. 4. Each unit contains a homologous peptide core segment having an MSH sequence or its analogue, which is followed by or lies within an individually different sequence. Furthermore, each of the repetitive units, as well as the MSH sequences themselves, is flanked on both sides by a pair of basic amino acid residues (or by one of the termini of the precursor molecule). The putative structure of the common precursor protein suggests that each of its units is destined to be separated from its neighbour by proteolytic processing and that further cleavage occurs within the unit to yield smaller peptides.

The peptide fragment consisting of amino acid residues -131 to -58 includes five cysteine residues, and the remainder of the precursor molecule contains no cysteine residue. Other peptide hormones, such as growth hormone and insulin, contain an even number of cysteine residues which form disulphide bonds³⁹. Thus, we can assume that the cysteine residue at position -127 is a constituent of the putative signal peptide, which is removed during the process of secretion, although we cannot exclude the possibility that an odd number of cysteine residues are encoded by the missing portion of the mRNA.

The repetitive nature of the amino acid sequence of the ACTH- β -LPH precursor molecule raises interesting questions concerning both the biological significance of its characteristic structure and the biological functions of possible peptide fragments that may be derived from it. The presence of several partly homologous units within the same molecule suggests that the functions of the component peptides may be related to or coordinated with one another. It is well established that ACTH is essential for the induction and maintenance of glucocorticoid production in adrenocortical cells⁴⁰. However, additional pituitary effects on adrenocortical cells, such as stimulation of mitogenic activity and mineralocorticoid production, cannot be accounted for solely by the action of ACTH⁴⁰. It is an intriguing hypothesis that other peptides which may be elaborated from the precursor molecule are involved in these actions. Another possibility is that these structurally related peptides are involved in neural functions. Several studies have demonstrated that not only endorphins and methionine-enkephalin⁴¹ but also MSHs and related peptides^{42,43} are present in various regions of the central nervous system. In addition to the potent opiate activity of endorphins and methionine-enkephalin⁴⁴, MSH-related peptides have been reported to exert certain effects on brain function, such as learning and retention of new behaviour⁴⁵. Thus, it is attractive to speculate that various peptides derived from the ACTH- β -LPH precursor may act together or antagonistically as modulators of neural functions.

Table 1 Codon usage in bovine ACTH- β -LPH precursor mRNA

Pro	UUU	1	Ser	UCU	1	Tyr	UAU	2	Cys	UGU	1
	UUC	8		UCC	4		UAC	4		UGC	4
Leu	UUA	0		UCA	0	Ter	UAA	0	Ter	UGA	1
	UUG	1		UCG	3		UAG	0	Trp	UGG	4
Leu	CUU	2	Pro	CCU	0	His	CAU	1	Arg	CGU	4
	CUC	4		CCC	11		CAC	3		CGC	10
	CUA	0		CCA	0	Gln	CAA	1		CGA	0
	CUG	13		CCG	7		CAG	9		CGG	5
Ile	AUU	0	Thr	ACU	1	Asn	AAU	1	Ser	AGU	3
	AUC	3		ACC	3		AAC	6		AGC	9
	AUA	0		ACA	0	Lys	AAA	1	Arg	AGA	1
Met	AUG	6		ACG	5		AAG	18		AGG	2
Val	GUU	1	Ala	GCU	3	Asp	GAU	2	Gly	GGU	3
	GUC	2		GCC	16		GAC	8		GGC	17
	GUA	0		GCA	0	Gln	GAA	7		GGA	1
	GUG	8		GCG	8		GAG	22		GGG	5

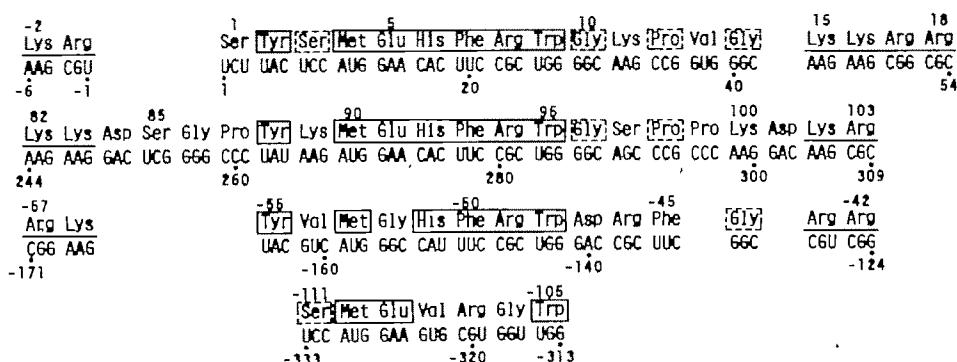
The amino acid sequence of the bovine ACTH- β -LPH precursor has been predicted solely from the nucleotide sequence of the mRNA, assuming that the translational initiation site is located at the methionine residue at position -131. Numbers next to codons indicate the numbers of amino acids using particular codons.

Characteristic features of ACTH- β -LPH precursor mRNA

We have previously observed that the coding regions for ACTH and the first segment of β -LPH contain an unusually large number of repeated nucleotide sequences, suggesting that these regions of the structural gene for the ACTH- β -LPH precursor have evolved by a series of genetic duplications¹⁷. In the present study, this observation has been extended to the remaining portions of the ACTH- β -LPH precursor mRNA. Comparison of the coding regions for α -MSH, β -MSH and γ -MSH strongly supports the view that DNA sequence duplications have occurred during the evolution of these genes (Fig. 3). The nucleotide sequences encoding the common amino acids are conserved, except for a very few silent codon changes; however, those coding for the amino acids that are different in the three peptides are drastically altered. In addition to showing the extensive nucleotide sequence homology just mentioned, computer analysis^{46,47} of the nucleotide sequence data presented in Fig. 2 reveals that the ACTH- β -LPH precursor mRNA contains other segments that seem to represent direct duplications; for example, residues -453 to -443 and 406 to 417; -93 to -82 and 235 to 246; -65 to -54 and 35 to 45; -11 to -2 and 299 to 308; 163 to 187 and 220 to 241; 400 to 411 and 506 to 517. Taken together, these findings support our concept that the structural gene for the ACTH- β -LPH precursor protein has evolved by a series of direct duplications of certain common ancestral DNA segments followed by substitution, addition or deletion of some regions.

Codon choices for the ACTH- β -LPH precursor mRNA have been assigned (Table 1), using the amino acid sequence shown in Fig. 2. No definitive conclusion can be reached concerning codon utilisation because the initiation site for translation has not been established. Nevertheless, codon usage seems to occur nonrandomly, as in the case of other eukaryotic mRNAs²¹⁻²⁵, and specific codon choices seem to be strongly preferred for

Fig. 3 Comparison of nucleotide and amino acid sequences in the regions including MSHs and their analogues. The amino acid sequences are lined up, introducing gaps so as to point out the homologous domains in the text, for the numbering of nucleotide and amino acid residues, see legends to Figs 1 and 2. The amino acid residues in solid-line boxes occur in at least three of the four structures shown and those in dashed-line boxes are found in two of them. Sequences of two or four consecutive basic amino acid residues are underlined. α -MSH³⁹ represents amino acid residues 1-13, of which the serine residue at position 1 is N-acetylated and the valine residue at position 13 is in the form of an amide, β -MSH³⁹ represents amino acid residues 54-



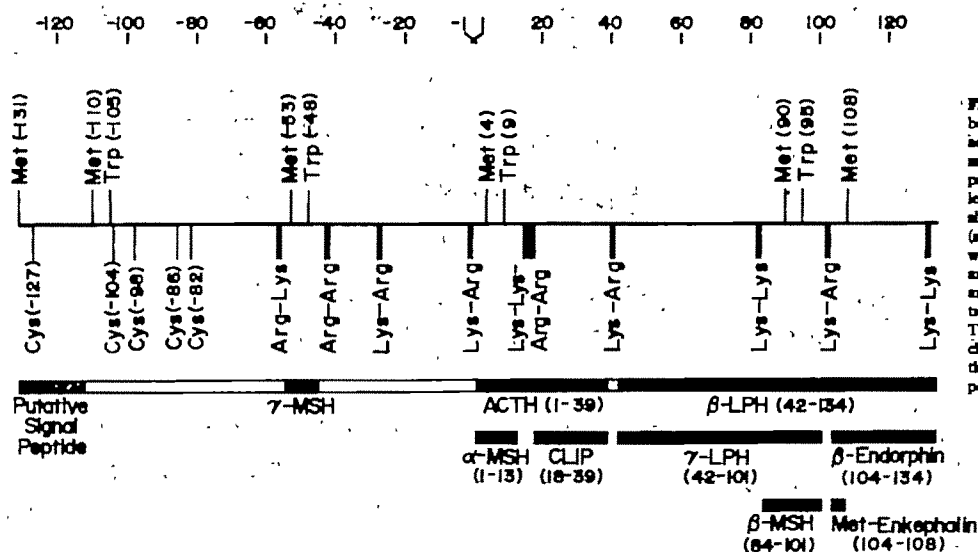


Fig. 4 Schematic representation of the structure of bovine ACTH- β -LPH precursor. Characteristic amino acid residues are shown, and the positions of the methionine, tryptophan and cysteine residues are given in parentheses, for the numbering of amino acid residues, see legend to Fig. 2. The location of the translational initiation site at the methionine residue at position -131 is assumed (see the text). The closed bars represent the regions for which the amino acid sequence was known, and the open and the shaded bars represent the regions for which the amino acid sequence has been predicted from the nucleotide sequence of the ACTH- β -LPH precursor mRNA. The locations of known component peptides are shown by closed bars; the amino acid numbers are given in parentheses. The locations of γ -MSH and the putative signal peptide are indicated by shaded bars; the termini of these peptides are not definitive.

some amino acids. Certain codons (for example, UUC for Phe, CUG for Leu, AAG for Lys) are used repeatedly, whereas other codon choices are either not made or are rare (for example, UUA for Leu, AUA for Ile, GUA for Val). This mode of codon selection reflects an apparent preference for G or C of the third position of mammalian codons²¹⁻²⁴. Assuming that the initiation site is located at the methionine residue at position -131, 116 codons (44%) terminate in G, 112 (42%) in C, 26 (10%) in U and 11 (4%) in A.

Reflecting the selective codon usage, the ACTH- β -LPH precursor mRNA (excluding the poly(A) region) has a high G+C content (65%) which exceeds that of total bovine DNA (39%)⁴⁸. Also, this mRNA exhibits nonrandom dinucleotide frequencies. The abundance of the dinucleotide CG in this mRNA exhibits a marked contrast to its deficiency in other eukaryotic mRNAs²¹⁻²⁵ as well as in total bovine DNA⁴⁹.

The 3' noncoding region of the ACTH- β -LPH precursor mRNA also shares some features with other eukaryotic mRNAs^{21-25,50}. A lengthy inverted repeat (that is, region of two-fold rotational symmetry) exists at nucleotide residues 469-

492 and 521-497, and this inverted repeat follows two in-frame copies of the nonsense codon UGA. Similar sequence features have been reported for the 3' noncoding end of human chorionic somatomammotropin mRNA⁵¹. Also, the sequence AAUAAA (residues 555-560), which is commonly present near the 3' noncoding end of eukaryotic mRNAs, is found in the corresponding region of the ACTH- β -LPH precursor mRNA.

The sequencing data are available from the authors on request.

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Organisation and sequences at the 5' end of a cloned complete ovalbumin gene

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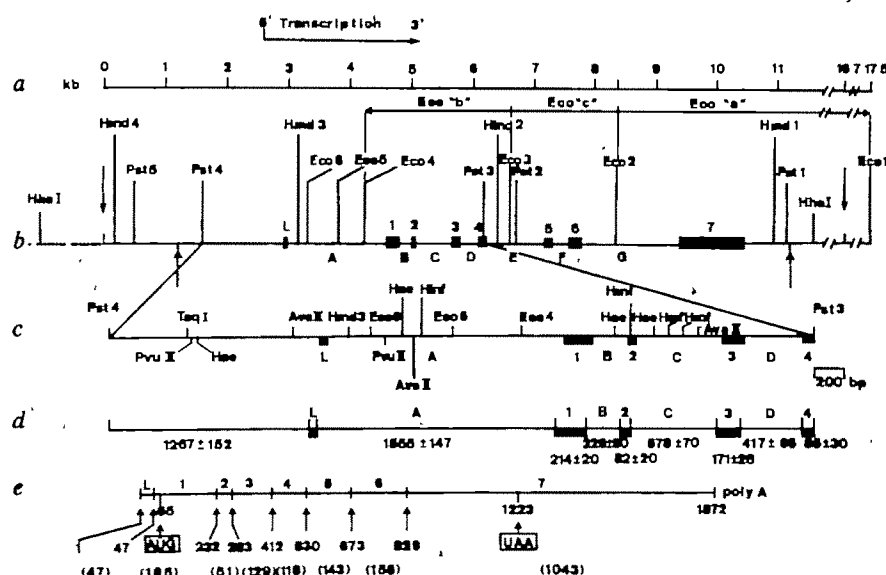
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A clone which contains the complete chicken ovalbumin gene, including its leader coding sequences, has been isolated. From electron microscopic analysis of this DNA we conclude that the minimal size of the transcriptional unit for ovalbumin is 7.7 kilobases. The DNA sequence of the region surrounding the 5' end of the ovalbumin gene is presented. Comparison of this sequence with those of other eukaryotic genes reveals striking similarities, possibly related to a promoter region, approximately 30 base pairs upstream from the site coding for the 5' end of the mRNA.

coding regions (exons 1 to 7) are contained in a DNA region of about 6 kilobases and are separated by six introns (B to G) (refs 6–8, and Fig. 1b). A similar conclusion has been reached by Dugaiczky *et al.*⁹. However, the first 45 nucleotides at the 5' end of the 1,872-nucleotide long ov-mRNA^{10,11} are not encoded by sequences contained in exon 1 or in *Eco*RI fragment 'b' upstream from exon 1 (ref. 12). We have called¹² this untranslated sequence of 45 nucleotides the ovalbumin leader, by analogy with the viral terminology. We have assumed that the leader-coding sequences are located upstream from the *Eco*RI site *Eco*4 and are separated from exon 1 by an additional intron A (ref. 12 and Fig. 1b). Thus, the determination of the localisation of the DNA segment containing the leader-coding sequences and its purification are prerequisites for the study of the molecular mechanisms involved in the transcription of the ovalbumin gene and its hormonal control. We report here the isolation of chicken genomic DNA clones which contain a complete ovalbumin gene including the region coding for the ov-mRNA leader. The position of the leader-coding sequences has been determined by electron microscopy and DNA

DUE to molecular cloning, our knowledge of the structure of the chicken ovalbumin gene has advanced rapidly since we first reported that this gene is split¹. The ovalbumin genomic *Eco*RI fragments (*Eco* 'a', *Eco* 'b' and *Eco* 'c', Fig. 1b) have been cloned and analysed^{2–3}. Seven ovalbumin mRNA (ov-mRNA)

Fig. 1 Organisation of the ovalbumin gene. a, Scale in kilobase pairs for (b). b, Localisation of the exons (1 to 7, heavy lines) and introns (A to G) of the ovalbumin gene in λ C4-ov5 and λ C4-ov6 within and upstream from the *Eco*RI fragments *Eco* 'a', *Eco* 'b' and *Eco* 'c'. The horizontal dotted line represents λ Charon 4A sequences. The uninterrupted and dashed arrows indicate the limits of the 16.7 and 9.8 kilobase genomic DNA fragments inserted in λ C4-ov5 and λ C4-ov6, respectively. L corresponds to the location of the leader-coding sequences as determined by electron microscopy and DNA sequencing (see text). The *Eco*, *Pst*, *Hind* and *Hha*I sites correspond to *Eco*RI, *Pst*I, *Hind*III and *Hha*I restriction enzyme sites which were mapped in this study or previously (refs 7, 12). c, Localisation of *Pvu*II, *Taq*I, *Hae*III (*Hae*), *Apa*II, *Hind*III (*Hind*), *Eco*RI (*Eco*) and *Hinf*I (*Hinf*) restriction enzyme sites within the *Pst*3–*Pst*4 fragment of b. L, A, B, C, D, 1, 2, 3 and 4 are as described for b. d, Schematic representation of the electron microscopic result shown in Fig. 3c and correlation with the restriction enzyme map shown under c. The heavy lines L, 1, 2, 3 and 4 represent the RNA-DNA hybrid regions corresponding to the leader-coding regions (L) and to exons 1 to 4, respectively. A, B, C and D correspond to the intronic single-stranded loops A, B, C and D, respectively. The numbers correspond to base pairs or nucleotides and were calculated from measurement of 51 molecules of the type shown in Fig. 3c. e, Schematic representation of ov-mRNA. The total length and the position of the AUG and UAA codons are taken from refs 10–12. L refers to the leader sequence and 1 to 7 correspond to the seven domains of ov-mRNA coded by exons 1 to 7. The arrows indicate the limits of these domains, assuming that the splicing events obey the GT-AG rule (see ref. 12 and text). Numbers in parentheses indicate the length (in nucleotides) of the domains.



sequence analysis. The sequences preceding those coding for the 5' end of ov-mRNA have also been determined and compared with those of other genes in a search for possible regulatory signals.

Isolation and mapping of ovalbumin clones containing sequences upstream from exon 1

A chicken erythrocyte DNA library has been constructed by J. Dodgson, D. Engel and R. Axel using a method described by Maniatis *et al.*¹³. Briefly, this approach consists of partially digesting the DNA by *HaeIII* and *AluI* restriction enzymes, selecting DNA fragments which are 15–20 kilobases long, ligating these fragments to the vector λ Charon 4A using *EcoRI* linkers, and infecting *Escherichia coli*. As the recognition sites for both *HaeIII* and *AluI* occur frequently, a partial digest by these enzymes is analogous to random shearing of the DNA. This library was made available to us, with the indication that it contained 400,000 independent clones.

To limit the number of clones analysed to those most likely to contain the 5' region of the ovalbumin gene, we screened² the library using a nick-translated ³²P-labelled *Eco4-Hind2* fragment (see Fig. 1b) as probe. Four clones containing ovalbumin sequences were isolated by screening about 2×10^6 plaques. Two of these (λ C4-ov5 and λ C4-ov6) were shown by preliminary mapping experiments to contain previously uncloned DNA, upstream from *EcoRI* fragment b, and were selected for further analysis.

We have previously shown^{1,7} that the ovalbumin sequences containing exons 1 to 7 are found in two *HindIII* fragments of about 4.6 and 3.2 kilobases (defined by the *HindIII* sites, *Hind1*, *Hind2* and *Hind3* in Fig. 1b) and in two major *PstI* fragments each of about 4.5–4.6 kilobases (defined by *PstI* sites, *Pst1-Pst2* and *Pst3-Pst4*, Fig. 1b). The DNA of λ C4-ov5 and 6 was digested with *HindIII*, transferred to nitrocellulose filters and hybridised with the ³²P-labelled ovalbumin double-stranded cDNA probe (*Hhaov*), as previously described¹. As shown in Fig. 2A (lanes 2 and 5), both clones contain the two *HindIII* fragments. Only one strongly hybridising band, corresponding to the two large *PstI* fragments, was seen (see legend to Fig. 2A) when the clones were digested with *PstI* (lanes 3 and 6). From these results we conclude that both clones contain all the DNA between sites *Pst1* and *Pst4* (Fig. 1b). In agreement with these results, *EcoRI* fragments 'b' (2.35 kilobases) and 'c' (1.75 kilobases) are present in both clones (Fig. 2A, lanes 1 and 4). However, the 9.2-kilobase fragment *EcoRI* 'a' was replaced in λ C4-ov5 by a 8.4-kilobase band (Fig. 2A, lane 4) and in λ C4-ov6 by a 2.8-kilobase band (lane 1). From these latter results we conclude that the limits of the inserted DNAs at their 3' end are as indicated by arrows at the right-hand side of Fig. 1b.

The DNA of the two clones was digested with *EcoRI* and the fragments stained with ethidium bromide after agarose gel electrophoresis. The results are shown in Fig. 2B. In addition to the two large arms of λ Charon 4A, (bands a and b), fragments of 8.4, 3.2, 2.35, 1.75, 0.5 and 0.45 kilobases were found in the digest of λ C4-ov5 (Fig. 2B, lane 3), whereas fragments of 2.8, 2.5, 2.35, 1.75 and 0.45 kilobases were present in the λ C4-ov6 digest (lane 2). The *EcoRI* fragments of 8.2 (λ C4-ov5), 2.8 (λ C4-ov6), 2.35 and 1.75 kilobases have already been mapped (see above and Fig. 1b). The above results indicate that the other *EcoRI* fragments are necessarily located on the 5' side of the *Eco4* site. Their relative positions in the λ C4-ov5 '*Pst3-Pst4*' fragment (Fig. 1c) which was sub-cloned in pBR 322, were derived by further mapping with *TaqI*, *PvuII*, *HaeIII*, *AvaiI*, *HinfI*, *HindIII* and *EcoRI* enzymes (not shown). We found that the *Pst3-Pst4* fragment contains, in addition to the already known *Eco4* site, two *EcoRI* sites, *Eco5* and *Eco6*, at 0.45 and 0.95 kilobases from *Eco4*, respectively (Fig. 1c). Therefore, there are 3.2 kilobases between *Eco6* to the 5' end of the inserted DNA (Fig. 1b, arrow) to which the *EcoRI* linker was added. Parallel experiments showed that the *EcoRI* site *Eco6* is missing in the DNA fragment inserted in clone λ C4-ov6, which

probably corresponds to an additional allelic form of the ovalbumin gene^{3-5,7,9}. From the size of the *EcoRI* fragments of clone λ C4-ov6, we conclude that the 5' end of the inserted DNA is 2.5 kilobases from *Eco5* (Fig. 1b, dashed arrow).

These analyses indicate that the sizes of the chicken DNA fragments inserted in clones λ C4-ov5 and 6 are 16.7 and 9.8 kilobases, respectively (Fig. 1b). In λ C4-ov5 there are about 10.3 kilobases separating the 5' end of the inserted fragment from the extremity of exon 7, which codes for the 3' end of ov-mRNA. The corresponding distance is 9.2 kilobases in clone λ C4-ov6. Considering these sizes, we expected that these clones should contain the sequences coding for the ov-mRNA leader.

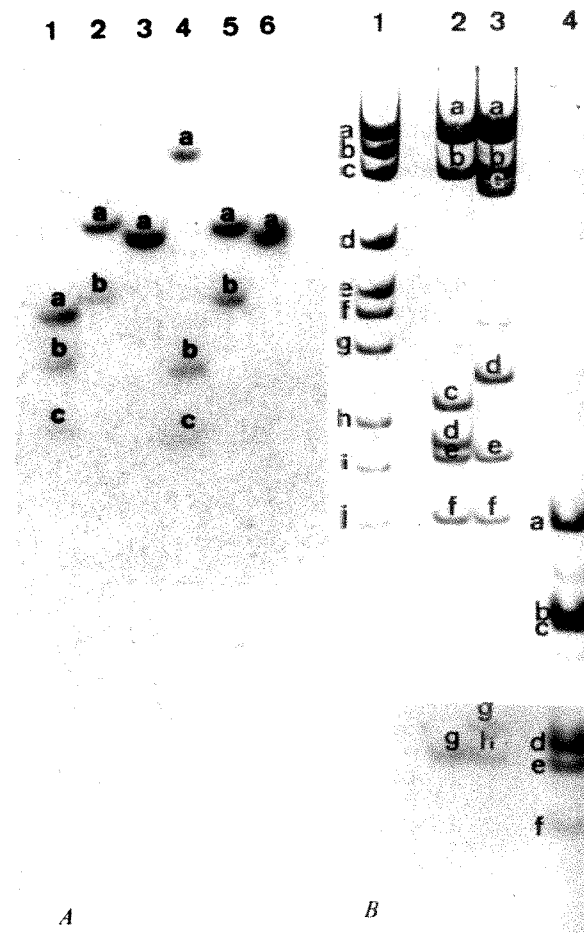
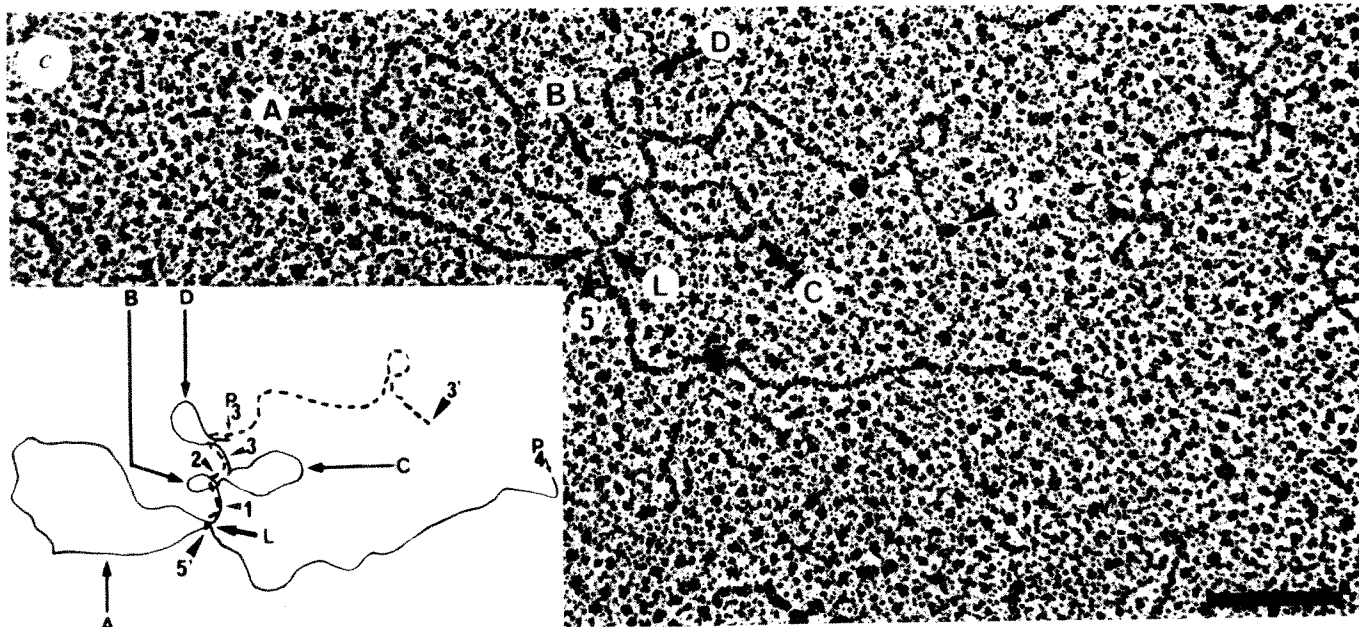
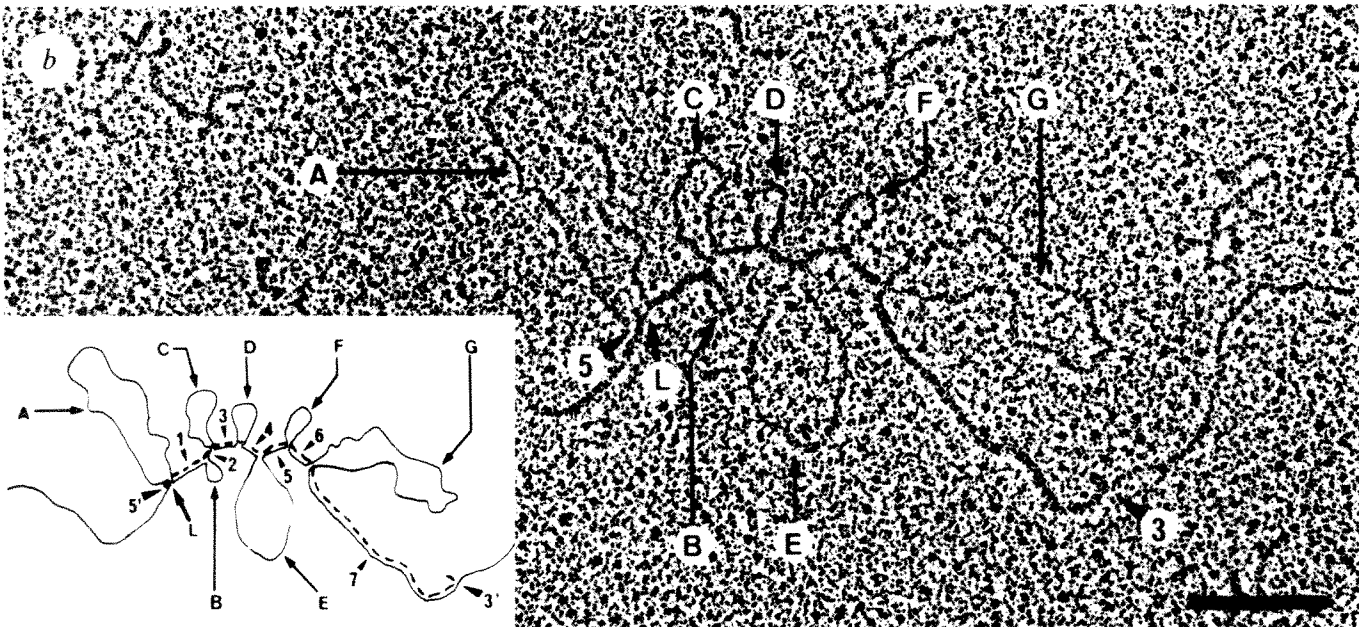
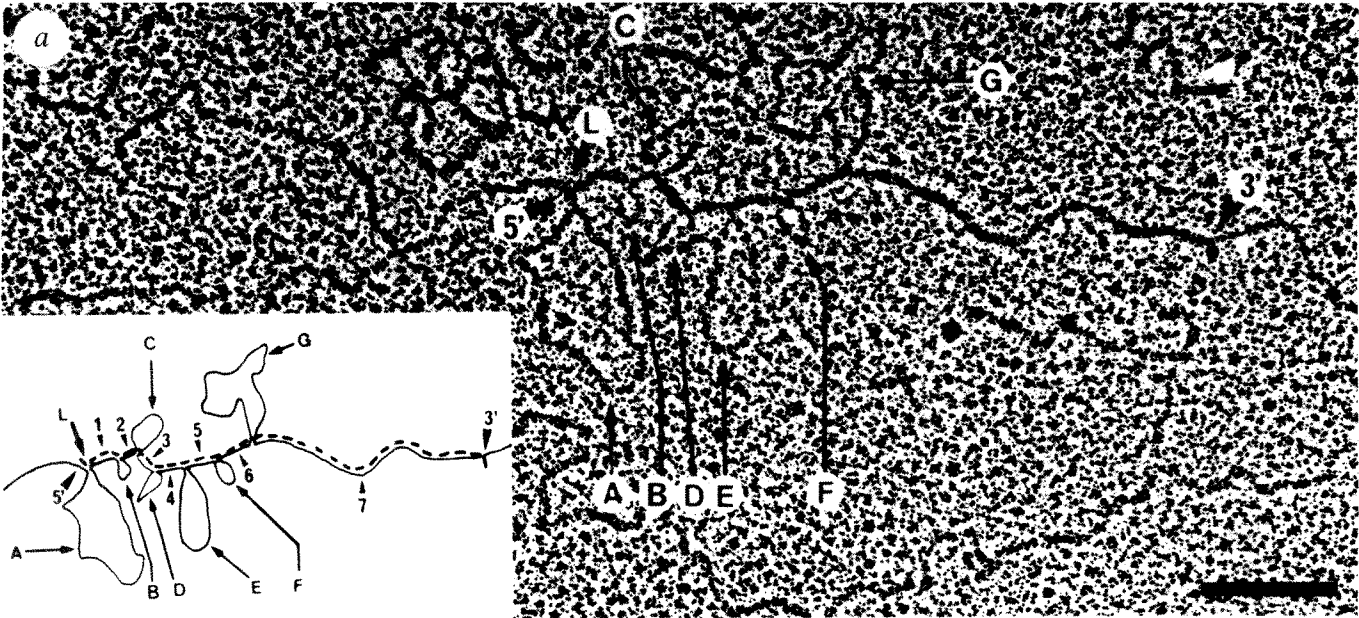


Fig. 2 A, Detection of restriction enzyme fragments which hybridise to the double-stranded ovalbumin cDNA probe ³²P-*Hhaov* (ref. 1), in *EcoRI*, *PstI* and *HindIII* digests of λ C4-ov5 and λ C4-ov6 DNAs. The DNA was digested, electrophoresed on a 1% agarose gel, transferred onto nitrocellulose filters, hybridised with a ³²P-*Hhaov* probe and revealed by autoradiography as previously described¹. Lanes 1, 2 and 3: *EcoRI*, *HindIII* and *PstI* digests of λ C4-ov6 DNA, respectively. Lanes 4, 5 and 6: *EcoRI*, *HindIII* and *PstI* digests of λ C4-ov5 DNA, respectively. Sizes of the hybridising fragments (in kilobases): lane 1, 2.8 (a), 2.35 (b) and 1.75 (c); lanes 2 and 5, 4.5 (a) and 3.2 (b); lanes 3 and 6, 4.5–4.6 (a) (the two fragments of 4.55 and 4.4 kilobases, which were clearly seen on the same gel after ethidium bromide staining, were poorly resolved on the autoradiogram); lane 4, 8.4 (a), 2.35 (b) and 1.75 (c). B, Detection of *EcoRI* restriction enzyme fragments of λ C4-ov5 and λ C4-ov6 DNAs by staining with ethidium bromide after electrophoresis on a 0.8% agarose gel. Lane 1, *BamHI* and *EcoRI* restriction enzyme fragments of adenovirus-2 DNA used as size markers (in kilobases): 21.05 (a), 14.6 (b), 10.4 (c), 6.3 (d), 4.7 (e), 4.4 (f), 3.71 (g), 2.9 (h), 2.05 (i) and 1.87 (j). Lane 2, *EcoRI* digest of λ C4-ov6 DNA; size of the fragments (in kilobases): 19 (a), 12 (b), 2.8 (c), 2.5 (d), 2.35 (e), 1.75 (f) and 0.45 (g). Lane 3, *EcoRI* digest of λ C4-ov5 DNA; size of the fragments: 19 (a), 12 (b), 8.4 (c), 3.2 (d), 2.35 (e), 1.75 (f), 0.55 (g) and 0.45 (h). Lane 4, SV40 DNA fragments of a partial *HindIII* digest used as size markers (in kilobases): 1.77 (a), 1.15 (b), 1.1 (c), 0.52 (d), 0.45 (e) and 0.22 (f). To show clearly bands g and h (lanes 2 and 3) the lower part of the figure was exposed for a longer time.



Electron microscopic mapping of the leader-coding sequences

The heat-denatured DNAs of λ C4-ov5 and 6 were hybridised to purified ov-mRNA at 54.3°C in the presence of formamide in conditions favouring the formation of RNA-DNA hybrids^{2-4,14}. Figure 3a and b are electron micrographs of the hybrid molecules obtained with λ C4-ov5 and λ C4-ov6, respectively. These micrographs show an RNA-DNA hybrid region of $1,893 \pm 114$ base pairs and seven single-stranded DNA loops. The same results were obtained with both clones. The size and relative positions of the DNA loops B to G and of the RNA-DNA hybrid regions 1 to 7 (see line drawings) show that they correspond to the region of the chicken genome which contains the ovalbumin exons 1 to 7 and introns B to G which we have described previously (refs 3-6, 12, and Fig. 1b). The additional single-stranded DNA loop, which was seen in most of the hybrid molecules, must therefore correspond to intron A which was expected to separate exon 1 from the DNA region coding for the ovalbumin mRNA leader.

To map the position of the DNA sequence coding for the leader segment, measurements were made on electron micrographs of hybrid molecules between the *Pst*3-*Pst*4 DNA fragment (see above) and ov-mRNA (Fig. 3c). These results are shown in Fig. 1d and are aligned with the corresponding restriction enzyme map (Fig. 1c). The size of loop A positions the leader-coding sequence at $1,555 \pm 147$ base pairs from the 5' end of exon 1 and $1,267 \pm 152$ base pairs from the *Pst*4 site. In addition, measurements of hybrid molecules similar to those shown in Fig. 3a, b indicate that there are about 7.7 kilobases between the 5' end of loop A and the 3' end of exon 7 (see legend to Fig. 3a).

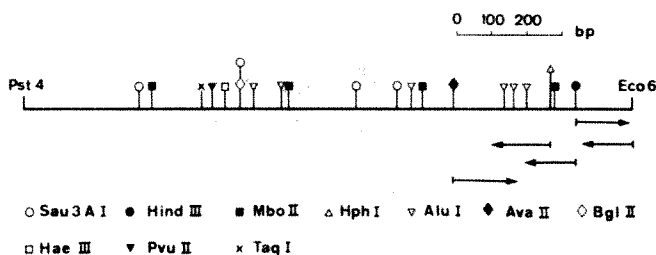


Fig. 4 Strategy for DNA sequencing in the leader-coding region. The detailed restriction map of the 1,730-base pair *Eco*RI-*Pst*I (*Eco*6-*Pst*4, Fig. 1c) fragment of clone (λ C4-ov5) was established using the technique of Smith and Birnstiel¹⁴ and by digestion of the fragment with various enzymes (indicated), either singly or in combination. The arrows below the sequence show the direction and extent of the sequence determinations.

Although the 5' end of the DNA-RNA hybrid region, responsible for the presence of loop A, was not actually resolved in electron micrographs, because of the small size of the leader segment, the consistency of the above measurements indicates the existence of a specific hybrid region. Moreover, this region must involve ov-mRNA, as no similar loops were obtained with the DNA alone. It is unlikely that the presence of this hybrid region is related to some DNA rearrangement which could have occurred during the cloning, because the same results were obtained with these two λ clones and with the cosmid clone



Fig. 5 Nucleotide sequence surrounding the ovalbumin mRNA leader-coding region and the 5' end of exon 1. The sequences surrounding the leader-coding region were established as outlined in Fig. 4 using the method of Maxam and Gilbert¹⁶ and exemplified in Figs 6 and 7. The sequence around the intron A-exon 1 junction was taken from ref. 12 and our unpublished results. The DNA sequence of the non-coding strand is aligned with the mRNA sequence¹⁰⁻¹². The positive numbers refer to the position of the nucleotides with respect to the 5' end of the mRNA. Negative numbers are given upstream from nucleotide number 1. The repeated dinucleotide AG found at the intron-exon junctions is boxed (see text). The vertical dashed line corresponds to the possible common excision-ligation point. The sequence with high complementarity to the 3' end of the 18S rRNA is indicated by a dotted line (nucleotides 25-33). The total length of intron A is about 1,500 base pairs. The *Alu*I sites predicted within the leader-coding regions from the ov-mRNA sequence are shown.

obtained independently by A. Roy *et al.*¹⁵. In addition, we did not detect any differences in the leader-coding region when we compared the restriction enzyme maps (*Hae*III, *Pvu*II and *Eco*RI/*Pst*I digests) of the cloned λ C4-ov5 and 6 DNAs with those of oviduct or erythrocyte cellular chicken DNAs (data not shown). These latter studies also indicate that no extensive sequences around the leader-coding region are repeated elsewhere in the chicken genome.

Sequences around the leader-coding region

Knowing the approximate position of the leader-coding sequences from electron microscopy, we have mapped in detail the *Pst*4-*Eco*6 region (Fig. 4). We looked for two neighbouring *Alu*I sites separated by about 20 base pairs, the existence of which is known from the sequence of the mRNA leader region¹⁰⁻¹². In fact, two such *Alu*I sites are found between the *Hind*III and *Ava*II sites (Fig. 4). We therefore sequenced this region using the technique of Maxam and Gilbert¹⁶ and our results are shown in Fig. 5. The accuracy of the sequence was ensured by sequencing both strands of the DNA, with the exception of nucleotides -60 to -50. Examples of autoradiograms are shown in Figs 6 and 7.

The sequence coding for the leader of the ovalbumin mRNA is clearly present within the sequenced region (Fig. 5), confirming the electron microscopic data. Furthermore, the results indicate that the leader-coding sequences are not interrupted in the chicken genome. This conclusion is based on the assumption that the ov-mRNA has been sequenced to its 5' extremity. However, this might not be the case, as it has not been

Fig. 3 Electron microscopy of RNA-DNA hybrids. The hybrid molecules were obtained as described in the text. a and b: electron micrographs of hybrids between ovalbumin mRNA and the DNA of λ C4-ov5 (a) and λ C4-ov6 (b). In the insets, ov-mRNA is represented by a dashed line, whereas the solid line corresponds to single-stranded DNA. 5' and 3' arrow heads: 5' and 3' ends of ov-mRNA, respectively, 1 to 7 (the thicker DNA-RNA hybrid regions) correspond to the ovalbumin gene exons, whereas A to G correspond to the seven introns. L is the hybrid region corresponding to the leader-coding sequences. The RNA-DNA hybrid region is $1,893 \pm 114$ base pairs long. The lengths of intronic loops A to G and of the exonic mRNA-DNA hybrid segments 1 to 7 are (in nucleotides or base pairs): 1,560 $\pm$ 147 (A), 238 $\pm$ 45 (B), 601 $\pm$ 35 (C), 411 $\pm$ 58 (D), 1,029 $\pm$ 71 (E), 323 $\pm$ 36 (F), 1,614 $\pm$ 88 (G), 197 $\pm$ 34 (1), 85 $\pm$ 14 (2), 164 $\pm$ 25 (3), 143 $\pm$ 18 (4), 154 $\pm$ 25 (5), 201 $\pm$ 28 (6) and 1,097 $\pm$ 51 (7), respectively. There are about 7.7 kilobases between the 5' end of loop A and the 3' end of exon 7. c: Electron micrograph of a hybrid between ov-mRNA and the cloned *Pst*3-*Pst*4 DNA fragment (see text and Fig. 1b, c). In the inset the dashed and solid lines, 5' and 3' arrow heads, L, 1, 2, 3, A, B, C and D are as described for a and b. P3 and P4 correspond to the *Pst*3 and *Pst*4 ends, respectively (Fig. 1c). The lengths (in base pairs or nucleotides) of the hybrid regions and of the single-stranded DNA loops and L-P4 segment are given in Fig. 1d. The bar corresponds to 0.1 μ m.

possible to sequence the mRNA directly from its 5' end¹⁷. The technique used by McReynolds *et al.*¹⁰ and by O'Hare *et al.*¹¹ to establish the mRNA sequence shown in Fig. 5 involves elongation of a DNA primer hybridised to the 5' end region of the messenger with reverse transcriptase in the presence of chain-terminating dideoxynucleotides. This technique could lead to premature termination if important secondary structure exists in the RNA.

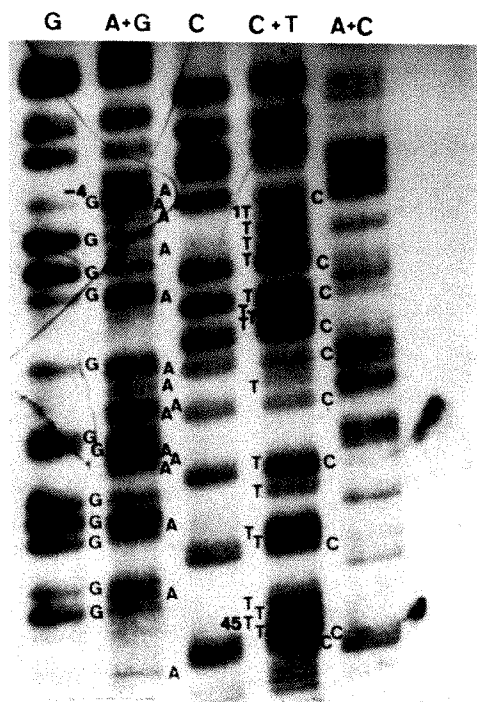


Fig. 6 Autoradiogram of sequencing gel showing the leader sequence. The sequence was read from the strand coding for ov-mRNA. An *HphI*/*HaeIII* fragment (Fig. 4) ³²P-labelled¹² at the *HphI* end was subjected to five base-specific cleavage reactions (G, A>G, C+T, C, A>C) according to the procedure of Maxam and Gilbert¹⁶. The products were analysed on a denaturing gel according to Sanger and Coulson⁴⁵. The numbers (45, 1, -4) indicate the position of the nucleotides corresponding to those shown on the non-coding strand in Fig. 5.

Sequences at the splice points

As shown in Fig. 5, the DNA sequences coding for the 5' end of ov-mRNA are interrupted at a position corresponding to nucleotide 47 of the messenger. This result agrees with our previous work which established that exon 1 does not code for the first 45 nucleotides of the mRNA¹². Nucleotides 46 and 47 (AG) of the messenger could be encoded either by the leader-coding region or by exon 1, due to the presence of the repetition of the dinucleotide AG at both ends of intron A. As previously discussed for the other ovalbumin introns¹², this prevents the definition of a unique splicing point. We have proposed that all intron-exon junctions are closely related and fit the general pattern 5'-TCAGGTA-3' (non-coding strand) at the 5' end of an intron, and 5'-TXCAGG-3' at the 3' end of the same intron¹². Others have stressed this similarity¹⁸⁻²⁰. The additional pair of junctions that we describe here is consistent with this proposal. Moreover, our present results also agree with the 'GT-AG rule' (ref. 12) which allows one to propose unique excision-ligation points common to all introns, despite the existence of the direct repeats. These points are indicated in Fig. 5 by the vertical dotted lines, with dinucleotides GT and AG at the 5' and 3' ends of intron A, respectively. Finally, as for other introns^{12,21}, intron A contains a pyrimidine-rich stretch at its 3' end on the non-coding strand.

The ovalbumin transcription unit

Assuming that no nucleotides were missed when sequencing the 5' end of ov-mRNA, our present studies establish that about 7.7 kilobases separate the genome regions coding for the 5' and 3' extremities of ovalbumin mRNA. Recent *in vivo*²² and *in vitro*²³ studies on primary transcripts from the major late transcription unit of adenovirus-2 strongly suggest that the cap site and the transcription initiation site are coincident and that the initiating residues of the primary transcript are conserved in mRNA as the capped 5' terminus. If, as for adenovirus-2, the ov-mRNA capped sequence identifies the site for the initiation of transcription, the minimal size of the ovalbumin transcription unit should be about 7.7 kilobases. A complete co-linear RNA transcript should then be in the range of 7,700 nucleotides, about four times the size of the mature mRNA. In fact, there is evidence for an ovalbumin gene transcript of approximately this size^{24,25}. Although there is no convincing evidence from these latter studies for ovalbumin RNA molecules longer than this transcript (our previous determination^{8,12} was overestimated due to a problem at that time with RNA size markers of high molecular weights), we cannot exclude the possibility that some processing has already occurred and that the ovalbumin transcription unit is longer than 7.7 kilobases.

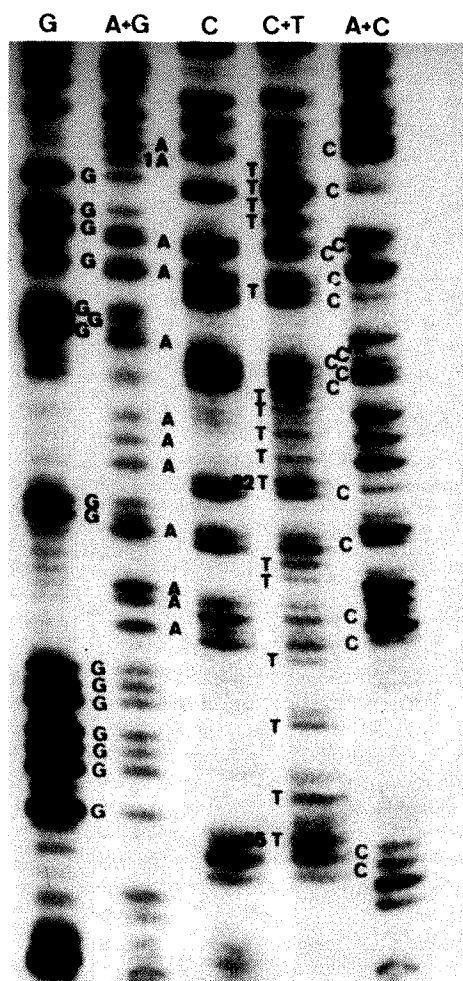


Fig. 7 Autoradiogram of sequencing gel showing the sequence upstream from the leader sequence. The sequence was read from the non-coding strand. An *AvaII*/*HindIII* fragment (Fig. 4) ³²P-labelled at the *AvaII* end¹², was sequenced as described in the legend to Fig. 6. The numbers (-55, -32, 1) correspond to the positions of the nucleotides of the non-coding strand as shown in Fig. 5. Cm corresponds to the position of a methyl C which belongs to an *EcoRII* site (G was unambiguously found at this position when the coding strand was sequenced).

Possible control regions

It has been suggested that complementarity between a purine-rich sequence, 3'-UAGGAAGGCGU-5', present at the 3' end of 18S ribosomal RNA and sequences of the 5' non-coding regions of many eukaryotic mRNAs might contribute to mRNA recognition by ribosomes²⁶. As previously noted²⁷, a sequence of nine nucleotides complementary (with one mismatch) to this 18S RNA region is found between positions 25 and 33 of the ovalbumin mRNA (Fig. 5, underlined by dotted line). This sequence is immediately preceded by a region (nucleotides 3-22) which can form a base-paired loop¹⁰. There is no such extensive complementarity with the 3' end of 18S rRNA in the messenger nucleotide sequence located between position 48 and the AUG initiation codon (position 65) and encoded for by exon 1. One can therefore speculate that one of the functions of splicing in the 5' non-coding region would be to transpose such a sequence to the vicinity of the initiation codon. A similar translocation has been described for the adenovirus tripartite leader^{22,28}.

A promoter is functionally defined in prokaryotes as the region of the DNA which precedes a transcription unit and indicates the startpoint for RNA polymerase. Most sequenced prokaryotic promoters have two common interacting sites, the Pribnow box²⁹ and the recognition site³⁰ (for other refs, see refs 31-33). The Pribnow box, which is of the form 5'-TATAATG-3', precedes the transcription-initiation site by five to seven nucleotides and belongs to the model sequence (ref. 32)



where the upper and lower case letters correspond to bases present at high and moderate frequency, respectively (the lower line shows the bases which are most often found as alternatives). The recognition site is a region centred at about 32 base pairs before the beginning of the mRNA and has a structure clearly related to the sequence 5'-TGTTGACATTT-3' (refs 30, 32, 33). Assuming that the ovalbumin mRNA cap site and the startpoint for transcription are coincident, sites analogous to the prokaryotic common interacting promoter sites could be contained within the region upstream from the leader-coding sequence. In a search for such sequences, we have compared the DNA sequences upstream from the cap site for ovalbumin, rabbit β -globin (J. Van der Berg *et al.*; A. Efstratiadis, T. Maniatis and L. Lacy; personal communications), mouse β -globin major³⁴ and adenovirus-2 major late²² genes. When these sequences are aligned so as to emphasise sequence homologies as shown in Fig. 8, there is clearly a 7-base pair AT-rich region of approximate homology centred at about 28 base pairs from the initial nucleotide of the mRNAs. This region of homology, which may be a common interacting promoter site, is very similar to the 'Hogness box' which is of the form 5'-TATAAATA-3' (D. Hogness, personal communication) and has been found in an analogous location upstream from the startpoint of transcription of several genes including the histone genes³⁵⁻³⁷. No similar sequence exists in analogous positions upstream from the 5' end of any of the other ovalbumin exons (ref. 12, and our unpublished results). Note that, in all four cases compared, the AT-rich region of homology is flanked by GC-rich segments (Fig. 8), suggesting that the 'Hogness box' could be extended by three or four residues. There is some resemblance between this eukaryotic region of homology and the prokaryotic model sequence which has the Pribnow sequence at one end (Fig. 8), suggesting that prokaryotic and eukaryotic promoters could have some common features. However, the 'Hogness box' is in a position more closely resembling that of the prokaryotic recognition site than that of the Pribnow box. In this respect, it is interesting that the recognition site of the *E. coli lac* promoter centred at about -30 is bounded on both sides by GC-rich sequences³⁸.

There is a striking similarity between the 10 residues which precede the first nucleotide of adenovirus-2 late major and

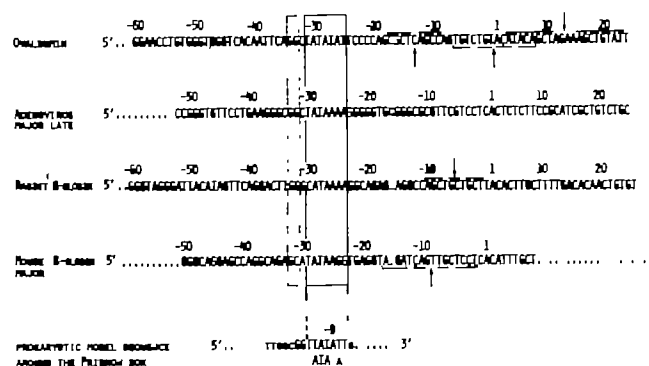


Fig. 8 Comparison between DNA sequences of ovalbumin, rabbit β -globin, mouse β -globin major³⁴ and adenovirus-2 major late²² genes upstream from the 5' end of the mRNAs. The sequences are aligned to show their similarities. Numbering is in base pairs and negative numbers are given to nucleotides upstream from the 5' end of the mRNAs (position 1). The AT-rich region of homology is boxed with a continuous line. The GC-rich region of homology is boxed with a dashed line. Lines above or below the sequences delineate regions of partial two-fold rotational symmetry. Centres of symmetry are indicated by arrows. The prokaryotic promoter model sequence³² which has the Pribnow box at one end is given for comparison (the upper and lower case letters correspond to bases present at high and moderate frequency, respectively).

mouse β -globin major mRNAs, as previously noted by Ziff and Evans²². However, this homology does not extend to the other genes included in Fig. 8. Additional sequence data are required to decide whether there is a second, but weaker, region of homology with a location similar to that of the Pribnow box.

Regions of partial (hyphenated) two-fold symmetry are found around the probable start of transcription of the ovalbumin gene downstream from the possible RNA polymerase interacting site centred at about -28 (Fig. 8). Regions of hyphenated two-fold symmetry are also present in a similar location in the rabbit and mouse β -globin genes, but are absent in the case of adenovirus (Fig. 8). Elements of complete or partial two-fold rotational symmetry are found in prokaryotic operators and at other sites controlling prokaryotic transcription (refs 30, 32, 38-43). Further studies will reveal whether specific regulatory proteins, possibly related to the steroid hormone induction system, could bind to these symmetrical sequences which surround the probable ovalbumin transcription initiation site.

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letters

GX339-4: a new black hole candidate

THE large area survey instrument on HEAO 1 has observed that the galactic X-ray source GX339-4 (4U1658-48) varies on time scales from tens of milliseconds to hundreds of days. Its long term variability was known from measurements made by OSO 7¹ and by Uhuru² but this is the first observation of aperiodic variability on time scales of tens of milliseconds to a few seconds. We show here that the overall characteristics of GX339-4 place it in the same class of X-ray sources as Cyg X-1 (ref. 3 summarises Cyg X-1) and Cir X-1^{2,4-6}.

The HEAO observations were made on 10-14 September 1977, 8-12 March 1978 and 10-14 September 1978. The average intensities in the 1-20 keV range of GX339-4 during these observations were ~ 0.25 , ~ 0.01 , and ~ 0.8 ct cm⁻² s⁻¹, respectively (1 HEAO ct cm⁻² s⁻¹ = 200 Uhuru flux units).

These values are approximate because the source was highly variable during the September 1977 observation, and because only a few quick-look observations were used to determine the March 1978 and September 1978 intensities. The first two intensities correspond to the 'low' and 'off' states defined by OSO 7¹. This is the first observation of detectable emission during the 'off' state. The third intensity falls between the 'low' and 'high' state values.

Detailed analysis has been made of ~ 200 s of data from the 'low' state observation in September 1977. GX339-4 was observed 20 times, ~ 10 s each, as the HEAO collimators ($1^\circ \times 4^\circ$ FWHM) scanned across the source. Fifteen of these scans were made with a time resolution of 320 ms and five were made with a resolution of 5 ms.

Figure 1 shows the intensity profile of GX339-4 observed with 320 ms resolution during three scans separated in time by 10 h and 18 h. The source intensities shown have been corrected

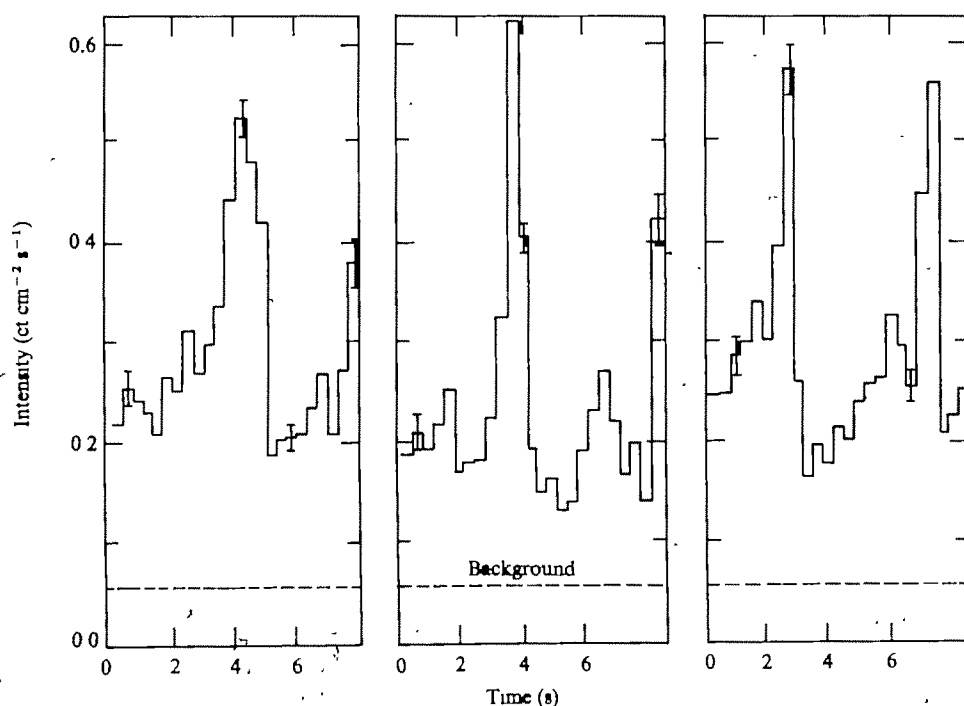


Fig. 1 Three scans through GX339-4 at 0.32 s resolution. Source intensities are plotted corrected for collimator response and with background subtracted. The background levels are shown for comparison.

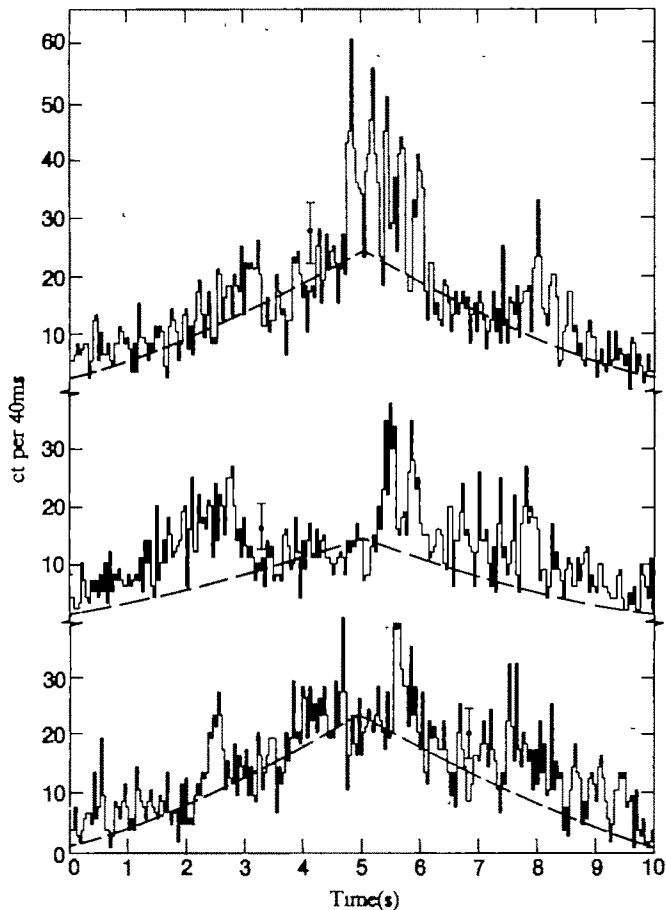


Fig. 2 Three scans through GX339-4 plotted at a resolution of 40 ms. No corrections have been made to the rates. The dashed lines show the collimator's response.

for collimator response and the background has been subtracted. The background (cosmic diffuse X-ray and particle) was determined from measurements before and after the source passed through the field of view. Flaring activity with changes in intensity by factors of 3 on time scales of 0.32 to 1.28 s is evident. A study of the ~ 150 s of data from the source indicates that these flares occur at a rate of ~ 0.3 s $^{-1}$, have an average duration of ~ 1 s and contain about 15% of the power emitted by the source. The intensity of the source averaged over 10 s is variable of a factor of ~ 2 on 5-h time scales.

Figure 2 shows three scans through GX339-4 with a resolution of 40 ms. The rates have not been corrected for collimator response; the dashed lines show the expected response of the collimator to a non-varying source. Flares exhibiting intensity changes of a factor of ~ 3 with rise times from 40 ms to 160 ms are evident. Of particular interest is the series of five sequential flares occurring in the top scan with separations of ~ 250 ms (the separation between the peaks of the first and second flares is ~ 350 ms). This type of sporadic quasi-periodic behaviour is similar to episodes observed in Cyg X-1³ and Cir X-1⁶, and will require more detailed investigation to assess its significance.

The time scales of the variability of GX339-4 are displayed in Fig. 3. This figure was obtained by grouping 40 s of high-resolution data into non-overlapping segments covering 1.6 s, 0.8 s, 0.4 s and 0.05 s. For each of these segments a value of χ^2 was calculated using 10 resolution elements (durations of 160, 80, 40 and 5 ms, respectively) within each grouping. Plotted are the normalised number of segments with $\chi^2/9$ (nine degrees of freedom) greater than the value given by the abscissa. The total number of segments used range from 20 for the 160 ms grouping, to 800 for the 50 ms grouping. The calculated distribution for random data is shown for comparison. This analysis indicates that the source is not significantly variable on time scales between 5 and 50 ms, but is highly variable on longer time scales. HEAO 1 data from Cyg X-1 analysed in the same manner exhibit similar variability⁷. The true characterisation of the variability of GX339-4 will require a larger data base. Unfortunately, the source was in its 'off' state during a pointed observation with HEAO 1 performed on 2 October 1978. Data from a second pointed observation have not yet been analysed.

Fourier analysis of the limited data base failed to reveal any significant periodic power from 10 ms to 2.5 s. In contrast, an autocorrelation analysis of the data reveals significant correlation times from 0.3 to 0.6 s. This suggests that a shot noise model, similar to that used for Cyg X-1⁸, may be the best way to characterise its variability.

The temporal characteristics of GX339-4 resemble those of Cyg X-1^{3,7,8} and Cir X-1^{4,6}, two X-ray sources which are candidates for black holes in a binary system. These characteristics are summarised in Table 1. Much of the information in Table 1 is contained in ref. 9. Cir X-1 and Cyg X-1 are known to be binaries, whereas the data on GX339-4 are insufficient to reveal a binary nature. With the optical counterpart of GX339-4 now identified^{10,11}, this information may soon become available. All three sources have X-ray spectra which are harder during their low emission states. In a study of 43 galactic sources with Uhuru¹² only Cir X-1 and Cyg X-1 exhibited this extreme bimodal spectrum. Their soft-mode spectra are the second and third softest in the sample, the softest of all being GX339-4. However, GX339-4 did not exhibit striking variability in the Uhuru data. Cir X-1 and GX339-4 have what

Table 1 Characteristics of black hole candidates

	Cyg X-1	Cir X-1	GX339-4	V861 Sco OAO1653-40
1. Variability				
Orbital period	5.60 d	16.6 d	—	7.85 d
Bimodal X-ray spectrum	Yes	Yes	Yes	Unknown
Extended 'off' states	No	Yes	Yes	Unknown
Correlation time	0.5 s	Variable	0.3-0.6 s	Unknown
Shortest time scale	≥ 1 ms	≥ 1 ms	≥ 10 ms	Unknown
2. Optical companion/ emission lines	HD226868 O9.7 Iab $m_V = 8.9$	Faint highly reddened $m_R = 16$ strong H α and HeI emission	$m_V = 16.6$	HD152667 = V861 Sco B0.5 Ia H α emission
3. Mass function	0.22 $M_\odot$	—	—	0.487 $M_\odot$
M_1	$\geq 10 M_\odot$	—	—	7-11 $M_\odot$
4. Distance	~ 2.5 kpc	≥ 8 kpc ¹⁴	≥ 4 kpc	2 kpc ¹⁵
5. Maximum luminosity (erg s $^{-1}$)	$\sim 2 \times 10^{37}$	$\geq 10^{38}$	$\geq 2 \times 10^{37}$	1.5×10^{35}
6. Other emission	Radio, IR	Radio	—	UV

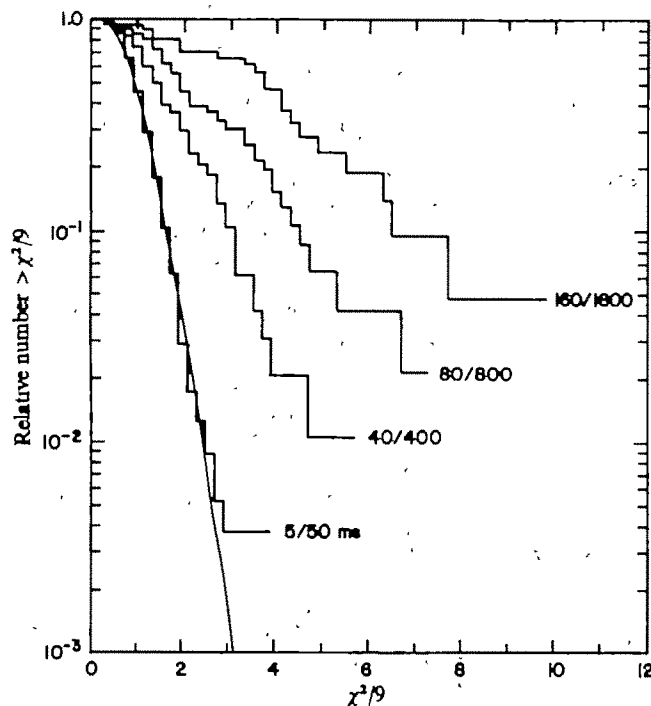


Fig. 3 The integral χ^2 distribution for corrected data sampled from GX339-4 on different time scales. The solid curve shows what is expected for random data. The numbers adjacent to the histograms give the range in time over which variability was tested (for example, the range 5-50 ms is indicated as 5/50). See text for details.

can be characterised as an 'off' state. We find that GX339-4 has an intensity of $\sim 0.01 \text{ ct cm}^{-2} \text{ s}^{-1}$ (~ 2 Uhuru flux units) while in this state. Cyg X-1 is not known to have a similar 'off' state. All three sources exhibit variability on time scales $< 100 \text{ ms}$.

Of the first three sources listed in Table 1, Cyg X-1 is the best black hole candidate. Its variable X-ray emission characterises it as a compact object. Furthermore, it is in a binary system for which both the orbital period and velocity are known³. From either the absence of eclipses or from photometric observations of the optical primary, it is possible to place a lower mass limit on the X-ray secondary. This is above the theoretical limit for neutron stars.

A complementary line of argument leading to a black hole interpretation of Cyg X-1 is derived from its variability over such a wide dynamic range of time scales, from milliseconds to years. No persistent periodic pattern has been observed; this is consistent with the central object being different from a pulsing neutron star. Other models have been suggested² but none seems more plausible than the black-hole model.

The case for regarding Cir X-1, and now GX339-4, as black holes rests on the similarity of their X-ray emission to that of Cyg X-1. They exhibit a similar range of time scales, essentially the same luminosity, and the same bi-modal spectral behaviour.

Table 1 includes the characteristics of V861 Sco which has been recently identified as an X-ray source¹³. The large mass of the non-optical companion and its compact nature, as inferred from the X-ray eclipses when it is occulted by the primary star, are strong evidence in favour of its identification as a black hole. Its luminosity is a factor of 100 less than the other candidates listed. This may be indicative of a fundamental difference in their natures. The determination of the characteristics of the variability of V861 Sco awaits future higher sensitivity studies in progress by satellites such as HEAO and SAS 3.

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Semi-periodic pulsations in circinus X-1

CIRCINUS X-1 (4U1516-56) was first detected in 1969 (ref. 1) and has since been studied in X-ray, radio, and optical wavelengths. Particular interest in this source has resulted from its resemblance to Cygnus X-1—a suspected black hole. Both objects have X-ray luminosities in the range 10^{37} – $10^{38} \text{ erg s}^{-1}$ (refs 2,3). Both exhibit rapid intensity variations with timescales as low as 1 ms (refs 4,5), and as long as seconds or days⁶⁻⁸. Both X-ray sources are in binary systems; the orbital period reported for Cir X-1 is 16.585 d (ref. 9). Cir X-1 differs from Cyg X-1 in that the total mass of the binary pair is unknown. While an optical candidate has been found¹⁰, no estimation of its mass has been made, so that the evidence for its being a black hole is not as strong for Cir X-1. Variable IR emission¹¹ has been reported from the companion. There have been conflicting reports of X-ray periods of the order of seconds: Margon *et al.* suspected a period of 0.685 s and Toor reported a possible 2.2 s period⁴. We report here our investigations, using HEAO A-1, of these semi-periodic pulsations in Cir X-1.

The main portion of the HEAO A-1 experiment consists of four large-area xenon-methane counters with a combined area of 6,800 cm^2 and an effective energy range of 0.5–25 keV. The counters have a $1^\circ \times 4^\circ$ collimator. The roll of the HEAO A-1 causes a point source to be scanned by these modules in 10 s, and a source remains in view for approximately 8 d. The scan modules are on the -y side of the spacecraft. On the opposite side is module 7, which has 1,900 cm^2 effective area. Its collimator, which is $8^\circ \times 2^\circ$, has the longer direction aligned with the scan, so that a source remains in view for about 80 s. Timing resolution is normally 320 ms for all modules, but 5 ms resolution is available.

Before 0200 UT on 28 August 1977, the observed intensity of Cir X-1 was low. At that time, there was a dramatic increase and the source continued in a high state for two more days, after which it left the field of view. Throughout this time the average intensity varied by as much as a factor of 2 during the 15–30-min intervals between successive scans.

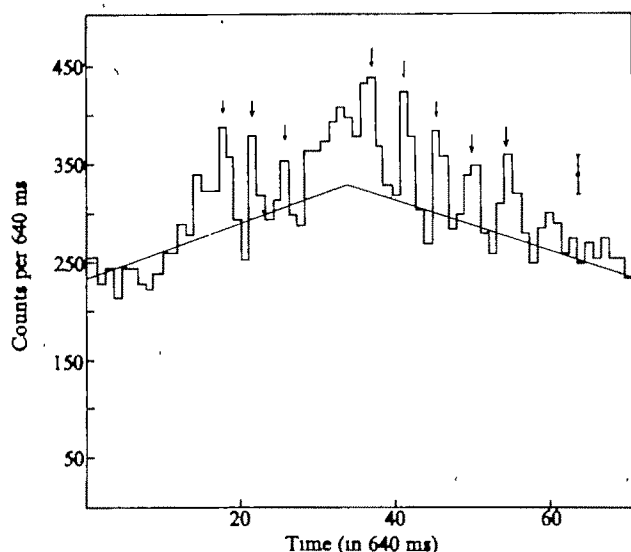


Fig. 1 A scan through Cir X-1 with module 7 using 640-ms sums. The triangular response of the collimators is represented by the two straight lines. A steady source output should follow these lines, but regularly spaced pulses appeared instead. The spacing seemed to be 2.5 s (there are 10 pulses in 39 bins, that is, the period is 2.50 ± 0.06 s).

The data base accumulated during the high state consists of 77 scans with modules 1-4, and eight good scans with module 7. Five of the scans with modules 1-4 used 5-ms timing resolution, but all of the module 7 scans had 320-ms timing resolution. The total observing time from all scans is 20 min.

Erratic intensity variations were observed on all scans, and on many scans no pattern in the variability was evident. A notable exception was the pass shown in Fig. 1, where a train of pulses lasting for 40 s was seen by module 7. The time between successive pulses was 2.5 s. On several scans, modules 1-4 observed similar pulsing. Figure 2 shows two scans, one with the pulsing and one without.

Although not all scans showed periodic pulsations, the pattern on the scan shown in Fig. 1 was sufficiently distinct (with individual pulses standing out at 5σ) for a more systematic search for periodicity to be undertaken.

In this search, four hypotheses were considered: (1) the pulsing in Fig. 1 was of instrumental origin; (2) Cir X-1 varies periodically with a 2.5 s period; (3) the source is not strictly periodic, but has some quasi-periodicity, such as a characteristic recovery interval following each burst or perhaps a periodic component which is sometimes clearly present and other times absent or masked by aperiodic variability; and (4) the intensity variations are aperiodic, that is, burst times are completely random (shot noise).

To test the third conjecture required high sensitivity, so that individual bursts could be isolated and their times could be determined.

Module 7 has intermittently exhibited a high, irregular count rate, thought to be due to electrical noise. At other times it responds normally to sources and has a low background rate. We have devised four data acceptance criteria: first, the count rate before and after a source must reach the normal background level; second, the count rate before and after a source must follow Poisson statistics; third, response to nearby constant sources must follow the known collimator response function; and finally response to Cir X-1 must not extend beyond the full width of the collimator. Eight scans of Cir X-1 on module 7 fulfilled these criteria. As we do not fully understand the nature of the malfunction, it is still possible that the periodicity in Fig. 2 is an instrumental effect. It should be kept in mind that scans from modules 1-4, which are free of this problem, show the same quasi-periodic pulses (Fig. 2).

The search for periodic pulsations was conducted using three methods. (1) Fourier analysis. Each of the scans from module 7 was Fourier analysed. Only one showed a significant peak at 2.5 s. The expectation value for this peak was 1 in 220. (To check for systematic effects, module 7 data at different times was Fourier analysed and no significant peak at 2.5 s was found). Scans from modules 1-4 were not Fourier analysed, there being only 32 points per scan, against 320 in module 7. (2) Epoch folding. The epoch-folding technique is more sensitive than Fourier analysis and can be used when the period is approximately known. Again, only the single pass from module 7 showed a significant peak. (3) Epoch folding of multiple passes. Data can be epoch folded from several scans of the source by inserting strings of zeros to fill the half-hour intervals between scans. A certain assumption needs to be made about unknown orbital parameters of the binary system which was that these effects were negligible. A search for periods near 2.5 s with this method failed to find a significant periodicity.

A comparative test of the hypotheses of quasi-periodicity and random bursting was made using the distribution of intervals between successive bursts. A rise in count was called a burst only if it stood out by at least 2σ above immediately adjacent data, that is, when there was a bin with high count which was both preceded and followed by bins with a count at least 2σ lower. The time of the burst was identified with the local maximum, and the inter-burst interval was the difference in time between two successive bursts. Figure 3 shows the distribution of such intervals.

If the bursts are randomly distributed in time, the distribution of intervals will be exponential, with a characteristic time which is the inverse of the mean rate. The circles show the distribution

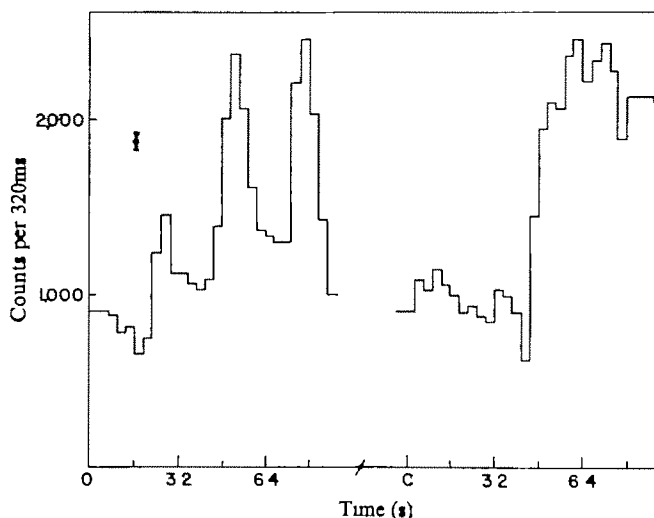


Fig. 2 Two consecutive scans from module 1-4 (corrected for collimator response). On the left one can see a train of three 2.5-s pulses; on the right, the normal erratic behaviour of Cir X-1.

expected if we fit the characteristic interval to the observed rate of bursting. There is a large excess in the number of intervals falling near 8 bins, or 2.5 s. The χ^2 value for the observed distribution is 57, giving a probability of 10^{-5} . The poor fit is due primarily to the contribution from intervals near 2.5 s. We therefore conclude that 2.5-s intervals from Cir X-1, as measured by modules 7 and 1-4, are more frequent than expected for randomly distributed events.

A random distribution of burst times fails to provide a good fit to the distribution of intervals between bursts, because there are many intervals near 2.5 s. Although this suggests a periodicity,

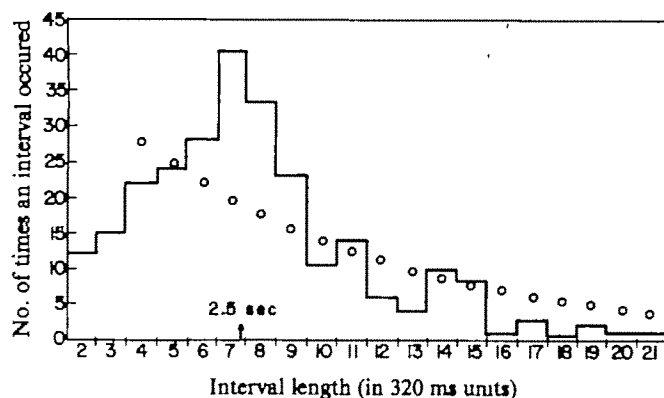


Fig. 3 The histogram shows the distribution of intervals between successive bursts seen in modules 1-4 and module 7. Circles represent the values that would be expected for a shot noise model.

and although highly periodic behaviour is seen on one scan, strict periodicity does not fit the observed intensity variations.

An alternative interpretation is that there is a characteristic delay following one burst before another can occur, and that in short data samples this sometimes gives the appearance of periodicity. This would mean that times of burst events are not completely independent, as in the shot noise picture.

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Structure of lithium-sodium β -alumina by powder neutron diffraction

THE first structural determination of a β -alumina compound containing two different alkali ions, lithium and sodium, in crystallographically distinct sites in the conducting plane is reported here. The lithium ions are displaced by about 1 Å from the mirror planes. Although such a displacement has been suspected it has not hitherto been confirmed.

Polycrystalline lithium-sodium β -alumina, with the two alkali species present in roughly equal proportions, has been shown to be a very good conductor of lithium ions¹. Although definitive

single crystal measurements have not been reported, the conductivity at room temperature, as measured by multi-frequency analysis of polycrystalline samples, is over 10^{-3} S cm⁻¹, the highest reported for any Li⁺ ion conductor. The ionic current is carried almost exclusively by lithium. The thermodynamic equilibrium is such that exchange of lithium for sodium above ~50–60% is difficult to achieve, and the ionic conductivity decreases if this is done. Such behaviour is in distinct contrast to the mixed alkali effect recently described² in sodium-potassium β -gallia, where, as in mixed alkali glasses, the ionic conductivity drops below that of either of the end members. This difference could arise if the structural distinction of the two alkali species, which we demonstrate in lithium-sodium β -alumina, is not a feature of most mixed alkali β -aluminas.

The structure of sodium β -alumina was determined in 1971 (ref. 3) and sodium atoms are found near the Beevers-Ross (BR) and mid-oxygen (MO) positions (Fig. 1). The structure of lithium β -alumina has not been reported, but the positive pressure coefficient of resistivity⁴ can be interpreted as indicating a displacement of lithium off the mirror plane at atmospheric pressure. Such a displacement would be expected from the known positions of the spinel block oxygen atoms and the effective ionic radii of lithium and oxygen⁵. When lithium is incorporated by ion exchange, as in the present work, it is expected to be present in the conducting plane. If incorporated during sintering and sample preparation, lithium will stabilise the β' phase⁶ and is then presumably present in the spinel block, although this has not been demonstrated by structure analysis.

Table 1 Lithium-sodium β -alumina, PANDA diffractometer, $\lambda = 1.534$ Å (atom positions at 4.2 K)

Atom	Position	x/a	z/c	B (Å ²)	Occupation
O(1)	12k	0.15655(40)	0.04957(12)	0.14(5)	6
O(2)	12k	0.50220(48)	0.14682(11)	0.33(6)	6
O(3)	4f	2/3	0.05751(27)	0.40(14)	2
O(4)	4e	0	0.14233(35)	0.87(15)	2
O(5)	6h	0.29673(142)	1/4	0.70(36)	1
Al(1)	12k	0.83148(81)	0.10632(20)	0.76(7)	6
Al(2)	4f	1/3	0.02430(45)	0.55(15)	2
Al(3)	4f	1/3	0.17575(48)	0.83(17)	2
Al(4)	2a	0	0	0.46(22)	1
Li	6h	0.834(17)	(0.2086(34)	1.0	0.57(11)
Na	6h	0.7182(41)	1/4	1.0	0.44(5)

Cell parameters: $a = 5.5923(4)$; $c = 22.5576(6)$.

Data were collected on the PANDA diffractometer, Harwell ($\lambda = 1.534$ Å). The starting material was Monofrax sodium β -alumina containing 6.4% Na₂O (1.24 Na₂O : 11 Al₂O₃) which had been 60% exchanged with lithium in molten lithium nitrate. It was maintained at liquid helium temperature to minimise thermal motion effects which are difficult to distinguish from atomic displacements at higher temperatures in materials such as the β -aluminas, where several sites in the mirror plane may be partially occupied by the same atom. The data were refined using the profile refinement method described by Rietveld⁷.

The final atomic positions and thermal parameters are given in Table 1. The weighted profile agreement factor was 0.069. The accuracy obtainable using the powder technique does not allow a very precise determination of the occupations of the alkali ions, but the occupation numbers determined are close to those expected from the chemical composition (0.74 Li, 0.50 Na). The temperature factors of both atoms were constrained at 1.0 Å². Nor can we determine unequivocally by powder diffraction the structural basis of the observed non-stoichiometry in β -aluminas. The refinement in Table 1 assumes a

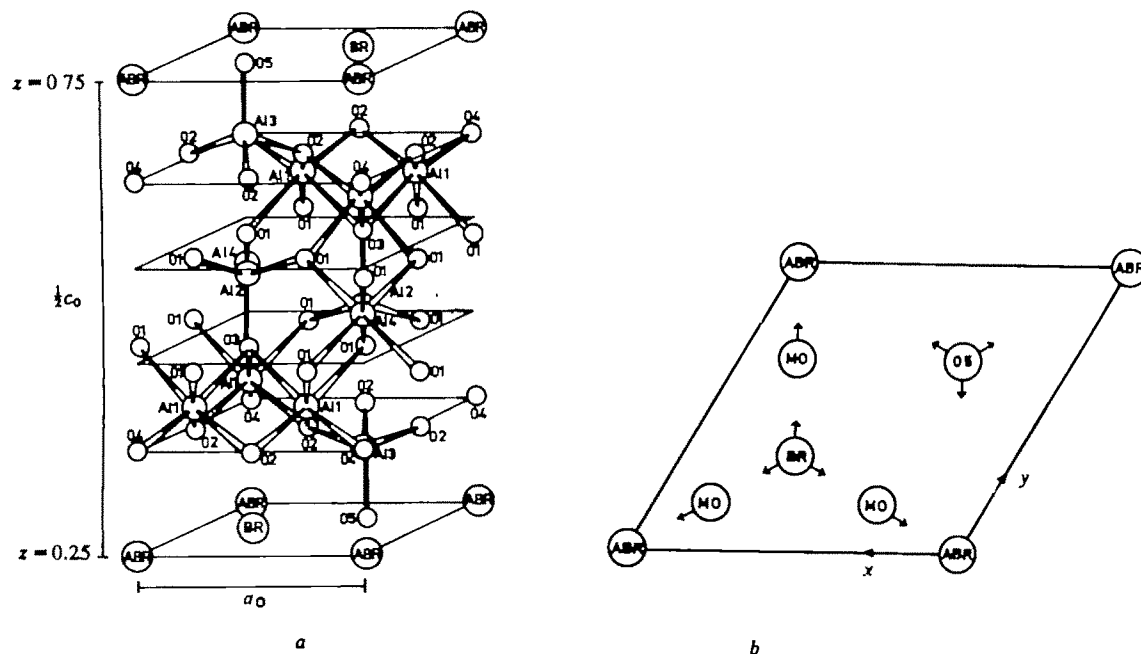


Fig. 1 The β -alumina structure. One half of the unit cell is shown in (a). A projection of the conducting plane is shown in (b) with the Beavers-Ross (BR), mid-oxygen (MO) and anti-Beavers-Ross (ABR) sites labelled. The directions of the small displacements often observed are indicated. For the sodium atom at the BR position in lithium-sodium β -alumina, there are three equivalent possible directions of displacement

stoichiometric spinel block. However, incorporation of 0.24 interstitial aluminium displaced from Al(1) sites, and 0.12 oxygen atoms in the mirror plane close to the mid-oxygen sites in accord with the observed non-stoichiometry, causes a small increase in the lithium occupation to 0.65(12). The sodium occupation (0.47(5)) is virtually unchanged as are all other parameters. Both alkali occupations are then within one standard deviation of the expected values. This model of defect compensation, first suggested by Roth *et al.*⁸ for sodium β -alumina, and found for potassium β -alumina by Collin *et al.*⁹ seems, therefore, to be applicable in the present compound.

The relatively weak scattering powers of lithium and sodium for neutrons entail only a weak (1%) dependence of the R -factor on their presence. We believe, nonetheless, that the results obtained are meaningful, both because of the concordance of the occupations and positions with expectation, and because satisfactory refinements were not obtained with the alkali ions in alternative locations in the conducting plane.

The important structural conclusions about lithium-sodium β -alumina which may be drawn from the present work are then that the lithium and sodium are localised on crystallographically distinct sites and that lithium is displaced off the mirror plane. The sodium location, about 0.5 Å from the BR site, is very close to the dominant sodium location observed in sodium β -alumina³. Na-O(2) distances are 2.722(7) and 3.130(8) Å. The nearest Na-O(5) distance is 2.805(11) Å.

Lithium is displaced ~1 Å off the mirror plane and is located almost exactly over the MO site. Li-O(2) and Li-O(4) distances are 2.126(52) and 2.198(71) Å respectively. This displacement is necessary to reduce the Li-O distance to a value close to the sum of the ionic radii ($O^{2-} + Li(IV) = 1.99$ Å (ref. 5)).

Although we have shown the first structural distinction of two different alkali ions in the conducting plane of β -alumina, a structural determination on its own cannot explain the observed conductivity data¹. Long conduction paths are probably present for lithium motion without blocking by static sodium atoms; the role of the β -alumina framework and the sodium ions in promoting lithium ion mobility might be revealed by lattice statics or molecular dynamics calculation. The observed Li-O

distance, and, particularly, the minimum ABR-O(4) distance (2.43 Å), are all greater than the sum of the ionic radii. Therefore, we still need to know the optimum size of hole or slot to expedite rapid Li^+ motion, if indeed such a parameter can be defined without reference to a particular structure type.

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Interconnected fibre masses precipitated from solutions of polymers

IN studies to increase the mechanical strength of polymeric encapsulants for high voltage electronic devices we have formed fibre masses of unusual structure from isotactic polypropylene. The method for producing these three-dimensionally interconnected fibrous structures is described here and involves agitation of a solution of the polymer at sonic frequencies with simultaneous cooling. This process has since been observed for polyethylene and polybutene-1. Furthermore, the process has

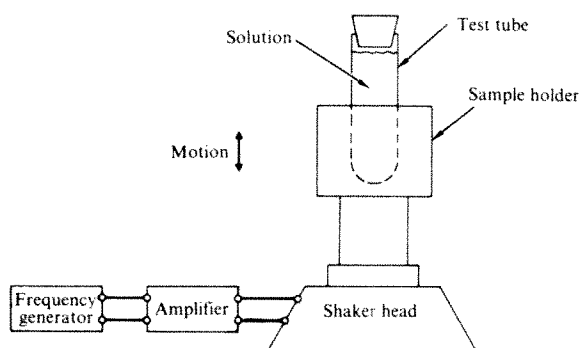


Fig. 1 Schematic diagram of experimental apparatus for low frequency agitation of polymer solutions.

been extended to other polymers, both crystalline and non-crystalline, through a process we call 'seeding'. Uniform reinforcement of encapsulating resins through the addition of high strength, short fibres, is not practical because of the complex shapes and numerous small interstices of the devices to be encapsulated. We therefore set out in 1973 to form fibres *in situ* by precipitation from low viscosity solutions which would readily impregnate a device of complex geometry. As a secondary objective, we hoped to polymerise the solvent to form a matrix around the fibres thereby producing a self-reinforced composite.

The fibrous crystal form of polymers was first reported in 1959 (ref. 1). This new crystal geometry, which consists of folded chain platelets regularly spaced on a central core of highly aligned polymer molecules, has been termed the 'shish kebab' structure. Pennings^{2,3} and Wikjord and St John Manley⁴ reported the formation of fibrous precipitates, almost entirely of the shish kebab structure, on the stirrer by stirring supercooled solutions of polyethylene and isotactic polystyrene respectively. Fibres have also been prepared by vigorous stirring of isotactic polypropylene, polyoxymethylene and polyethyleneoxide. Although the formation of high strength fibres by the creation of a sustained, essentially unidirectional, velocity gradient in a solution by stirring, high velocity jet, and other mechanical devices has become commonplace, there has been only one report of the use of high frequency agitation. Blackadder and Schleinitz⁵ found that irradiation of a dilute solution of polyethylene in *p*-xylene with ultrasonics from 85 to 190 kHz between 82 and 88 °C caused the formation of small fibrous crystals consisting entirely of the shish kebab structure.

Pennings⁶, in his study of the mechanics of fibrillar crystal growth, concluded that the creation of velocity gradients within the solution is the primary requirement. Extending this conclusion to the formation of fibre masses, we hypothesise that velocity gradients are generated in the solution by the sonic agitation. The hydrodynamic forces imposed on the coiled molecules by these velocity gradients cause them to extend completely or partially. Assuming that the thermodynamic conditions are suitable, the extended polymer chains and segments of chains have the opportunity to crystallise with each other in a substantially parallel or orientated manner. Segments of polymer coils which extend into the streaming solution, also undergo extension and orientation, thereby accelerating the growth process, and forming new branches of the original fibres. On cessation of the perturbing hydrodynamic forces, the segments of polymer chains extending from the partially orientated core of the fibre tend to crystallise in the usual folded chain configuration. They may account for the spherulitic and shish kebab-like structures which are often found mixed with the fibres.

In our initial experiments, we found that the stirring technique was an excellent way of establishing optimum conditions, such as

type of solvent, polymer concentration and temperature, for fibre deposition. Stirring, however, causes fibres to form on and extend from only the exterior surface of the rotating member and therefore was not suitable for forming fibres in and around complex shapes. Ultrasonic formation of fibres was ineffective; the yield of fibres was sparse and the fibres were discrete and very short. We then decided to try the effect of agitation at sonic frequencies on relatively concentrated polymer solutions. The apparatus used for these experiments is shown in Fig. 1. We placed a test tube of hot polypropylene solution in the sample holder and subjected it to low frequency agitation as it cooled. At a few degrees below the dissolution temperature dense fibre masses began to form in the solution. These masses grew until they filled the entire fluid volume of the container. The fibre masses were usually so densely interconnected that they had good mechanical integrity and retained the entire volume of the solvent.

Isotactic polypropylene proved to be most amenable to the formation of fibrous masses. Concentrations of 0.7–20% polypropylene were dissolved in *p*-xylene at 132 °C and allowed to cool while the frequency of vibration was scanned in the 80–1,000 Hz range. Dense fibrous mats were formed. In other experiments, fibre masses were formed from concentrations of 5% and 10% isotactic polypropylene in styrene monomer inhibited with 0.5% hydroquinone. The styrene-impregnated fibre plugs were then placed in an oven at 70 °C to polymerise to monomer into a plastic matrix of polystyrene to form a self-reinforced composite.

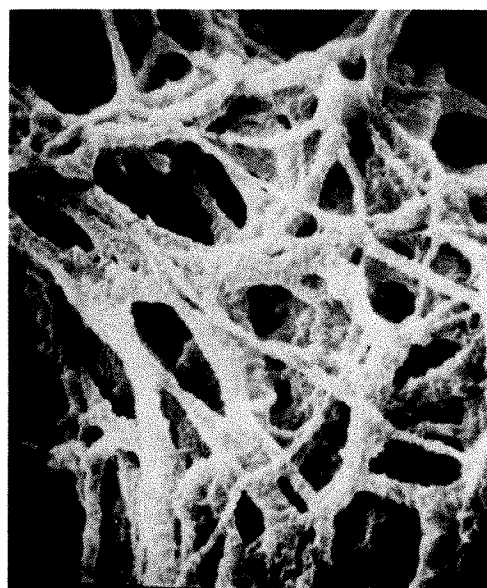


Fig. 2 Polypropylene fibre mass showing branching and interconnection. Scanning electron micrograph ($\times 4,800$).

Interconnected fibrous masses have been formed from polyethylene, isotactic polybutene-1 and isotactic poly-4-methylpentene-1. Polypropylene has also been used to induce other linear polymers, including Nylon 6, acrylonitrile butadiene styrene terpolymer, polystyrene, polyvinylidene fluoride, and tetrafluoroethylene hexafluoropropylene vinylidene fluoride terpolymer, to co-deposit in fibrous masses. This is the technique which we have called seeding. It is possible that the phenomenon of fibre mass formation under sonic agitation is general for isotactic crystalline polymers provided the optimum solvent system, concentration range and precipitation temperature is selected. Furthermore, the technique can be extended by seeding to many other linear polymers.

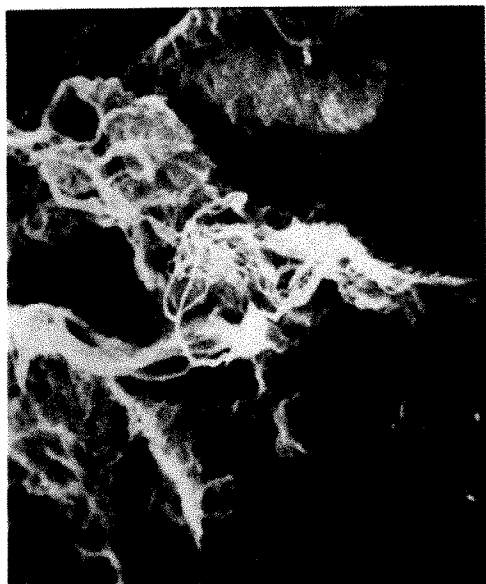


Fig. 3 Fibrillar structure of fibres from fibre mass scanning electron micrograph ($\times 11,000$).

The morphology of the fibrous masses depends on many factors including concentration, rate of cooling, solvent type and the frequency and effectiveness of the agitation. In general, the most uniform, highly orientated, fibrous and highest strength fibre masses are produced from fairly low concentrations at very slow rates of cooling during precipitation. The precise mechanism of fibre mass formation by sonic agitation is not understood. We do not know whether the hydrodynamic shearing forces necessary for initial fibre formation are produced by cavitation, interaction of the solution with the walls of the container or some combination of perturbations. The following general observations may be helpful. Isotactic polypropylene very readily forms fibre masses from several solvents such as *p*-xylene, styrene, toluene, and mixtures of styrene, acrylic acid and methylmethacrylate. Lower viscosity solutions form fibres more readily than viscous, concentrated solutions. Efficient coupling between the container of the polymer solution and the shaker is important for the formation of uniform fibre masses. Frequency of agitation in the sonic range does not seem to be important except that those frequencies which are resonant with the container of polymer solution will cause the most efficient agitation and result in the most uniform fibrous masses. Also, a small air-filled space between the cap of the container and the surface of the solution seems to be necessary. Fibre plugs will not form in a full test tube which is sealed with an impervious rubber stopper but will form when the rubber stopper is replaced with a cork.

The structure of a well formed fibrous mass is shown in Fig. 2. If the solution is cooled too rapidly, or is too concentrated, the fibres will be co-deposited with spherulites and flocculent material. The fibres vary from 300 Å to about 0.3 mm in diameter and are variously branched or bifurcated to form an interconnected, three-dimensional network. Examination of the fibres at high magnification reveals that they seem to be made up of essentially parallel arrays of fibrils (Fig. 3).

We and our co-workers have subsequently formed fibre masses by a variation of the sonic agitation technique which permits more precise control of the experimental conditions. Fibres from these experiments have the shish kebab morphology and greatly improved strength over the bulk polymers. This work will be reported elsewhere.

This study was supported as a Hughes Aircraft Company Independent Research and Development Program and has

resulted in the filing of several patent applications to cover fibre masses and the 'seeding' technique. We thank C. G. Walance and G. R. Hokenstad for their support and R. E. Kelchner for his technical advice.

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Sea-level changes during the late Pleistocene and Holocene in the Strait of Malacca

CURRENT concepts of late Pleistocene sea level history, generally referred to the ^{14}C time scale, differ considerably¹. Some authors^{2,3} assume that the sea level at about 30,000 BP was comparable with that of the present and others^{4,5} assume a considerably lower sea level at that time. We have now obtained ^{14}C dates from *in situ* roots and peat which indicate that the sea level was lowered eustatically to at least 40–60 m below the present level between 36,000 and 10,000 BP. The sea level rose from –13 m to about +5 m from 8,000 to 4,000 BP and then approached its present level.

A combined coastal and offshore survey in the Strait of Malacca between Port Dickson and Singapore yielded 33 reliable ^{14}C ages obtained from autochthonous organic material such as peat and roots (Table 1). In the coastal region, samples were collected by hand augers which could penetrate up to 18 m of unconsolidated sediments. In the offshore area a shipboard-operated vibrocorer was used which recovered cores up to 3 m long. Samples were obtained between 75 m below and 6 m above mean sea level.

The geological settings of the dated layers cannot all be used as direct indicators of sea level but can serve as indirect indicators. In the offshore zone, the substratum of the peats is mostly unknown, because the vibrocorer did not penetrate the peat layers. Some of these peats may have been formed in association with freshwater lakes, as indicated by the presence of a diatom ooze. The upper parts of the peat and their possible sedimentary cover were eroded during the Holocene transgression or by sub-Recent tidal currents. Today the peats, which have a fresh and unweathered appearance, are mostly overlain by sandy marine sediments. Data obtained from this type of peat indicate only that the sea was below the sampled level during the time of peat formation. The same conclusion can be drawn from *in situ* roots in terrestrial sediments.

The Holocene basal peats of the coastal sedimentary sequences were formed on top of terrestrial deposits usually with no erosional hiatus between them and the overlying marine sediments. In this case it can be assumed that the sea invaded the area shortly after the phase of peat formation.

In wide areas of the coastal lowlands, peat occurs as an organic cover on the top of clastic deposits. This overlap of a semi-terrestrial facies over brackish and marine facies indicates the beginning of regressive tendencies in the coastal development. Mangroves are direct indicators of the tidal zone between mean neaps high and mean springs high (at present ~1.3 m).

Depth distribution of ^{14}C ages of autochthonous organic material of the sampled core sections (Figs 1, 2) shows that

Table 1 ^{14}C dates from *in situ* roots and peat

^{14}C age BP	Lab. no	$\delta^{13}\text{C}$	Geographical coordinates		Relation to mean sea level (m)	Dated material
1,055±85	Hv 7598	-27.6	2°31' N	101°46' E	-0.65 to -0.75	Mangrove wood on top of terrestrial deposits
1,145±80	Hv 7603	-27.3	2°25' N	101°58' E	-0.60 to -0.63	Mangrove wood on top of marine sediments
2,130±155	Hv 7588	-27.7	1°46' N	103° 1' E	+1.80 to +2.00	Basis of peat layer on top of brackish sediments
4,040±100	Hv 7583	-29.9	1°37' N	103°25' E	+5.85 to +5.90	Mangrove and peat above terrestrial deposits
4,135±90	Hv 7589	-31.8	2° 1' N	102°47' E	+4.95 to +5.00	Peat on top of brackish deposits
4,275±70	Hv 7587	-29.5	1°48' N	103°15' E	+4.70 to +4.95	Peat on top of brackish deposits
4,335±105	Hv 7586	-31.0	1°43' N	103°18' E	+2.75 to +2.80	Peat to fresh water deposit on top of brackish sediments
4,980±75	Hv 7580	-29.5	1°29' N	103°23' E	+3.70 to +3.80	Basis of peat layer on top of brackish deposits
5,975±75	Hv 7594	-28.4	2°19' N	102° 5' E	+1.10 to +1.25	Mangrove wood at the base of coastal barrier sand
6,260±75	Hv 7582	-29.2	1°32' N	103°28' E	+0.63 to +0.67	Peat to mangrove above terrestrial deposits
6,610±70	Hv 7581	-27.8	1°32' N	103°28' E	+0.80 to +0.95	Mangrove wood
6,945±120	Hv 7584	-27.2	1°37' N	103°25' E	-0.35 to -0.50	Peat on top of terrestrial deposits
6,985±180	Hv 7601	-28.8	2°22' N	102° 0' E	-3.70 to -3.80	Mangrove wood
7,015±80	Hv 7592	-29.3	2°10' N	102°36' E	-2.55 to -2.90	Peat on top of terrestrial deposits
7,175±70	Hv 7595	-27.9	2°25' N	101°56' E	-5.00 to -5.10	Wood from a thick sequence of mangrove deposits
7,340±100	Hv 7591	-27.5	1°59' N	102° 40' E	-5.00 to -5.10	Peat on top of terrestrial deposits
7,440±175	Hv 7596	-30.2	2°25' N	101°56' E	-5.80 to -5.85	Wood from a thick sequence of mangrove deposits
7,560±135	Hv 7597	-29.0	2°25' N	101°56' E	-7.50 to -7.55	Wood from a thick sequence of mangrove deposits
7,985±220	Hv 7593	-29.5	2°12' N	102°18' E	-12.65 to -12.85	Wood from a peat on top of terrestrial deposits
8,490±85	Hv 7650	—	1°51' N	102° 9' E	-21.10 to -21.20	Peat on top of marine muds
9,265±105	Hv 7644	-28.7	1°13' N	103°12' E	-33.41 to -33.42	Woody peat overlain by marine mud
9,840±330	Hv 7669	-26.5	2°20' N	101°44' E	-53.40 to -53.70	Peat overlain by marine sand
10,590±190	Hv 7646	-14.2	1°16' N	103°11' E	-33.65 to -33.75	Clayey peat with <i>in situ</i> roots on top of terrestrial silty clay
12,440±705	Hv 7643	-23.7	1°14' N	103°14' E	-38.45 to -38.65	<i>In situ</i> roots in weathered quartzite
17,170±110	Hv 7662	-30.4	2°39' N	101°28' E	-56.95 to -57.09	Woody peat overlain by marine mud
23,800±2,550	Hv 7663	-21.3	2°36' N	101°31' E	-54.00 to -54.10	<i>In situ</i> roots within terrestrial clay
1,930						
26,330±3,410	Hv 7653	-31.7	1°59' N	102°17' E	-35.30 to -35.60	Leaves and wood from a fresh water diatom ooze
2,380						
27,400±1,000	Hv 7665	-29.8	2°34' N	101°30' E	-65.60 to -66.00	<i>In situ</i> mangrove (?) roots
890						
32,800±2,170	Hv 7648	-30.2	1°30' N	103° 7' E	-34.70 to -35.60	Leaves from freshwater (?) silty clay
1,700						
33,670±2,100	Hv 7664	—	2°35' N	101°30' E	-67.20 to -67.25	Peat overlain by marine sand
1,660						
37,700±1,810	Hv 7640	-30.3	0°44' N	103°18' E	-16.50 to -16.52	Sand impregnated with humate
1,480						
41,650±3,850	Hv 7641	-25.5	0°43' N	103°20' E	-17.60 to -17.70	Peat overlain by marine sand
2,600						
>44,200	Hv 7652	-27.9	2° 0' N	102°20' E	-36.13 to -36.15	Peat overlain by marine mud

between approximately 40,000 and 10,000 BP continental conditions prevailed in most parts of the southern Malacca Strait. Between 40,000 and 38,000 BP peat was formed at 17 m below present sea level, and around 34,000 BP at -67 m. Leaves and wood found at -36 m in freshwater diatom oozes gave ^{14}C ages of 32,800 and 26,300 BP. *In situ* roots, presumably from a former mangrove swamp at -65 m and with a ^{14}C age of 27,400 BP, give the only direct time-depth position of a Pleistocene sea level. *In situ* roots from terrestrial sediments and peat grew at -55 m between 23,800 and 17,200 BP. Three additional peat dates from the Strait of Malacca^{6,7} and the southern Gulf of Siam⁸ supplement these dates. From 8,000 to 6,000 BP the sea level rose from -12.8 m to +1.2 m relative to the present level.

Sea level indicators for the time interval between 5,000 and 4,000 BP have been found at +2.5 m to +5.8 m, which is the highest dated level. The subsequent lowering of the sea level to its present value is indicated by a peat sample at +2.0 m with a ^{14}C age of 2,000 BP and two mangrove samples at -0.8 m, dated at 1,000 BP. This tendency is consistent with the results of Tjia *et al.*⁹. However, our data do not show whether the lowering of the sea level took place steadily or through an oscillatory process.

The reliability of conclusions concerning sea level history is limited by the errors involved in ^{14}C dating, and in correlating

the sample sites with the corresponding sea levels. For the dating of our offshore peat samples, only contamination by younger roots need be taken into account. Because of the fresh appearance of the peats and their stratigraphical position below the marked marine erosional horizon, such roots were not expected and they were not found. These roots would also have to be older than 10,000 yr because of the Holocene transgression, and in our experience contamination by finely dispersed macroscopically invisible roots is generally lower than 3%. With the most unfavourable of these assumptions, samples with actual ages of 100,000, 40,000, 30,000, and 20,000 yr would correspond to ^{14}C ages of 38,000, 33,600, 27,700 and 19,500 yr, respectively. In other words, the ^{14}C time scale seems to be increasingly compressed towards the earlier end of the scale.

The amount of compaction which may have changed the depths of the sea level indicators is unknown for the offshore area. For the coastal area it can be estimated roughly to be up to 5.5 m. This is the height difference between the highest sea level indicator in the sedimentary sequences and the corresponding level of a marine abrasional platform on weathered granite.

The curve for Holocene sea level changes in the Strait of Malacca compares well with those areas which are considered to

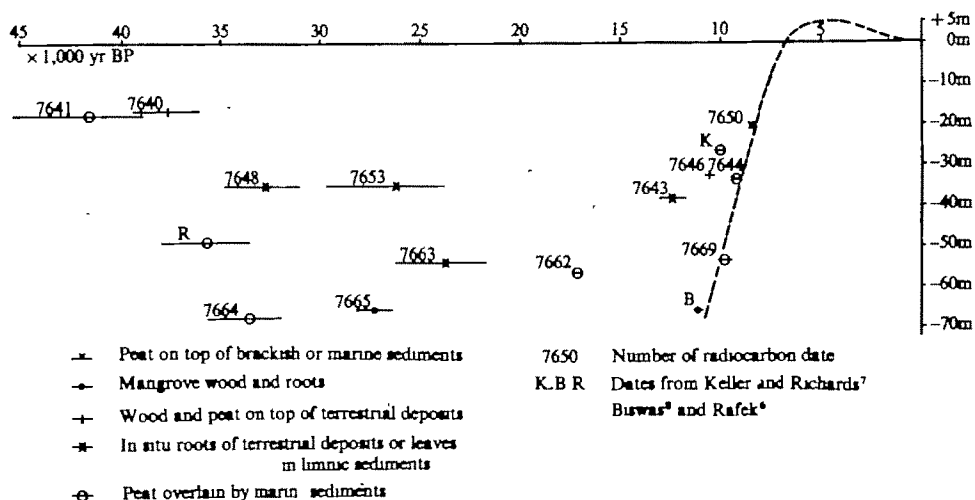


Fig. 1 Depths and ^{14}C ages of sea level indicators. The dotted line shows the change of the sea level for the past 10,000 yr (see also Fig. 2).

be tectonically stable, like Mauretania¹⁰ and Mozambique¹¹. This similarity indicates that at least during the Holocene the Malacca Strait area was not influenced by tectonic subsidence or uplift. These conditions seem to have prevailed over a longer period. From the palaeogeographical pattern of marine Tertiary sediments¹², it is obvious that the Malaysian Peninsula was either terrestrial or has been uplifted since that time.

Because secondary effects such as compaction and tectonic subsidence as well as contamination affected our data on the original depth and age very little, the formation of Pleistocene peat and mangroves at the bottom of the present Malacca Strait are explained by eustatic lowering of the sea level. Although the Pleistocene dates are not suitable for construction of a sea level curve, they support the conclusion that the sea level was lowered worldwide by at least 40–60 m from 36,000 to 10,000 BP.

Our conclusions are supported by a date from the shelf off the Ivory Coast¹³, where peat at –65 m indicates continental conditions at about 23,000 BP. The unpublished date of $36,150 \pm 3,000$ BP (Hv 6280) from a humous sand taken at –38 m from the shelf of northern Mozambique also corresponds to continental conditions. ^{14}C and $^{230}\text{Th}/^{234}\text{U}$ dates from New Guinea also indicate a –40 m level at 30,000 and 40,000 BP¹⁷.

In contrast, the often-reproduced sea level curves from Curray² and Milliman and Emery³ show a high sea level for around 30,000 BP, levels that are comparable to the present level. The discrepancy between their results and ours is surely due mainly to their use of mobile carbonate material such as shells, ooids, and pieces of carbonate cemented sand, which would result in dubious ^{14}C ages and erroneous sea level indications. This material could have been reworked¹⁴ or rejuvenated by contamination during several thousand years of sub-aerial weathering¹⁵. Consequently, the ^{14}C time scale used by Curray² and

by Milliman and Emery³ seems much more compressed at the earlier end of the scale than ours. Accordingly, the high sea level postulated by these authors most probably belongs to an older warm period, presumably to the last interglacial period. Our interpretation agrees well with the sea level reconstructions based on global climatic history^{4,5} and the changes in the oxygen isotope ratio in deep-sea sediment cores¹⁶, which assume low sea levels for the period around 30,000 BP.

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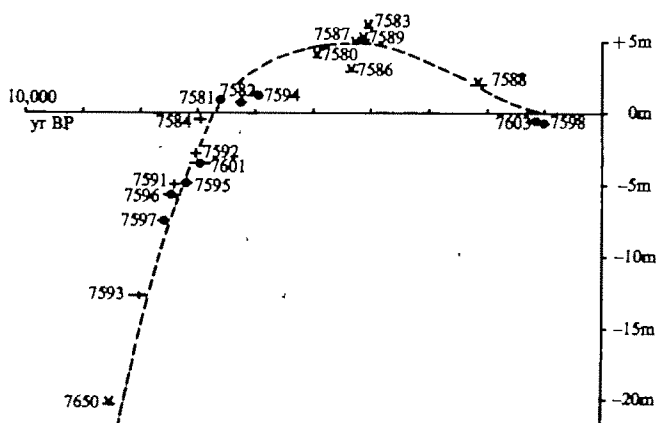


Fig. 2 Sea level curve for the past 7,900 yr.

Maximum density of random placing of membrane particles

THE apparently simple problems of (1) how many short lines ('cars') of the same length can be randomly placed ('parked') in non-overlapping fashion on a line ('street'); (2) how many squares (Fig. 1) or disks (Fig. 2) of the same size can be randomly placed in an area without overlapping; (3) how many solid spheres (atoms) can be randomly packed in a given volume, are of interest from both physical and mathematical points of view, and occur in various situations¹. Our interest in the two-dimensional problem arises from a computer simulation^{2,3} of particles

Table 1 The maximum fraction f of an area covered by randomly placed particles

Square particles		Method, coarse mesh (except as indicated)							
Palásti ⁵ 0.5589		Periodic boundary				Rigid boundary			
Computer simulation		Current work				Current work		Akeda & Hori ⁷	
Size of area ($N \times M$)	Number of trials	Average lower bound	Average upper bound	Standard deviation of upper bound*	Average lower bound	Standard deviation	Mean	Standard deviation	
20 × 30	40	0.5653	0.5677	0.0089	0.5509	0.0140	0.5473	0.0096	
31 × 31	40	0.5633	0.5652	0.0089	0.5525	0.0090			
31.5 × 31.5	20		0.5538†	0.0080†					
40 × 40	40	0.5629	0.5649	0.0053	0.5537	0.0061	0.5534	0.0043	
60 × 60	40						0.5567	0.0040	
100 × 100	40						0.5592	0.0023	
200 × 200	10						0.5610	0.0012	

Circular particles

Method, fine mesh; periodic boundary conditions; size of area, 1,024 × 1,024 fine squares; particle diameter, 27.5 fine squares; 10 trials.

* Standard deviation of lower bound was virtually the same.

† Fine mesh; not an upper bound, but a mean.

Mean 0.5027. Standard deviation 0.0033.

cell membranes. When the membrane bilayer is split into two leaflets by freeze-fracture method⁴ and an area of the split membrane is visualised by electron microscopy, then the imbedded membrane particles (believed to be predominantly roteineaceous) are seen as circular shapes. In natural membranes the unaggregated particles seem to be random in position, and the areal density of the particles may be high. It seems that nature has provided us with a two-dimensional sample of (nearly) maximum random parking. Hence we have estimated the maximum fractional area covered by circular disks in a two-dimensional membrane, using a computer simulation with periodic boundary conditions. The correspondence with articles in a biological membrane has implications for membrane biogenesis.

The two-dimensional random sequential placing ('car parking') problem may be described as follows. Cars, each of the same size, are dropped from the air one at a time into the empty space of a parking lot. The place to park each car is chosen so that there is a (uniform) probability of parking on the remaining empty space. When no more cars can be parked (without overlapping one another) then the parking lot is 'saturated', and the fractional area f of the lot which is occupied by cars is the quantity of interest. Palásti⁵ conjectured from the one-dimensional case that in two dimensions $f \sim 0.5589$. Previous simulations of the two-dimensional case^{1,5-7} have taken the cars to be square, with sides always parallel to those of a square parking lot of finite extent ('rigid boundary'). Here, Monte Carlo analogues are used to find f for circles and for squares, with periodic boundary conditions being novel in the present context. The choice of computing technique is most important, as it is affected by the expense of computation time.

We used two basic methods for placing particles by computer: (1) the coarse-mesh method for square particles only; and (2) the fine-mesh method for square and disk particles. They will be described in detail elsewhere³.

Method (1) is an improvement of that of Akeda and Hori^{6,7}. Some results are shown in Table 1. The principle of the fine-mesh method is that the parking lot is covered by a fine mesh of squares which are much smaller than a particle: a particle (disk) is centred on a square, and the particle is then represented by the minimum set of fine squares which will completely cover the area of the particle. To obtain reasonable accuracy (to three significant figures in placement) the square 'parking lot' was divided into a mesh of 1024 × 1024 fine squares: the

availability status (whether or not a fine square was available for a particle centre) was stored as a bit in a single megabit array. Square particles (used for checking the fine-mesh method against the large-mesh method) were $(65/2) \times (65/2)$ fine squares in size; circular particles were of diameter 55/2 fine squares.

The essence of the fine-mesh method is that, by careful counting, the problem of making many trials to avoid overlaps is eliminated, which is unavoidable in the large-mesh method. As the centre location for each particle is randomly selected directly from those fine meshes available, then the neighbouring region thereby excluded to another particle is also determined and recorded in the megabit array: this region will be a square of 65×65 or a circle of diameter 55 fine meshes. The areal error due to representing the smooth outline of a circle by an outline made from many small squares is much less than 1.5%.

To identify directly the next vacant fine squares on which a particle centre might be randomly placed, two additional 1,024 long integer arrays are used (that is, two integers for each row of the 1,024 × 1,024 array) for the book-keeping purposes.

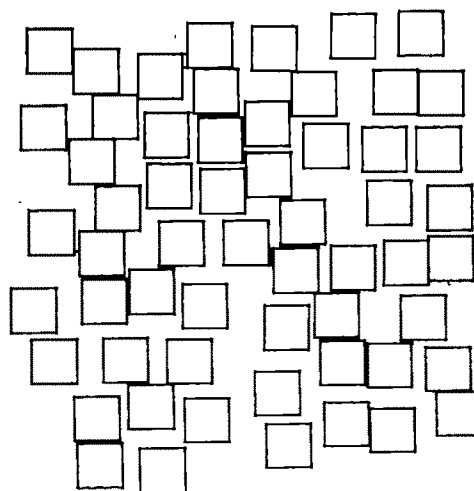


Fig. 1 Sample of large mesh method for randomly sequentially placing square particles into a square region.

To produce plots such as Fig. 2, an integer array is used to record the coordinates of the particle centres. To reduce the particle placement time, the shape of one-quarter of a particle is stored in an integer array (further details required to make the method feasible are given in ref. 3).

A great advantage of the method is that the computer time required to find a location for each particle is the same for each placement (parking). Particles are placed until there is no space available for a particle centre. While the time required to place a particle is quite long (48 ms to place each $(65/2) \times (65/2)$ square particle, including all book-keeping, on IBM 370/168), it remains independent of the number of particles placed. (The time and memory requirements of the program increase drastically with the accuracy of the placement desired. To add one

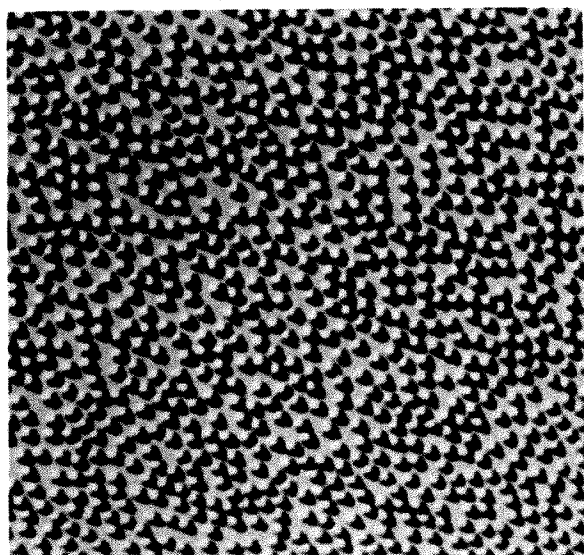


Fig. 2 Sample of fine mesh method for randomly sequentially placing circular particles (disks) into a square region. (The area shown is covered by a square mesh—not shown—of $1,024 \times 1,024$. Also, the disks have been synthetically shadowed so as to simulate the appearance of freeze-fractured particles, which usually seem to be hemispherical.)

significant figure of accuracy in the two dimensions would increase both the time and memory requirements of the computer program 100-fold.)

The new result for the fractional area covered by maximally packed disks is 0.5027 ± 0.0020 . We are not aware of any theoretical (or previous experimental) estimate (and simply note that this result is not inconsistent with the difference in shape between circles and squares, and that the squares were all orientated with sides parallel). For the maximum fractional area f covered by non-overlapping orientated square particles on a surface, in addition to Palásti's⁵ conjecture of 0.5589 and Akeda and Hori's⁷ extrapolation of a simulation with rigid boundary conditions of 0.5626 ± 0.0006 , we now have simulations with more realistic periodic boundary conditions which do not require extrapolation. These new results are for the coarse-mesh method $f = 0.5629 \pm 0.0016$ (lower bound) and $f = 0.5649 \pm 0.0016$ (upper bound) (errors are 95% confidence limits about the estimated mean), and for the fine-mesh method $f = 0.5538 \pm 0.0035$. (The nominally low error quoted⁶ is statistical, due to taking many samples, and will not affect the methodological problem associated with rigid boundaries in their simulation.) These new results bridge Palásti's conjecture⁵. Hence, in contradiction to other simulations⁷ these new results together show that simulations give no reason to reject her⁵ conjecture for orientated squares.

The areal density of particles in the human erythrocyte membrane² is substantially close to that of the maximum placing

of disks. As during membrane biogenesis^{8,9} proteins may be inserted into the membrane in a sequential process, it seems that to avoid the time and energy costs of many insertion attempts, the membrane must permit the protein to move laterally after insertion, that is, there must be at least short-range membrane fluidity⁹.

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The greening of polar bears in zoos

POLAR BEARS (*Thalarctos maritimus*) normally have creamy-white fur, presumably an adaptation for camouflage in a snowy environment. However, during the summer of 1978, the fur on the back and sides of three adults in the San Diego Zoo turned green, though the animals remained otherwise healthy. (Of these bears, one female was born in the zoo in Calgary, Alberta, Canada, in 1966 and was transferred to the San Diego Zoo in 1969; a second female was born in the wild, having been caught at Spitzbergen in 1951; the third, a male, was born in the San Diego Zoo in 1970.) This phenomenon, though less marked, has been noted in several previous summers, both here and in zoos elsewhere, for example, in Cologne, Germany (C. Hill, personal communication). The coloration was particularly evident on the flanks, on the outer fur of the legs and in a band across the rump: fur on the head and belly and inner sides of the legs was white. We first supposed that the colour was due to green algae such as *Chlorella* or *Scenedesmus* on the surfaces of hairs, growth of such algae being promoted by the presence of nitrogenous wastes in the waters of the bears' pool. (The pool in the exhibit area, which contains 12,500 gallons of tap water, is drained and cleaned twice weekly.) However, microscopic examination of samples of hair taken from the three San Diego bears and from a similarly green polar bear in the zoo at Fresno, California, revealed that this was not so. The outer surfaces of the hairs appeared clean and smooth, except for the normal squamation. The coloration was clearly attributable to the presence of algae inside the hairs, specifically in the hollow medullae of many of the wider (50–200 μm), stiffer guard hairs of the outer coat. (The thinner (<20 μm) and more undulant hairs of the under coat, which were not hollow, were colourless.) Some of the lumina were apparently filled with air, but many of these hollow spaces were partly occupied by masses of small greenish cells, which we describe here.

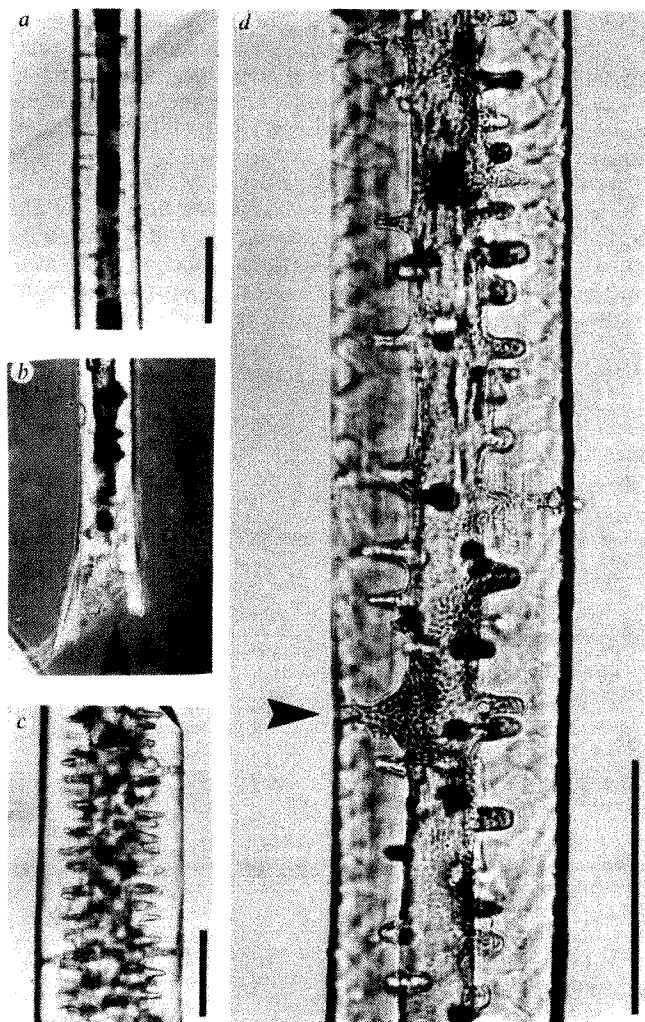


Fig. 1 *a*, Portion of green polar-bear hair, showing medulla containing air cavities and sections filled with algal cells. Note lateral ducts. *b*, Broken end of green polar-bear hair, showing medulla containing algal cells. *c*, Portion of green polar-bear hair, showing medulla with numerous lateral ducts. *d*, Portion of green polar-bear hair, showing medulla with lateral ducts (some communicating with outer surface: arrow) containing algal cells. Phase-contrast; scale bar, 100 μm .

The interior surfaces of the lumina of the narrower guard hairs were smooth. Many of those of intermediate width had pits and channels, more or less perpendicular to the hair axis, extending part of the way to the surface. Some were expanded into chambers parallel with the hair axis, especially near the broken distal end. Those of the stoutest hairs had very many such transverse channels (5–10 μm wide), some extending fully to the outer surface. These features, visible by light microscopy (Fig. 1*a, b*), could be clearly seen in stereoscan electron micrographs of sectioned hairs (Fig. 1*c, d*), which also showed pits in the hair surfaces where some of the side channels opened to the outside (Fig. 2*a, b*).

We have been unable to find any reference to such lateral ducts in the hairs of any mammal¹. Hairs of comparable size from the head, shoulders and flanks of two wild polar bears from Spitzbergen, killed at Isfjord in May 1912 and at Svalbard in July 1967 were white, as were those of three bears obtained this year at L. Churchill, Manitoba: they had similarly hollow medullae but lacked any signs of lateral channels or of algae. (Hairs from four other marine mammals, a sea-otter (*Enhydra*), a northern fur seal (*Callorhinus*), a California sea-lion (*Zalophus*) and a harbour seal (*Phoca*), appeared solid: they too were alga-free.) It is possible that the transverse ducts were formed by keratolytic bacteria, which initially entered the lumina through

broken distal ends. However, in the sections of green bear hairs examined by electron microscopy we saw little evidence of bacterial degradation of the keratin (Fig. 2*c, d*).

The fact that some of these lumina were in connection with the external air or water could explain how the algal cells could have entered the hairs in the first place, and how exchange of O_2 and CO_2 and uptake of water and mineral salts would be facilitated and could permit growth of the algae if suitably illuminated. Indeed, such a habitat has certain advantages, being warm and protected from most kinds of potential predators.

The algal cells from the green zoo bears were spherical, 2–4 μm in diameter. They were clearly prokaryotic; no plastids or nuclei could be distinguished, either by phase-contrast microscopy or in thin sections examined under the electron microscope. They divided by equatorial constriction; no other mode of reproduction was seen. In size and form they resembled *Aphanocapsa montana* Cramer, a blue-green alga in the order Chroococcales, though they appeared more green than blue-green.

Pure cultures were obtained by grinding a washed sample of the green hairs with sterile mineral nutrient medium in a Potter-type homogeniser and streaking a drop of the supernatant algal cell suspension on a 1% agar medium containing mineral nutrient salts². The cells were constantly illuminated at ~2,000 lux from cool-white fluorescent lamps at 27°C. After a week, individual algal colonies were visible under low magnification. Virtually all colonies were of the same size and form, confirming what had already been indicated by microscopic examination of the hairs, that is, that their algal cells were almost all of one type. (Among hundreds of thousands of colonies, only a dozen were of an evidently different kind: a filamentous blue-green alga, similar to *Phormidium*, with cells <2 μm wide.) Apparently clean colonies were selected, transferred to liquid Maertens' medium and illuminated in the same way. When tested on medium supplemented with 0.1% yeast extract, although most were contaminated with bacteria, a few were axenic. Pure cultures were subcultured in Maertens' medium in flasks on a shaker; they grew well, and were comparable in colour, cell size (2.5–4.5 μm), form and fine structure with those observed in the bear hairs (Fig. 3*a*). They required no organic factors for growth. In older cultures, possibly depleted

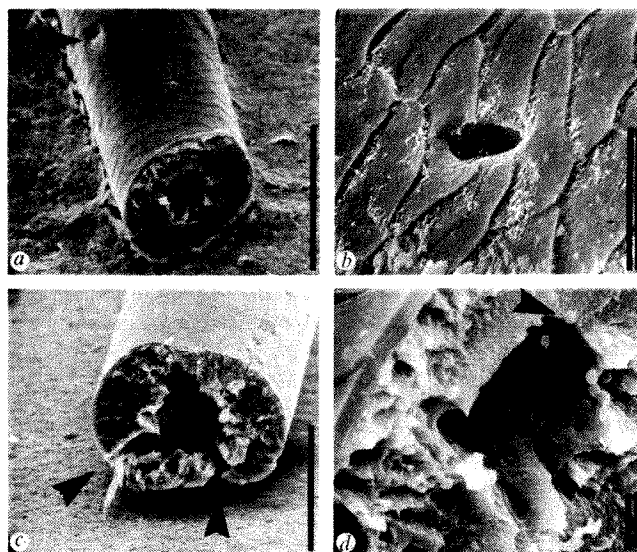


Fig. 2 *a*, Cut end of green polar-bear hair, showing hollow medulla and lateral pore (arrow) among imbricate scales. Stereoscan electron micrograph (SEM); scale bar, 100 μm . *b*, Pore among imbricate scales on surface of polar-bear hair. SEM; scale bar, 100 μm . *c*, Cut end of green polar-bear hair, showing hollow medulla with lateral ducts opening to surface (arrows). SEM; scale bar, 10 μm . *d*, Cut end of green polar-bear hair, showing algal cells (arrow) in lumen. SEM; scale bar, 10 μm .

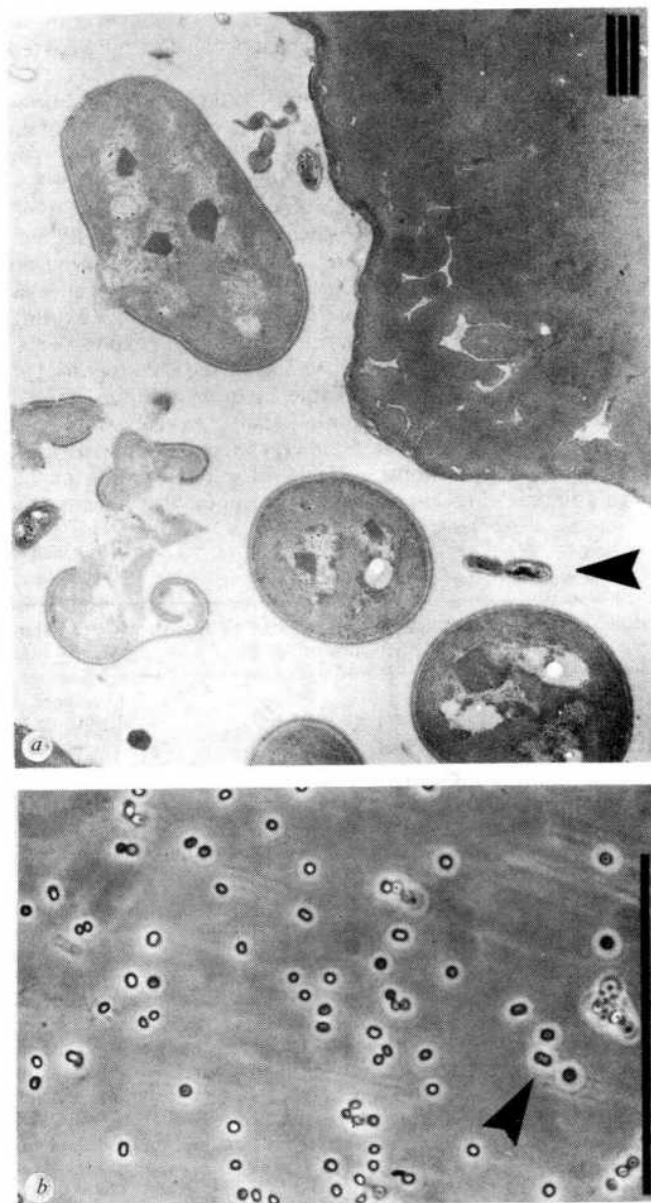


Fig. 3 *a*, Thin section of green polar-bear hair, showing cells of cyanophytes and bacteria (arrow) in medullary space. Transmission electron micrograph (TEM); scale bar, 1 μ m. *b*, Cells of *Aphanocapsa montana* (?), showing division stages (arrow), in clonal culture isolated from green polar-bear hair (San Diego Zoo). Phase-contrast; scale bar, 100 μ m.

of a fixed source of nitrogen, the cells became enveloped in colourless mucilaginous sheaths (Fig. 3*b*) which often developed asymmetrically. Growth was most rapid at 33 °C; above 36 °C the algae did not grow. When tested in a range of salinities they behaved like many freshwater species; at salinities below one-third sea water they grew well, but at higher concentrations growth was inhibited. Although we know of no record of keratin digestion by any alga, we tested for this by incubating sections of white polar-bear hairs with a pure culture of this alga in a mineral medium constantly shaken with air at 27 °C in white light. After 12 weeks, although cells grew in the lumina, we could see no indication of erosion of the hairs.

The cultured algal cells were usually bright green. When extracted with non-polar solvents they were found to contain chlorophyll *a* (but no chlorophyll *b*) and various carotenoids. When grown in white light, the cells produced no detectable phycobilin pigment; but when grown in red light they developed a distinctly bluish tinge and formed appreciable amounts of phycocyanin (tested by *in vitro* absorption and fluorescence spectra). We therefore concluded that the alga is a cyanophyte.

Similar cultures were isolated in the same way from hairs of the green polar bear at Fresno, mentioned above. Apparently the alga commonly infests the hairs of polar bears in zoos. It is a freshwater species, the cells of which probably originate from the pond water in the bears' enclosures and enter the medullary lumina of the guard hairs through broken distal ends or through the patent lateral ducts.

Few other chordates are known to harbour algae on their surfaces. Certain colonial ascidians in the family Didemnidae are associated with prochlorophytes³. Freshwater turtles may bear branched filamentous chlorophytes in the genus *Basiacladia*⁴. Unidentified green algae may colour the pelage of monk seals (*Monachus*)⁵, while brown or red algae (*Ectocarpus*, *Erythrocladia*) have occasionally been found growing on the hairs of fur seals⁶. On two-toed and three-toed sloths (*Bradypus* and *Choleopus*) grooves on the outer surfaces of their guard hairs bear algae of two kinds; filamentous chlorophytes assigned to the genus *Trichophilus* (Chaetophorales) and purple cyanophytes which have been called *Cyanoderma* (Chamaesiphonales)⁷. Several genera of pennate diatoms grow on the skin of various kinds of whales, including the 'sulphur-bottomed' whale⁹. These algae are all quite unlike the species found in hairs of captive polar bears, described here. The African prosimian primate *Galagoides* has been reported to have green algae associated with its fur⁸, but this could not be confirmed.

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Receptor for IgG(Fc) and human β_2 -microglobulin on *S. mansoni* schistosomula

HOST antigen uptake by schistosomes is generally considered from circumstantial evidence to be a potent mechanism by which schistosomes can evade immune response and has therefore stimulated several investigations on the chemical nature of the molecules as well as their possible mode of incorporation¹. These studies have indicated that the parasite can acquire host material in the form of glycolipids of erythrocyte origin² and gene products of the murine major histocompatibility complex³. As an alternative to host antigen incorporation, the possibility of immunological blockade has been suggested. Host immunoglobulins have been demonstrated to be associated with the tegumental surfaces of *Schistosoma mansoni* from mice and baboons, and recent experiments^{4,5} have shown the presence of

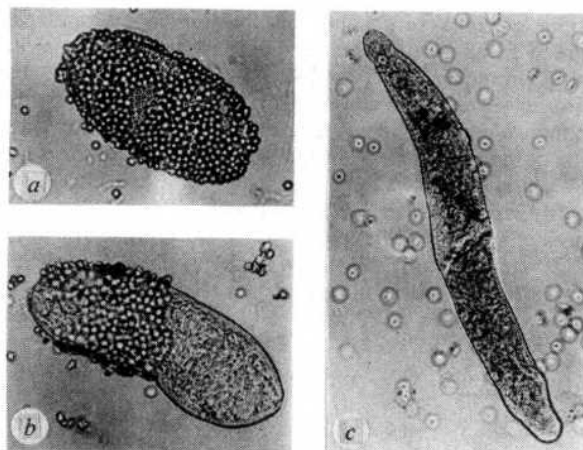


Fig. 1 Photomicrographs ($\times 100$) of characteristic rosette formation (EA) with sensitised sheep RBC on the surface of different life stages of schistosomula. Schistosomula collected *in vitro* after skin penetration show complete rosetting EA (a). Schistosomula obtained after mechanical disruption of the cercarial tail show EA rosettes capped (b) at the anterior part of the parasite. No rosette formation is observed when 5-d-old schistosomula collected by lung recovery are used (c).

heterospecific antibody on the tegumental surface of *S. mansoni* adult worms. The ability of adsorbed antibodies to bind their respective antigens indicates that some of these immunoglobulins are orientated so as to present at least one free Fab region of the antibody molecule. Although this could merely represent a nonspecific adsorption, it could also represent the specific binding of the immunoglobulin on the parasite's surface. Using a rosette test we have now demonstrated the presence of receptors for both IgG(Fc) and human β_2 -microglobulin.

Sheep erythrocytes (RBC) were sensitised with mouse anti-sheep erythrocyte antibody (EA) to form rosettes, after which the cells were washed and placed in contact with schistosomula obtained *in vitro* after skin penetration according to Clegg and Smithers⁶. In other experiments schistosomula were incubated for 30 min with mouse anti-sheep RBC antibody, washed and placed in contact with normal sheep erythrocytes. In these two experimental conditions, rosette formation was observed around schistosomula which seemed to be almost totally coated with erythrocytes (Fig. 1a). When cercariae collected before skin penetration or 5-d-old schistosomula (Fig. 1c) collected by lung recovery in infected mice were used, no rosette formation was observed (Table 1). Schistosomula, obtained without skin penetration, after mechanical disruption of the cercarial tail⁷,

Table 1 Rosette formation at different life stages of *Schistosoma*

	Sensitised sheep RBC	Sheep RBC
Cercariae	—	—
3-h skin preparation schistosomula	+++	—
Artificially transformed schistosomula	++	—
5-day lung schistosomula	—	—
Adult worms	—	—

Sheep erythrocytes stored in Alsever's solution were washed three times with 0.15 M NaCl and suspended to 3×10^8 cells ml^{-1} in phosphate-buffered saline (PBS), pH 7.2. Equal volumes of 1/1,000 diluted mouse anti-sheep erythrocyte serum were added to the red cell suspension and incubated at 37 °C for 60 min. The sensitised cells were washed three times with PBS and used for the EA-rosette formation. 50 washed larvae or 10 adult worms were incubated with 5×10^4 sensitised red cells in a final volume of 1 ml hydrolysate lactalbumin Earle's glucose (HLEG) at 37 °C for 30 min. After gentle resuspension, rosette formation was observed. Larvae or worms surrounded by more than five red cells on the surface were scored as positive. The intensity of the rosetting was graded from ++ (partial erythrocyte binding, >30 RBC) to +++ (totally coated with erythrocytes).

showed a partial erythrocyte binding (Fig. 1b) usually starting at the anterior end of the parasite, which becomes totally coated within 1 h of *in vitro* incubation.

The specificity of the schistosomula binding site for immunoglobulins was assessed with various heterologous proteins of an approximately similar molecular weight. Previous incubation at a concentration of 1 mg ml^{-1} for 30 min at 37 °C with ribonuclease I (Worthington), cytochrome *c* from horse heart (type VI, Sigma) or lysozyme from egg white (grade I, Sigma) did not inhibit rosette formation, whereas inhibition was observed on incubation with immunoglobulin from different animal species (man, rabbit, mouse, rat) (Table 2). Specificity for IgG binding was similarly studied by various inhibition experiments. Incubation of schistosomula with unaggregated (100 $\mu\text{g ml}^{-1}$) or aggregated (1 mg ml^{-1}) IgG (Table 2) from different animal species (man, rat, mouse and rabbit) entirely inhibited subsequent rosette formation, whereas incubation with IgA, IgM or IgE had no inhibitory effect (Table 2). Evidence for the specific binding of IgG to a possible Fc receptor was indicated by two series of experiments.

Table 2 Specific inhibition of EA rosettes by IgG or Fc γ on skin schistosomula

Schistosomula	Sensitised sheep RBC
Non-incubated	+++
Previously incubated with:	
Human, rat, mouse and rabbit Ig	—
Human IgM, IgA, IgE (50 $\mu\text{g ml}^{-1}$)	+++
Unaggregated (100 $\mu\text{g ml}^{-1}$) or aggregated human IgG (1 mg ml^{-1})	—
Fc fragments of human IgG (40 $\mu\text{g ml}^{-1}$)	—
F(ab') ₂ fragments of rabbit anti-human IgG (10 $\mu\text{l ml}^{-1}$)	+++

In each experiment, 50 larvae were preincubated with components adjusted to appropriate concentrations with 1 ml HLEG at 37 °C for 30 min and washed three times with HLEG before addition of sensitised sheep red blood cells. The intensity of the rosetting was graded as in Table 1. The purity of IgA (α I subclass) or IgM each with λ light chains was verified by the Ouchterlony technique. Fc fragments were prepared according to the method of Porter⁹ with papaine, and purified on DEAE-cellulose (0.3 M NaCl). F(ab')₂ fragments of rabbit prepared by pepsin digestion (ratio: 1/50, 10 mg ml^{-1} proteins in 0.1 M sodium acetate, pH 4.5, 24 h, 37 °C) were purified by gel filtration through Sephadex G-200. The purity of fragments was verified by immunoelectrophoresis against specific antisera.

When schistosomula were incubated with Fc fragments of human IgG, total inhibition of rosette formation was observed, whereas incubation with rabbit IgG F(ab')₂ anti-human IgG did not affect erythrocyte binding (Table 2). On the other hand, no rosette formation was observed when schistosomula were incubated with ox erythrocytes sensitised with an F(ab')₂ fraction prepared by pepsin digestion from rabbit anti-ox RBC IgG (Table 3) and with an anti-ox RBC 19S fraction purified from rabbit (Table 3).

Enzyme sensitivity of the Fc receptor was also estimated by rosette inhibition experiments. Fc receptors on schistosomula were found to be trypsin resistant (trypsin TRTPCK, Worthington) (0.5 mg ml^{-1} for 30 min at 37 °C) and pronase (Calbiochem) (0.5 mg ml^{-1} for 30 min at 37 °C) sensitive. Results of binding of radioiodinated IgG to Fc receptors on schistosomula will be published elsewhere (G.T., in preparation).

The experiments reported here indicate the existence on schistosomula of a specific binding site for the Fc portion of IgG possessing the specificity of Fc γ receptors described on eukaryotic cells. The appearance of the above described receptors seems to follow a dynamic process during the early stages of the parasite life cycle. It was observed that although free-living

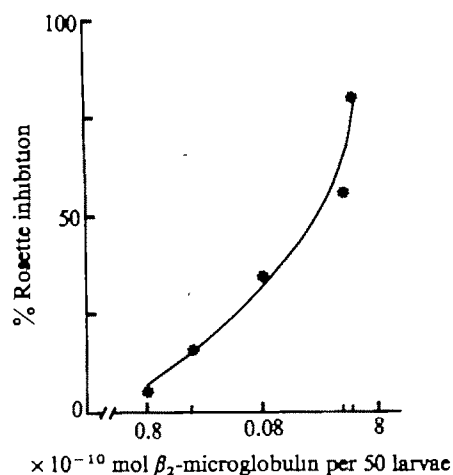


Fig. 2 Inhibitory effect of human β_2 -microglobulin on EA-rosette formation. Schistosomula collected *in vitro* after mouse skin penetration were washed three times with large volumes of HLEG. For use in the inhibition assay, 50 larvae were incubated in 1 ml HLEG with different amounts of β_2 -microglobulin with frequent mixing at 37°C for 30 min. Schistosomula were then washed in HLEG and the rosette formation was observed with sensitised sheep RBC (EA) prepared as described in Table 1. Larvae surrounded by less than five red cells on the surface were counted as negative and their percentages were calculated from at least 30 schistosomula.

cercariae did not express these surface receptors, maximum density of binding sites was observed by rosette formation on 3-h-old schistosomula collected after skin penetration. Strikingly, newly transformed schistosomula collected after mechanical tail disruption showed a progressive appearance of binding starting at the anterior end of the parasite, near the glandular apertures, and extending to the whole body within 1 h of *in vitro* incubation. This dynamic process might be related to the excretion of parasite enzymes known to occur during this period⁶. In the later life stages of the parasite, 5-d-old schistosomula and adult worms no longer express the ability to form rosettes.

The binding of IgG to the Fc receptor on eukaryotic cells is not inhibited by β_2 -microglobulin despite the homology between this molecule and the third domain of the γ chain. As histocompatibility antigens are known to bind to schistosomula³ we investigated whether β_2 -microglobulin interfered with EA rosetting. When schistosomula were first incubated with human β_2 -microglobulin (Pharmacia), total inhibition of rosette formation was observed at a concentration of 5 ng ml⁻¹ for 30 min at 37°C (Fig. 2).

The present results indicate that IgG binds to *S. mansoni* schistosomula. The affinity of the receptor for β_2 -microglobulin suggests two possibilities: (1) a receptor for IgG which, because of the phylogenetic distance between trematodes and mammals presents some unusual features and is mostly for the third domain of the γ chain, or (2), a receptor for β_2 -microglobulin

Table 3 Rosette formation on schistosomula using ox RBC and anti-ox RBC antibody

Ox RBC sensitised with:	Rosetting
Anti-ox RBC IgG (rabbit)	+++
Anti-ox RBC 19S (rabbit)	—
F(ab') ₂ fragments of rabbit anti-ox RBC IgG*	—

Ox erythrocytes stored in Alsever's solution were washed three times with Hank's balanced salt solution (HBSS) and suspended to 2×10^8 cells ml⁻¹ in HBSS. Sensitised ox RBC (EA) were prepared in the usual way with rabbit IgG anti-ox-RBC isolated by column chromatography with DEAE Sephadex A-50, rabbit 19S anti-ox RBC passed through a Sephadex G-200 column and an F(ab')₂ fraction prepared by pepsin digestion from rabbit anti-ox RBC IgG.

binding IgG through the homology with its third domain. In any case, the receptor might be involved in the specific binding of murine major histocompatibility antigens³ on schistosomula in addition to that of IgG. The precise relationship between these two host antigens is still under investigation.

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Demonstration of MHC-specific haemolytic plaque-forming cells

It would be convenient to be able to measure the antibody response to alloantigens of the major histocompatibility complex (MHC) at a cellular level. As MHC alloantigens are expressed on erythrocyte membranes in mice one might suppose that a conventional Jerne plaque assay with enumeration of haemolytic plaque-forming cells (allo-PFC) against a lawn of

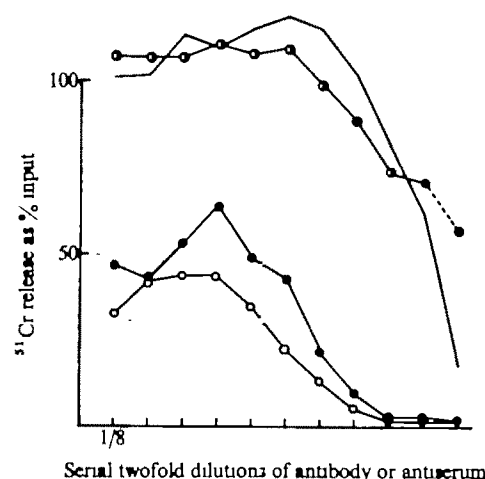


Fig. 1 Synergistic lysis of DA erythrocytes by two monoclonal AO anti-DA alloantibodies, R2/15P (HLGK) and R2/15S (HL) compared with the AO anti-DA antiserum S.006. (—) R2/15P (○) and R2/15S (●) were titrated alone, and R2/15P titrated additionally in the constant presence of a 1/32 dilution (final) of R2/15S (○). Marked synergy between the two monoclonal antibodies is apparent. Their activity together resembles that of the antiserum. Fresh DA erythrocytes were labelled with ⁵¹Cr-Na₂CrO₄ as described previously⁴. Guinea pig serum used as a source of complement at 1/8 (final) was absorbed with 2×10^6 packed rat blood cells before use. The assay mixture was incubated for 1 h at 37°C in V-bottomed microtitration trays.

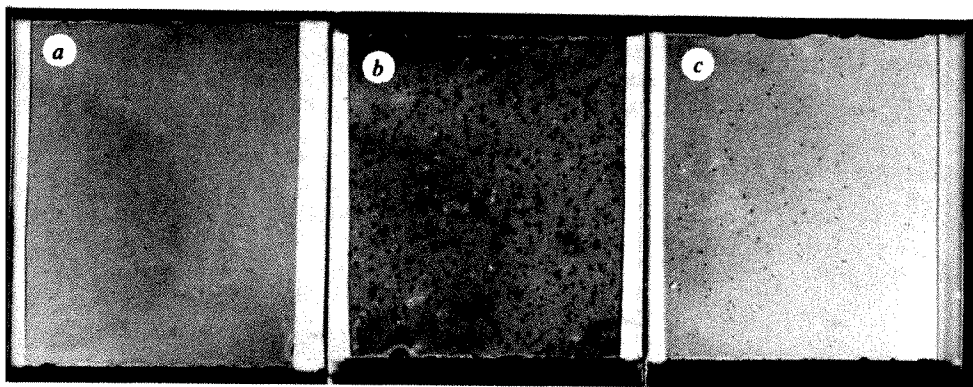


Fig. 2 Plaque formation by spleen cells from an AO rat immunised against DA lymphoid cells (AO 1; see Table 1 legend for immunisation and technical details). 5×10^5 cells per Cunningham chamber: a, No additional reagents added to the assay; b, R2/10P (HL) monoclonal anti-DA (H-1A^a) antibody added at 1/30 final; c, R3/47 (HL) monoclonal anti-DA antibody added at 1/10 final. ($\times 2$.)

suitable allogeneic erythrocytes would be straightforward. In fact such assays have proved difficult, with very few plaques being detected^{1,2}. We have found the same problem in detecting allo-PFC in the spleens of alloimmunised rats. Although alloantisera raised in appropriate strain combinations have a high titre in a direct complement dependent haemolytic assay (Fig. 1) emphasising the potential value of this convenient assay for alloantibody in the rat^{3,4}, very few allo-PFC were found in spleen cell suspensions from hyperimmunised donors. We show here that the discrepancy may be due to the homogeneity of the antibody product of a single PFC compared with the heterogeneity of serum antibody.

During a study of the haemolytic properties of monoclonal anti-rat MHC (H-1) alloantibodies generated by somatic cell hybridisation^{4,5} we observed that of six monoclonal anti-H-1A^a IgG antibodies studied none was able to lyse more than a proportion (<50%) of DA (H-1A^a) erythrocytes at saturation, whereas mixtures of appropriately chosen pairs of monoclonal antibodies would regularly lyse 100% of erythrocytes (Fig. 1). The principal factor limiting cell lysis by a single monoclonal alloantibody was determinant density on the target cell⁵. Recent data demonstrate that erythrocyte populations are heterogeneous in H-1A^a determinant density and that cells with high determinant density are lysed preferentially by a single monoclonal alloantibody (unpublished results). The determinant density dependence of lysis is radically reduced when appropriate mixtures of monoclonal antibodies are used. The definition of 'appropriate' in this context is that the two monoclonal antibodies used should bind noncompetitively to different determinants on the same target molecule. In these conditions, for reasons which are not yet clear, the two antibodies synergise strongly in lytic activity⁵.

The failure to demonstrate allo-PFC in the spleens of hyperimmune donors may therefore have been due to the fact that only a small proportion of erythrocytes was lysed by the monoclonal antibody product of each potential plaque-forming cell resulting in 'plaques' that were too faint to be seen. It therefore seemed likely that if one component of a synergistic pair of monoclonal antibodies was added to the plaque assay system in excess, clear plaque formation would occur around a cell secreting a complement fixing antibody that was not competitive with the exogenous antibody. Spleen cells taken from AO (H-1^a) rats 6 and 7 days after an intravenous boosting immunisation with DA (H-1^a) lymphoid cells showed the characteristic low but measurable allo-PFC activity in conventional Cunningham⁶ plaque chambers. When exogenous monoclonal anti-H-1A^a antibodies were added to the chambers an enormous increase in plaque count was seen (Table 1, Fig. 2). The size of the increment was a function of the monoclonal antibody added: three monoclonal antibodies probably directed against the same determinant⁵ (R2/10P, R2/15P and R3/13) were highly efficient in developing plaques. Monoclonal antibodies directed against another part of the molecule (R2/10S, R2/15S and R3/47) were less efficient. This order has been maintained (with the single exception of AO4) in all animals assayed (Table 1 and unpublished results). It was also noticeable that plaques forming in the presence of R2/10P, R2/15P and R3/13 were larger than those forming with the other antibodies (Fig. 2). It is therefore possible that the differential efficiency is a reflection of differential visibility of plaques. Alternatively, each group of antibodies might allow a distinct subset of the plaque-forming cell response to be seen (that is those producing antibody not competitive with the added monoclonal antibody) and that the subsets are regularly of different sizes.

Table 1 Demonstration of allo-plaque-forming cells using synergistic lysis with monoclonal alloantibodies

Antibody added*	Plaque-forming cells per 10^6 spleen cells						
	Unimmunised AO rats		Immunised AO rats				
	AO(N1)	AO(N2)	1	2	3	4	5
0	<0.1	<0.1	43	97	54	40	20
R2/10P (HL)	—	—	1,950	5,226	3,677	—	—
R2/15P (HLGK)	—	—	2,100	4,548	—	—	—
R3/13 (HL)	0.6	1.8	2,000	3,742	—	480	540
R2/10S (HLK)	—	—	43	742	—	—	—
R2/15S (HL)	<0.1	0.2	825	2,338	2,290	640	240
R3/47 (HL)	—	—	545	1,323	—	—	—
Anti-rat IgG†	—	—	>500	48	26	400	600

AO ♀ rats at 3–4 months old were immunised against DA spleen and lymph node cells by primary subcutaneous and intraperitoneal injection on day 1, followed by an intravenous boosting injection of DA lymphoid cells at day 142 (rat 1), day 43 (rats 2 and 3), or days 43 and 72 (rats 4 and 5). Spleen cells were taken 6 days (rats 1–3) or 7 days (rats 4 and 5) after the i.v. boost, suspended in Dulbecco's phosphate-buffered saline containing 2% fetal calf serum (DAB/FCS), and diluted to an appropriate concentration for assay. Fresh DA erythrocytes were washed in phosphate-buffered saline and diluted to a 25% suspension in DAB/FCS for use. Guinea pig serum absorbed 1:4 with DA blood cells was used as a source of C' at a final concentration of 1/10. Plaque assays were incubated in Cunningham chambers for 60 min at 37°C.

* Monoclonal antibody supernatants were used at final concentrations between 1/10 and 1/30 in the plaque-forming cell assay with the exception of R2/10S on AO 2. In this case the supernatant was concentrated 10-fold by $(\text{NH}_4)_2\text{SO}_4$ precipitation between 50% and 60% saturation and used finally at 1/10, equivalent to undiluted supernatant.

† Anti-rat IgG was a rabbit anti-rat IgG2 antiserum (JH 1/7) used at a final (optimal) dilution of 1/30. Other anti-Ig reagents were also used in some experiments. Plaques detected with anti-Ig reagents were always small and hard to score.

Synergistic lysis by monoclonal antibodies is usually a more efficient method of demonstrating allo-plaque formation than is the addition of heterologous antiglobulin antisera (Table 1). Plaques developed by anti-IgG reagents were usually small and hard to score. This low efficiency is presumably partly responsible for the variations recorded in anti-Ig plaque scores in Table 1. We have no direct evidence that the plaques seen in the synergy experiments are produced by IgG antibody. However, since the synergistic lysis phenomenon is only known by us from IgG monoclonal antibodies, and since the assays were performed relatively late (6 or 7 days) in a secondary or tertiary response it seems probable that the exogenous monoclonal antibodies do in fact reveal IgG plaque-forming cells. It may be that the small numbers of plaques seen without the addition of a synergistic monoclonal antibody represent IgM secreting cells. We do not have an IgM anti-H-1A^a monoclonal antibody but such an antibody would be expected to be less dependent on determinant density for its lytic activity. It may be that the H-1A^a specificities are particularly suitable targets for demonstrating the synergistic development of PFC by monoclonal alloantibody, as there is evidence that these specificities are unusually abundant on erythrocytes of the DA strain⁷. However, haemolytic antisera may be raised against other haplotypes³.

The phenomenon of synergistic lysis explains why only very few allo-PFC could be detected in animals producing high titre haemolytic antisera: a lytic alloantiserum is a synergistic antibody mixture.

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The beige mutation in the mouse selectively impairs natural killer cell function

GERM LINE mutations affecting defined cell populations are often valuable tools in elucidating the function of these cells in complex biological systems such as tumour rejection. We report here that a mutant gene in the mouse called *beige* (*bg*⁺), leads to a complete and selective impairment of naturally occurring killer lymphocytes, whereas all other forms of cell-mediated lysis are apparently normal. The defective gene product may lie within the lytic pathway subsequent to tumour cell contact. Because many cell types, including natural killer (NK) cells, T cells and macrophages, may be involved in tumour resistance *in vivo*¹, these mice will provide a critical test of the hypothesis that it is NK cells which provide a first line of defence against neoplasia². It is likely that this mutant will be invaluable for further investigations in tumour immunology just as the nude mouse has been indispensable in evaluating the role of the

thymus in the development of the T-lymphoid system and the role of T cells in the rejection of tumours.

The *beige* mutation in C57BL/6 mice occurred spontaneously in an autosomal recessive gene on linkage group XIV (ref. 3) and is thought to provide an accurate model of the Chediak-Higashi syndrome in man⁴. Humoral immunity and delayed-type hypersensitivity are normal and the chief cause of mortality is an increased susceptibility to infection⁵ and a lymphocytic infiltration which may be malignant⁶.

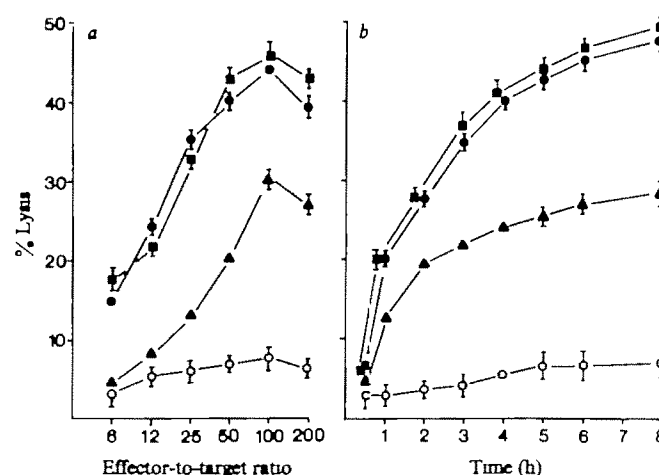


Fig. 1 The absence of NK-mediated cytotoxicity in *beige* mice. Nylon wool column-passed spleen cells from 4-week-old mice were incubated with ⁵¹Cr-labelled YAC tumour cells at various effector-to-target ratios in a 4-h assay¹¹ (a) or for various periods of time at a 100/1 effector/target ratio (b). ▲, A/Sn; ○, C57BL/6, *bg*⁺/*bg*⁺; ●, *+/bg*⁺ littermates; ■, age-matched *+/+* controls. Values represent the mean % specific lysis ± s.e. in quadruplicate wells.

To investigate NK cell function in mutant mice, spleen cells were passed over nylon wool columns, a procedure which enriches for NK cells (Fig. 1). Compared with one of the lowest responder strains known (A/Sn), it can be seen that the homozygous *beige* mutants (*bg*⁺/*bg*⁺) were completely deficient in their ability to lyse YAC cells, a Moloney lymphoma which is the most sensitive NK target yet described. The heterozygous littermate controls (*+/bg*⁺) responded as well as the wild type (*+/+*). The low response of the *bg*⁺/*bg*⁺ mice was not altered by prolonged incubation times or high effector-to-target ratios. In additional experiments (unpublished observations) the absence of NK cytotoxicity was apparent in all the NK-bearing lymphoid organs (spleen, lymph node, bone marrow and peripheral blood) and at all points in the life cycle whether tested against YAC or other NK-sensitive targets (RBL-5, MPC-11, 136-6, P-52, Molt-4). In addition, interferon or interferon-inducing agents (poly I:C, tilorone) failed to augment lysis in *beige* mice (submitted for publication) whereas these agents greatly augmented NK function in low responder strains such as A/Sn and in very young or old mice of the same strain (Gidlund, personal communication). Therefore, the deficient NK response in *beige* mice cannot be accounted for by an altered tissue distribution, age-dependent maturation or a shift in target selectivities. It was then relevant to consider if the defect in cytotoxicity was selective for NK cells or whether it involved a general failure of all forms of cell-mediated lysis.

As shown in Table 1 (lines 4-6), cytotoxic T lymphocytes generated by alloimmunisation of *+/bg*⁺ or *bg*⁺/*bg*⁺ mice *in vivo* or by one-way mixed lymphocyte cultures (MLC) *in vitro* were equally capable of lysing P815 cells (a completely NK-insensitive target). In addition, spleen cells from *bg*⁺/*bg*⁺ mice alloimmunised *in vivo* responded to secondary challenge in MLC cultures by mounting a strong response on day 3. Peak responses in primary MLC cultures were not obtained until days 5 or 6. Further experiments revealed identical kinetics of cytotoxic

Table 1 The selectivity of the NK defect

Effector cell*	Generation	Target†	Maximum lysis	Lytic units per 10 ⁶ ‡
1 +/bg ^j NK	None	YAC	53 (100/1)§	10.1 ± 0.8
bg ^j /bg ^j NK	None	YAC	5 (100/1)	0.0 ± 0.0
2 +/bg ^j ADCC	None	CRBC	45 (100/1)	1.8 ± 0.2
bg ^j /bg ^j ADCC	None	CRBC	48 (100/1)	2.0 ± 0.1
3 +/bg ^j ADCC	None	P815	25 (50/1)	8.3 ± 0.4
bg ^j /bg ^j ADCC	None	P815	2 (50/1)	0.0 ± 0.0
4 +/bg ^j CTL	<i>In vivo</i>	P815	55 (100/1)	8.3 ± 0.6
bg ^j /bg ^j CTL	<i>In vivo</i>	P815	47 (100/1)	7.8 ± 1.1
5 +/bg ^j CTL	1 st MLC	P815	60 (25/1)	41.4 ± 2.7
bg ^j /bg ^j CTL	1 st MLC	P815	64 (25/1)	50.2 ± 3.8
6 +/bg ^j CTL	2 nd MLC	P815	66 (25/1)	86.5 ± 4.9
bg ^j /bg ^j CTL	2 nd MLC	P815	80 (25/1)	99.1 ± 6.0
7 +/bg ^j CTL	Con. A	P815	85 (100/1)	7.8 ± 2
bg ^j /bg ^j CTL	Con. A	P815	70 (100/1)	5.0 ± 1
8 +/bg ^j Macrophage	<i>In vitro</i>	P815	30 (4/1)	33.5 ± 2.3
bg ^j /bg ^j Macrophage	<i>In vitro</i>	P815	25 (4/1)	28.7 ± 2.1

Lines 1, 2, 3: spleen cells from untreated mice were tested in a 4-h ⁵¹Cr-release assay against normal YAC targets (Moloney lymphoma from A/Sn, H-2^{kd}) or P815 cells (DBA/2 mastocytoma, H-2^{dkd}) and chicken erythrocytes treated with a predetermined optimum concentration of specific antibody (1/80 A.BY × C57Bl/6 F₁ anti-A/Sn and 1/200 mouse anti-CRBC, respectively). P815 and CRBC targets treated with normal serum or untreated yielded <2% lysis. Line 4: mice were injected intraperitoneally on day -10 with 30 × 10⁶ P815 cells (passage 25 *in vitro*) and pooled spleens were assayed in a 2.5-h ⁵¹Cr-release assay. Lines 5, 6: spleen cells from untreated mice or mice immunized *in vivo* with P815 (line 4) were cultured with mitomycin C-treated DBA/2 spleen cells at a 5/1 responder/stimulator ratio. Primary (1st) and secondary (2nd) cultures were collected on days 5 and 3, respectively, and assayed in a 2.5-h test. Non-stimulated control cultures yielded <15% lysis of P815 and stimulated cultures failed to lyse RBL-5 cells (H-2^{dkd}). Line 7: mice were injected intravenously at -24 h with 250 µg Con A and spleen cells were assayed in a 16-h ⁵¹Cr-release test in the presence of 10 µg ml⁻¹ Con A. Con A did not affect target cell survival and was necessary for maximum lysis *in vitro* as described previously. Line 8: bone marrow cells were cultured as previously described and on day 3, non-adherent cells (promonocytes) were removed and cultured with lymphokine derived from the supernatants of 24 h, Con A-stimulated C57Bl/6 spleen cells. The 'activated' macrophages which adhered to glass were cultured for 16 h with ⁵¹Cr-labelled P815 target cells. Non-activated control cultures yielded <5% lysis.

* Cytotoxicity by effectors designated as CTL was inhibited over 90% by anti-θ serum and guinea pig complement whereas NK cells were inhibited <10%. Neither CTL nor NK was affected by pretreatment with carbonyl iron and a magnet. Incorporation of heat-aggregated mouse gamma globulin (1–500 µg ml⁻¹) into the cytotoxic assay blocked 70% of ADCC against chicken erythrocytes (CRBC) but had no effect on lysis by NK cells, CTL or ADCC against P815. All responses of age-matched +/+ mice were similar to those of heterozygous littermate controls, +/bg^j, and therefore the wild-type values have been omitted for clarity.

† All targets were maintained by continuous *in vitro* culture except CRBC.

‡ A lytic unit was defined as the number of effector cells required for 50% maximum lysis and was calculated from parallel effector titration curves over the entire range of lysis from 0 to maximum.

§ The values given represent the mean % lysis in quadruplicate wells at the effector/target ratio given in brackets. These levels of lysis were designated as the maximum attainable, as they occurred on the plateau of effector titration curves.

T-lymphocyte generation in MLC cultures and the kinetics of target cell lysis in the cytolytic assay also overlapped (unpublished data). In addition, the normal level of lysis in *beige* mice by T cells activated with concanavalin A (Con A) indicates that the total pool of cytotoxic T cells is normal, as this technique is thought to measure all polyclonally distributed killer T cells regardless of their specificity⁸. These results strongly suggest that T cells from *beige* mice are capable of exerting normal cytolytic function.

Tumour cell destruction by other cells types also seemed normal in *beige* mice (Table 1, lines 8, 9). Bone marrow-derived macrophages activated *in vitro* with a lymphokine⁹ lysed P815 cells, as did peritoneal macrophages when activated with lipopolysaccharide¹⁰ (unpublished observation). Antibody-dependent cell-mediated cytotoxicity (ADCC) against antibody-coated chicken erythrocytes (CRBC) was also normal in *beige* mice (Table 1, line 2). As ADCC against CRBC in the mouse is mediated largely by Fc⁺, adherent cells^{11,12}, this population seems to be normal. In addition, the killing capacity of monocytes and granulocytes may also be normal in *beige* mice, as these effectors are involved in human ADCC against CRBC¹³. ADCC against tumour cells, however, is mediated by different lymphocytic effectors called K cells¹. As shown in Table 1, line 3, the function of these cells is completely deficient in the homozygous *beige* mice; this provides the strongest evidence that murine K cells and NK cells are identical, as suggested by Ojo and Wigzell¹². These workers showed that antibody-coated, NK-insensitive P815 cells competed with non-antibody-coated, ⁵¹Cr-labelled YAC cells in a cytolytic assay¹². However, murine NK and K cells directed against tumour cells do not possess readily detectable Fc_γ receptors, as direct lysis of antibody-coated P815 cells is not inhibited by aggregated gamma globulin, as reported earlier¹¹ and confirmed here (Table 1). It is possible, therefore, that non-antibody-dependent contact with a target

triggers the expression of Fc receptors in the NK cell which can then kill by either an antibody-dependent or antibody-independent mechanism, depending on (1) the availability of antibody on the target cell and (2) the NK sensitivity of the target.

The detailed nature of the NK defect in *beige* mice is not known, but may lie within the lytic machinery of the cell rather than at the level of population size or recognition receptors. As shown in Table 2, nylon-passed spleen cells from *bg^j/bg^j* mice were completely normal in their capacity to bind to YAC targets. We have previously shown that this assay is a reliable measure of the frequency of NK cells in a mixed population^{14,15}. The target-binding lymphocytes from *beige* mice were selective,

Table 2 The frequency of target-binding cells in *beige* mice

Genotype	Preincubation of effectors*	% Target-binding cells
C57Bl/6 (+/+)	None	20.4
	NK-TS	8.1
C57Bl/6 (+/bg ^j)	None	19.8
	NK-TS	9.2
C57Bl/6 (bg ^j /bg ^j)	None	21.3
	NK-TS	7.9
A/SN	None	7.6
	NK-TS	3.4

Nylon wool column-passed spleen cells from age and sex matched mice were labelled with fluorescein and incubated with a 10-fold excess of YAC cells. The number of lymphocytes binding to YAC was then determined by counting 500 cells in an ultraviolet microscope¹⁴. Additional spleen cells were preincubated for 1 h at 4 °C with 1 µg of 140 K glycoprotein isolated from YAC membranes and purified by SDS-polyacrylamide gel electrophoresis¹⁶. The inhibition of subsequent binding to intact YAC was dose dependent and was not observed when similar concentrations of purified H-2^{kd} antigen was used. NK-TS, natural killer cell-target structure, a glycoprotein isolated from YAC cells¹⁶.

as they could be inhibited by preincubation with solubilised and purified NK target antigens from YAC but were not inhibited by H-2 antigen prepared in the same manner, as previously documented in other strains¹⁶. Further experiments are necessary to localise the defective gene product in the lytic pathway subsequent to target cell contact.

In conclusion, we have described a genetic impairment in the lytic machinery of NK cells which totally prevents these cells from initiating antibody-dependent or antibody-independent cytotoxicity of tumour cells. This defect was selective for NK cells, as lysis by immune T cells, macrophages and cells participating in ADCC against a non-tumour target was normal. Additional experiments, designed to test oncogenesis and spontaneous tumour development in these mutant mice, will provide a critical test of the hypothesis that NK cells are involved in surveillance against neoplasia.

The data showing normal macrophage killing in *beige* mice has recently been confirmed and extended by Marie-Luise Lohmann-Matthes.

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Amyloid resistance in A/J mice is determined by a single gene

WIDESPREAD tissue deposition of the unique fibrous protein amyloid occurs in a variety of human diseases¹. 'Secondary' amyloidosis arises as a complication of chronic infectious or inflammatory disease and an identical syndrome can be induced in mice by repeated injections of casein². Little genetic work has been done in this area, probably because development of amyloidosis is often variable, even with a standard induction regimen, and results are inconsistent³. Furthermore, because of the many complex immunological aberrations occurring during the

amyloid induction period⁴, the various experimental manipulations which can affect it⁴, and the wide range of induction times seen in different murine strains², one would expect that multiple genes and alleles would influence amyloidogenesis. Nevertheless, the discovery of the uniquely resistant A strain⁵ suggested the possibility of isolating at least part of the genetic basis for amyloid susceptibility or resistance. We now report a genetic analysis of amyloid induction in two inbred strains, CBA/J and A/J, differing markedly in susceptibility to amyloidosis⁵. We show for the first time that a single gene confers resistance on the A/J strain. Our results imply that genetic factors may also define subpopulations of chronic disease patients who are at greater risk from amyloid disease.

Table 1 No. of mice in each group resistant to a 6-week period of amyloid induction by azocasein

Group	No. tested	No. resistant
CBA/J	20	0
(CBA × A) _{F1}	20	0
A/J	20	20
F ₁ × A/J	43	19

Although we have generally used casein in our experimental protocols, the more potent, predictable amyloidogen azocasein⁶ was chosen for these experiments. We find that five subcutaneous injections per week of 10% azocasein induces amyloid in 100% of the highly susceptible CBA/J strain within 2 weeks. Induction time has been consistent with different batches of azocasein and in different experiments, in contrast to our experience with casein. F₁ hybrid mice were produced by mating CBA/J females with A/J males. Preliminary work established that although all CBA/J mice had amyloidosis after 2 weeks of azocasein, 3-4 weeks were required to achieve a 100% incidence in F₁ hybrids. In contrast, the earliest appearance of amyloid in A/J mice was at 7 weeks, with a less than 30% incidence after 8 weeks of injections. We tentatively concluded that although the F₁ heterozygous mice had a slightly prolonged amyloid induction period, the marked resistance of the A/J strain was a recessive trait.

For the definitive genetic experiment, females of the F₁ hybrid group were mated to A/J males. CBA/J, F₁ hybrid, backcross and A/J mice were subjected to serial azocasein injections, five per week, and congo red-stained spleen sections were then scored for the presence or absence of amyloid. F₁ hybrid mice were studied after 2 or 4 weeks of injections. An induction period of 6 weeks was selected for backcross mice, as this is well beyond the period required to induce a 100% incidence of amyloid in F₁ hybrid mice, but too soon to produce amyloid in any A/J mice. Groups of A/J mice were studied after 6, 7 or 8 weeks of injections. Under this protocol we expected that only those backcross mice with resistance similar to that of the A parent would remain free of amyloid. The percentage of amyloid-resistant mice in the backcross group would allow an estimate of the number of genes involved in determining resistance. The results are shown in Table 1. In agreement with preliminary experiments, 100% of CBA/J mice had amyloid when studied at 2 weeks and 100% of F₁ hybrids had amyloid at 4 weeks. After 6 weeks, no amyloid was found in A/J mice, although one of six A/J animals had amyloid after 7 weeks (not shown), demonstrating that 6-7 weeks is the threshold for amyloid induction in the A strain in these laboratory conditions. In the backcross group, 19 of 43 animals were resistant to 6 weeks of azocasein injections. This is close to the 50% incidence of amyloid resistance expected if it were the expression of a single gene, and significantly greater than the 25% incidence expected for a trait requiring two genes.

In contrast to other groups, there was a different incidence of amyloid in males and females of the backcross group; 13 of 22

females but only 6 of 21 males were resistant (data not shown). In addition, male spleens contained histologically more amyloid than female spleens. A simple explanation for this is that males of this group in particular demonstrated considerably more fighting than other groups of mice. As wounds from fighting alone are a major cause of amyloidosis in male mice⁷, and as inflammation correlates with amyloidosis⁸, the biting and clawing of injection sites observed in these mice would be expected to increase amyloid production. Therefore, the incidence of resistance in 13 of 22 female mice probably represents the 'true' incidence.

We are not proposing that a single genetic locus alone governs susceptibility to amyloid. The genetic data presented here, however, demonstrate that a single gene with a major influence on amyloidogenesis can account for the extreme degree of resistance seen in the A strain, compared with CBA mice.

The mechanism of amyloid resistance remains unknown, but several possibilities are suggested by previous work. The inducing agent casein has been demonstrated to be a polyclonal B-cell activator in the susceptible CBA strain⁹. Although genetic analysis of this B-cell response was not carried out, the genetics of B-cell response to lipopolysaccharide, another amyloid-inducing agent, has been extensively studied; a single gene has been shown to account for variation in macrophage and B-cell responsiveness to this agent¹⁰. The role of B-cell activation in amyloid production is not known, but differences in B-cell function during amyloid induction in CBA and A mice have been demonstrated¹¹. Thus, a single gene determining B-cell responses to azocasein is plausible by analogy to other systems.

A very different hypothesis is suggested by recent work on the cellular degradation of the AA protein, a major amyloid component, and SAA protein, its serum precursor¹². Although three different patterns of breakdown of SAA were observed, one particular pattern of enzymatic breakdown was found in all amyloid patients but only some normal controls¹². If a single enzyme system involved in degradation of SAA protein is indeed a critical factor in amyloidogenesis, then a single genetic locus could very well have a major influence on amyloid susceptibility, as suggested by the data presented here.

The present data do not allow us to choose between these or other hypotheses accounting for the unusual amyloid resistance of the A/J strain mouse. However, the demonstration that a single genetic locus may be the basis for the resistance offers the hope of elucidating one of the critical steps in the pathogenesis of amyloid.

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Histamine-coated particles generate superoxide (O_2^-) and chemiluminescence in alveolar macrophages

We have recently shown that histamine bound to red cells forms 'rosettes' with various guinea pig leukocytes and that these 'histamine receptors' are particularly well expressed on the alveolar macrophage¹. As the lung is a major site of histamine storage and high concentrations of histamine are present in the sputum in certain respiratory diseases such as chronic bronchitis and asthma^{2,3}, we have performed experiments to determine whether free histamine, or histamine bound to particles, interacts with its membrane receptor to initiate the respiratory burst in alveolar macrophages. Activation of the respiratory burst in phagocytic cells is achieved by a number of agents, both soluble

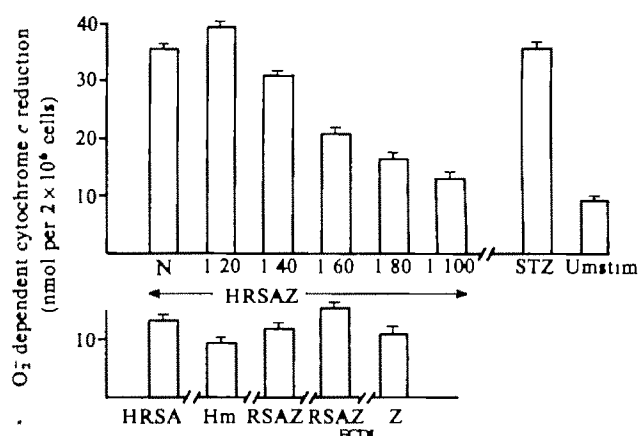


Fig. 1 The generation of O_2^- by alveolar macrophages activated with histamine bound to zymosan (HRSZ). Guinea pig alveolar macrophages, obtained by broncho-tracheal lavage, were purified by centrifugation on Ficoll-Hypaque, washed three times with the balanced salt solution (BSS⁺⁺) described previously⁴ and adjusted to a concentration of 2.4×10^6 cells ml^{-1} . This procedure was repeated if there were any contaminating granulocytes. These procedures gave alveolar macrophages of 90-95% purity which were free of granulocytes and red cells, the contaminating cells being virtually all lymphocytes. The cells (0.8 ml) were incubated with 0.1 ml of the various agents indicated or with BSS⁺⁺ to obtain the resting or unstimulated (Unstim.) values. Following addition of cytochrome c (100 nmol in 0.1 ml) in the presence or absence of superoxide dismutase (4.2 nmol in 50 μ l), the mixtures were incubated at 37°C for 30 min, transferred to an ice-bath and centrifuged at 1,000g for 5 min. The O_2^- activity was measured as cytochrome c reduction (calculated from the decrease in absorbance at 550 nm on addition of potassium ferricyanide as described previously⁵) using a Pye Unicam SP 1800 UV spectrophotometer. Any reduction occurring in the presence of superoxide dismutase was subtracted from these values. Histamine dihydrochloride (1.4 g) was conjugated to 200 mg rabbit serum albumin (HRSA) with 1.2 g of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (ECDI) contained in 20 ml phosphate buffered saline (PBS) and extensively dialysed as described by Kedar and Bonavida¹⁰. For neat (N) HRSZ, zymosan (10 mg) was incubated with 1 ml of HRSA for 45 min at 37°C, washed twice and resuspended in 1 ml of PBS at pH 7.2. Dilutions of HRSA from 1 in 20 to 1 in 100 were also incubated with 10 mg of zymosan and similarly incubated and washed. For HRSA alone, zymosan was omitted; for RSAZ, histamine and ECDI were omitted and histamine was omitted in the preparation of RSAZ_{ECDI}. Serum treated zymosan (STZ) was prepared by incubating 1 ml fresh guinea pig serum with 10 mg zymosan and incubating and washing as for HRSZ. Hm, histamine dihydrochloride (10^{-3} mol l^{-1}), Z, zymosan (10 mg in 1 ml PBS). When unstimulated cells or cells with treatments were incubated in the presence of superoxide dismutase, values obtained were less than 2.00. Each test was performed in duplicate and the results represent the mean $\pm$ 1 s.e.m. of four experiments.

and particulate, many of which bind to the plasma membrane (for example, particles opsonised with complement⁴) through cell surface receptors. This suggests that there may be a close association between 'membrane recognition' of certain exogenous stimuli and the enzyme which initiates the primary oxygen-consuming reaction (also thought to be membrane bound and probably 'NADPH oxidase'⁵). In the experiments reported here superoxide anion formation (O_2^-) and chemiluminescence have been measured as these are recognised features of the respiratory burst of phagocytic cells⁵.

O_2^- generation by alveolar macrophages was measured by dismutase-inhibitable cytochrome *c* reduction. When histamine in free solution was incubated with purified alveolar macrophages, free of other phagocytic cells, the amounts of O_2^- formed were no more than those obtained with resting (unstimulated) cells. In contrast, when histamine was bound to particles such as zymosan (a yeast cell-wall extract) large amounts of O_2^- were generated. The binding of histamine (H) was achieved by first coupling the amine to rabbit serum albumin (RSA) (see Fig. 1) and then allowing this HRSA conjugate to adsorb on to the particle. Histamine coupled in this way is known not to be substantially altered chemically⁶. Also, we were able to show that bound histamine retained its biological activity in terms of its capacity to contract the isolated guinea pig ileum. The results shown in Fig. 1 were with zymosan although several other particles which absorbed the coupled histamine also generated O_2^- . These included a cross-linked dextrose gel (Sephadex G-10), a bead-formed agarose gel (Sephacrose 4B), a cross-linked polystyrene polymer (Amberlite XAD-2) and latex (polystyrene) beads. By themselves, or following addition of RSA alone, these particles did not generate appreciable amounts of O_2^- , indicating that the observed effect was independent of the chemical properties of the carrier and was a result of the histamine conjugated to RSA. The O_2^- formed was directly proportional to the quantities of HRSA offered to the particles (Fig. 1). Zymosan which had adsorbed a 1 in 20 dilution of HRSA generated as much O_2^- as zymosan incubated with fresh serum. Serum (as a source of complement) coated zymosan (STZ) is known to be particularly effective in initiating the respiratory burst in many phagocytic cells, including the alveolar macrophage⁷. When alveolar macrophages were incubated with the various controls shown in Fig. 1, the amount of O_2^- generated was similar to that in unstimulated cells. These results indicate that the observed effect was attributable to bound histamine and not to the zymosan alone or zymosan coated with the carrier protein in the presence (RSAZ_{BCDI}) or absence (RSAZ) of the coupling reagent (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide). Of these controls RSAZ_{BCDI} gave the highest values but this was not significantly different from RSAZ, HRSA, Hm or Z.

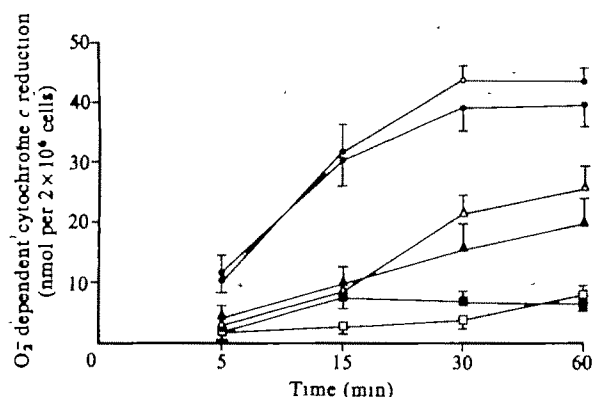


Fig. 2 The time course of generation of O_2^- by alveolar macrophages activated with histamine bound to zymosan (HRSAZ). STZ (○), HRSAZ (●), RSAZ (△), HRSA (▲) and Hm (■) (10^{-3} mol l⁻¹) were prepared as in Fig. 1. □, unstimulated (resting) cells. Each time point was performed in duplicate and results represent the mean $\pm$ s.e.m. of four experiments.

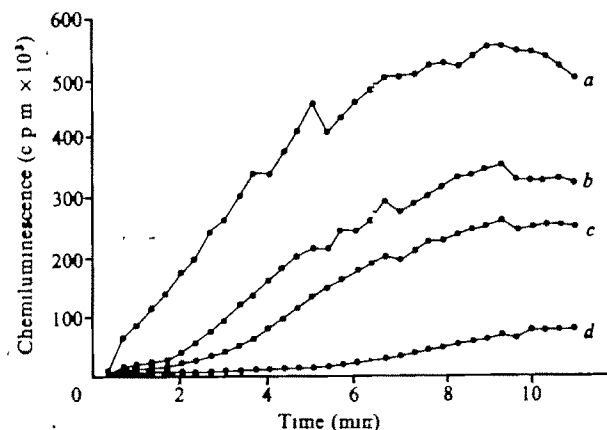


Fig. 3 Chemiluminescence by alveolar macrophages activated with histamine bound to zymosan (HRSAZ). HRSAZ (0.1 ml) was incubated with 0.5 ml of 0.2 mM solution of luminol (Sigma) dissolved as described¹¹, and contained in BSS⁺⁺. Two million alveolar macrophages contained in 0.4 ml were added to this mixture and the counting commenced immediately. Experiments were performed in polypropylene minivials (Searle) contained in glass scintillation vials with pre-bored screwcaps (Koch-Light) which were pre-heated at 37 °C for 13 h. All manipulations were performed in the dark at 37 °C. Counts were recorded at room temperature in a Beckman LS-250 liquid scintillation counter, operating in the 'out of coincidence' mode¹², using a minimal iso-set module adjusted to detect approximately the lower third of the tritium energy spectrum. The counting mode was set to record infinite sequential, 0.2 min total count readings at a present error of 0.2%. HRSAZ dilutions: a 1:10; b, 1:20; c, 1:40; d, 1:80. The figure is representative of six experiments. Peak luminescence with the controls, HRSAZ+superoxide dismutase, RSAZ_{BCDI}, RSAZ, HRSA, RSA, Z, Hm (10^{-3} mol l⁻¹) and unstimulated gave values <73 c.p.m. $\times 10^3$. STZ gave peak luminescence of 617 c.p.m. $\times 10^3$.

The time course of O_2^- production by alveolar macrophages stimulated with histamine bound to zymosan by the RSA conjugate (HRSAZ) is shown in Fig. 2. This was compared with STZ, RSAZ, free histamine, the histamine conjugate in the absence of particles (HRSA) and the amounts formed by unstimulated macrophages. Maximal O_2^- generation with HRSAZ and STZ was achieved within 30 min whereas with RSAZ and HRSA the amounts detected were much less and did not 'plateau', even after 1 h. The relative differences between O_2^- produced by alveolar macrophages following activation by the various agents and the amounts produced by unstimulated cells were similar to those obtained in Fig. 1. These experiments (Figs 1 and 2) indicate that bound histamine is comparable to bound complement in its capacity to initiate the respiratory burst in alveolar macrophages and that the effects are both dose- and time-dependent.

We were also able to show that O_2^- production by bound histamine was an H_1 - and not an H_2 -receptor dependent effect. This was determined in seven experiments in which the alveolar macrophages were preincubated for 30 min with the appropriate drugs at various dilutions. The H_2 -receptor antagonists, burimamide and metiamide, at doses of 5×10^{-5} , 2×10^{-5} and 0.8×10^{-5} mol l⁻¹, produced insignificant inhibition of O_2^- production, the values being $8.3\% \pm 3.0$, $9.5\% \pm 2.9$, $10.5\% \pm 3.8$, and $3.4\% \pm 1.7$, $2.2\% \pm 1.6$, $8.4\% \pm 4.3$, respectively. In contrast, the H_1 -antagonists, chlorpheniramine and mepyramine at the same concentrations, gave linear dose-dependent inhibitions of $22.3\% \pm 3.8$, $13.8\% \pm 3.6$, $10.7\% \pm 4.4$ and $24.4\% \pm 5.0$, $19\% \pm 4.8$, $12.8\% \pm 3.3$, respectively. The inhibition produced by the highest doses of the H_1 -receptor antagonists were highly statistically significant ($P < 0.005$). The drugs had no significant effect on the resting values of O_2^- production or on O_2^- generated by STZ indicating that HRSAZ probably initiates the respiratory burst by the H_1 -dependent histamine receptor that we previously described using the 'rosette technique'¹.

Chemiluminescence, which also accompanies the respiratory burst of phagocytic cells⁵, was initiated by histamine bound to zymosan. As shown in Fig. 3 this effect was both time- and dose-dependent. This experiment was repeated five times and gave similar results. Chemiluminescence induced by HRSAZ or STZ was completely inhibited by superoxide dismutase indicating that light emission by these cells was closely associated with O_2^- production.

These results suggest that there may be a direct relationship between histamine and lung macrophages. We have established that histamine, when bound to a number of different particles, initiated the respiratory burst in these cells. The response, like the 'histamine rosettes' described previously¹, was H_1 -receptor dependent.

Although histamine alone was without effect it is known to bind to a number of plasma proteins ('histaminopexy'⁶) and so our observation may be of significance both physiologically, in the regulation of oxygen-dependent microbial killing by lung macrophages, and in disease states such as asthma and chronic bronchitis^{2,3}. In these latter situations high sputum concentrations of histamine may adversely influence the well recognised defensive role of the alveolar macrophage and so contribute to respiratory infections.

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Anti-inflammatory steroids induce biosynthesis of a phospholipase A_2 inhibitor which prevents prostaglandin generation

ASPIRIN prevents prostaglandin (PG) generation by directly inhibiting the cyclo-oxygenase enzyme responsible for PG biosynthesis¹⁻³. In addition, there is now conclusive evidence that anti-inflammatory steroids can also prevent PG generation⁴⁻¹³. Unlike the aspirin-like drugs, steroids have no anti-cyclo-oxygenase activity but exert their action by preventing the release from phospholipids of the fatty acid substrates required for PG biosynthesis^{4-9,12,13}. We have shown that stimulation of thromboxane A_2 (TXA₂) release by agents such as histamine, 5-hydroxytryptamine and rabbit aorta contracting substance-releasing factor (RCS-RF) (but not arachidonic acid) is inhibited

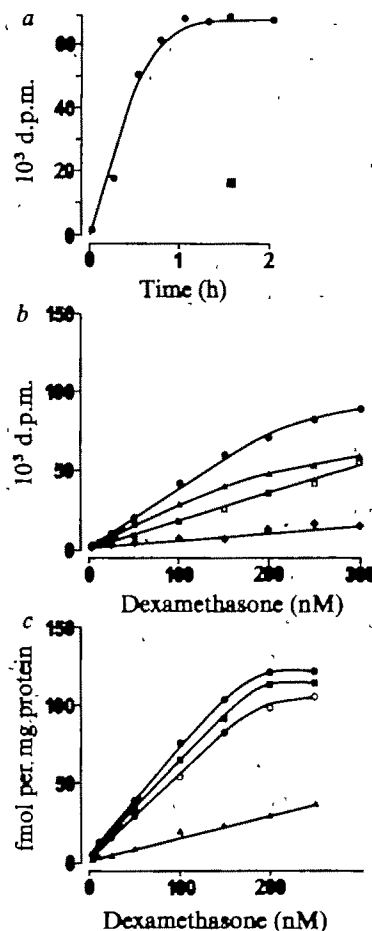


Fig. 1 Male guinea pigs (250-300 g) were killed by cervical dislocation and exsanguination, and lungs were removed and washed in ice-cold saline and then homogenised in 10 ml Tris buffer (pH 7.5, 20 mM containing 2 mM $CaCl_2$, 1 mM Na_2MoO_4 and 1 mM $MgCl_2$). The homogenate was centrifuged at 100,000 g for 90 min at 2 °C. The supernatant was retained on ice until use, (usually within 2-3 h of preparation). Protein content was estimated by the Biuret method. The binding assay used reaction sets of 0.1 ml Tris buffer (20 mM pH 7.5), 0.3 ml cytoplasmic fraction, 0.05 ml unlabelled steroid or saline control and 0.05 ml ^3H -dexamethasone, incubated for 2 h at 4 °C. Unbound dexamethasone was adsorbed by adding acid-washed Norit charcoal (5 mg) and vortex mixing for 20 s. Charcoal was removed by centrifugation (10,000 g for 10 min). Supernatant (0.3 ml) was removed for estimation of bound dexamethasone using liquid scintillation counting. **a**, Total binding of dexamethasone by lung supernatant as a function of time. ^3H -dexamethasone (20 pmol) was used for this experiment; binding was maximal by 90 min, also shown as the uptake in the presence of 10^{-5} M unlabelled dexamethasone. **b**, Total binding of dexamethasone by lung supernatant (ordinate) as a function of the free steroid (abscissa). Curves represent one of four experiments. Binding varied between lungs, and although results were clear at <300 nM dexamethasone, the binding above this concentration became erratic and difficult to reproduce. The 'control' curve is derived from samples containing only ^3H -dexamethasone. The ^3H background curve is derived from samples containing no cytoplasmic protein. The other curves show displacement of the ^3H -dexamethasone from the receptor by cortisone (10^{-5} M) and 10^{-5} M unlabelled dexamethasone; this concentration gave a maximal displacement of the labelled drug. The protein concentration was 6.2 mg ml⁻¹. **c**, 'Specific' binding of dexamethasone is defined here as that binding resistant to the addition of 10^{-5} M unlabelled drug, and is calculated by subtracting the binding in the presence of 10^{-5} M dexamethasone from the binding found in its absence. Figures are expressed as fmol dexamethasone bound per mg cytoplasmic protein. Also shown is the displacement of the binding curve by 10^{-7} M dexamethasone, 10^{-6} M hydrocortisone and 10^{-5} M cortisone. Results are calculated from an experiment similar to that shown in **b**. The binding varied between experiments and a range of 100-400 fmol dexamethasone per mg cytoplasmic protein was observed in the four experiments carried out. ●, Control; ■, dexamethasone; △, cortisone; ○, hydrocortisone; ♦, ^3H background.

by anti-inflammatory steroids, and that their potency in this action closely parallels their anti-inflammatory activity^{12,13}. Furthermore, their mechanism of action involves the inhibition of phospholipase A_2 activity, and thus of arachidonate release within the lung^{12,13}. In several other tissues, the mechanism of steroid hormone action depends on the combination of the

steroid with a cytosolic receptor protein, the translocation of this drug-receptor complex to the nucleus and the initiation of protein biosynthesis¹⁴⁻¹⁶. We now show that similar events occur in the guinea pig perfused lung before inhibition by steroids of phospholipase A₂ activity (and thus TXA₂ generation). We have discovered a steroid-induced factor which mimics the anti-phospholipase effects of these anti-inflammatory agents.

Dexamethasone was used for most of the experiments, although results were confirmed with triamcinolone and hydrocortisone. Dexamethasone was used because it is a potent inhibitor, available as the soluble sodium phosphate salt (Decadron, Merck), and can be obtained in labelled form ([1,2(n)³H]dexamethasone, 39 Ci mmol⁻¹, Radiochemical Centre). We used this tritiated steroid to look for a steroid receptor protein in lung cells. The method was that of Baxter and Tomkins¹⁷ (see Fig. 1) which shows that there is a time-dependent, saturable 'specific' binding of labelled dexamethasone by the crude cytosol fraction of lung homogenate. This binding was reduced or prevented by unlabelled dexamethasone (10⁻⁷-10⁻⁵ M), higher concentrations (10⁻⁶-10⁻⁴ M) of the weaker anti-inflammatory steroid hydrocortisone, or by the 'anti-glucocorticoid' corticosterone (10⁻⁶-10⁻⁵ M), which binds to other steroid receptors¹⁸. The binding capacity of the cytosolic supernatant was abolished by pretreatment with papain (100 µg ml⁻¹ protein), or by heating the preparation to 40 °C for 60 min. The bound steroid could be removed from the binding protein by vigorous shaking with *n*-heptane. The radioactivity thus liberated co-chromatographed with authentic dexamethasone on TLC (developing solvent, chloroform:methanol:water, 9:1:0.1, v/v), indicating that no metabolism of dexamethasone had occurred. These results show that dexamethasone is bound to a receptor protein in one or more cell types in the lung.

In the second series of experiments, we investigated the activity of actinomycin D (an inhibitor of RNA but not protein biosynthesis)¹⁹, cycloheximide (an inhibitor of protein and RNA biosynthesis)²⁰ and puromycin (an inhibitor of protein biosynthesis)²⁰ on the steroid-induced suppression of phospholipase activity. Guinea pig isolated lungs were prepared²¹, and perfused with oxygenated Krebs' solution (37 °C) at 10 ml min⁻¹. First, we measured the release from the perfused lung of TXA₂ in response to stimuli (such as RCS-RF) which act through phospholipase activation¹². TXA₂ release was detected by a superfused rabbit aortic strip, and the TXA₂ generated by a standard stimulus was compared with that produced when a known amount of sodium arachidonate was injected through the lung. Infusion of steroids through the lung selectively abolishes stimuli which act through phospholipase activation without affecting arachidonate stimulation. Thus, the ratio between the two responses is a measure of phospholipase activity^{12,13}.

Second¹³, a specifically labelled phospholipid (2-[³H]-oleoyl]phosphatidylcholine) was injected through the lung (with a small amount of [¹⁴C]-oleic acid to act as an internal standard). Free oleic acid liberated by the action of phospholipase A₂ within the pulmonary vascular bed is measured by liquid scintillation counting of the extracted effluent. The hydrolysis of this phospholipid is suppressed by steroids and the results closely parallel those obtained by bioassay^{12,13}. Figure 2a shows that in the untreated lungs, dexamethasone (1 µg ml⁻¹) markedly reduced phospholipase activity as estimated by the release of TXA₂ or by hydrolysis of the radioactive phosphatide. This inhibition was reversed after the steroid infusion was discontinued, and responses had fully recovered after 1 h (not shown). Figure 2b-d shows that cycloheximide (2 µg ml⁻¹), actinomycin (1 µg ml⁻¹) or puromycin (5 µg ml⁻¹) either abolished or reduced the inhibitory effect of the steroid. Indeed, a potentiation of phospholipase activity was sometimes detected. The blockade of the inhibitory activity of steroid by inhibitors of RNA or protein synthesis implies that the action of the steroids depends on a transcriptional as well as a translational step. Cycloheximide pretreatment of the lung did not diminish the number of steroid receptors in cytosolic preparations.

Having demonstrated that corticosterone displaced dexamethasone from the cytoplasmic receptor, we tested its effect in an intact perfused lung. Corticosterone (25 µg ml⁻¹) given with or during an infusion of dexamethasone (1 µg ml⁻¹) reduced the inhibitory effect of the latter on phospholipase activity (as estimated on both biological and radiochemical assays) by up to 85%. Corticosterone itself had no intrinsic activity at these concentrations.

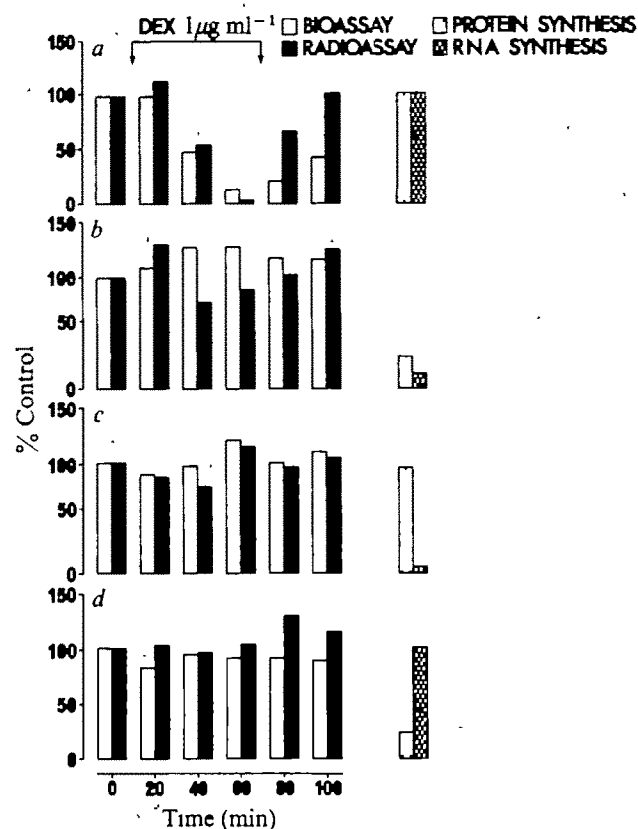


Fig. 2 The effect of dexamethasone (1 µg ml⁻¹) on lung phospholipase activity and the release of TXA₂, and the abolition of this action by protein and RNA synthesis inhibitors. The guinea pig isolated perfused lungs were prepared, and the bio- and radioassays carried out as described^{12,13}. All data have been reduced to the same format and are expressed as a per cent change from the control response. For the bioassay the anti-phospholipase activity was calculated as a decrease in the ratio between the release of TXA₂ by a standard dose of RCS-RF and a standard dose of arachidonic acid. Phospholipase activity is expressed as the per cent change in hydrolysis (pmol oleic acid released) occurring on injection through the lung. The data shown here represent one of 3-5 experiments. Protein and RNA synthesis was estimated by infusing 0.150 µCi [¹⁴C]-lysine or 12.0 µCi [³H]-uridine into a perfused lung over 1 h. Some lungs received saline only, others actinomycin (1 µg ml⁻¹), cycloheximide (2 µg ml⁻¹) or puromycin (5 µg ml⁻¹). After 1 h the lung was chopped with scissors and homogenized in a glass homogenizer. The proteins and nucleic acids were precipitated with 5 vol ice-cold 1 M perchloric acid and the precipitate was sedimented in a centrifuge. This precipitate was washed twice more with 5 vol 0.5 M perchloric acid and the precipitate was sedimented in a centrifuge. This precipitate was then washed with 5 vol ethanol:ether (1:1) and the final pellet solubilized with concentrated NaOH. The radioactivity was estimated by liquid scintillation counting after neutralization with acetic acid, and the protein content was estimated by the Biuret reaction. Results are expressed as per cent changes from control d.p.m. per mg protein. a, Control; b, + 2 µg ml⁻¹ cycloheximide; c, + 1 µg ml⁻¹ actinomycin; d, + 5 µg ml⁻¹ puromycin.

Clearly, steroids act in the perfused lungs in much the same way as they act to produce metabolic effects in other tissues. Could this effect be produced by stimulating the biosynthesis of an inhibitory factor, perhaps a polypeptide? We have now demonstrated the presence of such a factor using perfused, cycloheximide-treated lungs which are thus resistant to the inhibitory action of steroids. First, effluent from a perfused lung (generator lung) was re-oxygenated and pumped directly through another perfused lung (test lung) which also received

cycloheximide ($2 \mu\text{g ml}^{-1}$) to render it insensitive to steroids. Anti-inflammatory steroids were infused either through the generator lung (TL) or into the effluent leaving the generator lung (IE). Thus, the only difference in the effluent perfusing the test lung was whether or not the steroid had passed through the first lung. Bioassay or radiochemical assays were as described earlier.

When steroids were infused into the test lung (Fig. 3) they did not affect phospholipase activity. When steroids were infused into the generator lung, however, phospholipase activity of the test lung (estimated by either technique) was greatly reduced. Similar results were obtained when the steroids were perfused TL first and then changed to IE. In this case the depressed phospholipid hydrolysis and responses to RCS-RF returned when the steroids were infused IE.

Second, two lungs were perfused simultaneously, one received steroid TL the other IE. The only difference in the perfusates was that in one case the steroid had perfused through the lung and in the other it had gone directly into the effluent. After storage at 4°C overnight or for longer periods of time, the flasks of effluent were re-oxygenated and alternately perfused through a lung receiving cycloheximide. Some results obtained with this technique are shown in Fig. 4. When perfused with fresh Krebs' solution, or the effluent containing steroid-infused IE, there was no inhibition of phospholipase activity, but when perfused with effluent from steroid-treated lungs the basal phospholipid hydrolysis was depressed or abolished and the responses to RCS-RF attenuated. Return to normal occurred when the steroid was omitted from the ordinary Krebs' solution or when effluent was used from lungs where steroids were infused IE.

No metabolism of dexamethasone occurred in the lungs, as infusing the tritiated drug, extracting it from the Krebs' solution with *n*-heptane and analysing the product by TLC showed that all the radioactivity co-chromatographed with authentic dexamethasone. Triamcinolone acetonide and hydrocortisone hemisuccinate gave similar effects.

We conclude that the anti-phospholipase action of steroids is induced by a mechanism essentially similar to that mediating their other metabolic actions. First, the steroid combines with a receptor located in the cell cytoplasm of one or more cell types in the lung. The amounts of steroid required to saturate the receptors in the lung (10–20 pmol per mg protein) are higher than those reported for some other tissues (see refs 17, 22), and

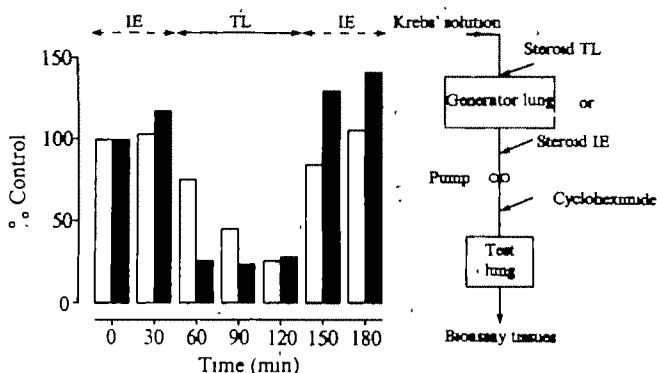


Fig. 3 A factor induced by steroids is released into the effluent of the lung and can have an anti-phospholipase effect in a second lung. Two lungs were perfused in series as shown. The re-oxygenated effluent from the 'generator lung' was pumped into the test lung which also received cycloheximide ($2 \mu\text{g ml}^{-1}$) to prevent the action of steroids. Dexamethasone ($1 \mu\text{g ml}^{-1}$) was infused either directly into the effluent from the first lung (IE) or first through the generator lung (TL). During the equilibration period the steroid was infused IE and there was no change in the activity of the enzyme; however, when infused TL there was an immediate fall in phospholipase activity of the second lung showing that a factor was formed in the first lung which had an anti-phospholipase effect in the second. When the steroid was again perfused IE the phospholipase activity returned to control values. Similar results were obtained in five other experiments. $\square$, Bioassay; $\blacksquare$, radioassay.

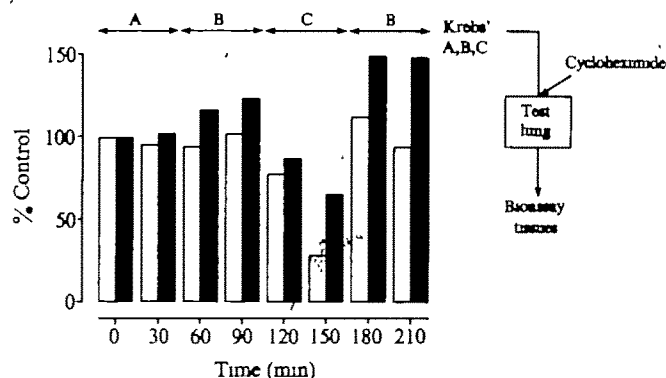


Fig. 4 Steroids induce the biosynthesis of a stable anti-phospholipase factor. The assay was with a single perfused lung receiving cycloheximide ($2 \mu\text{g ml}^{-1}$) to prevent the action of steroids. The lung is perfused with one of three batches of Krebs' solution: a, normal Krebs' solution, b, the lung effluent to which dexamethasone ($1 \mu\text{g ml}^{-1}$) had been added, c, the effluent from a lung in which dexamethasone ($1 \mu\text{g ml}^{-1}$) had passed through the lung. Only during an infusion of solution c was there a fall in the phospholipase activity of the recipient lung. The steroid-induced factor was stable in Krebs' solution at 4°C for at least 7 but less than 14 d. $\square$, Bioassay, $\blacksquare$, radioassay.

the actual binding (fmol per mg protein) is much less. Perhaps there are more receptors per mg lung protein, or they have a much lower affinity than those of other tissues studied. Scatchard plots of the binding data were non-linear, perhaps indicating the presence of more than one receptor population. Certainly, combination with the receptor is important for the action of steroids, as cortisone displaced labelled dexamethasone from the receptor and blocked the inhibitory action of this steroid in the perfused lung system. Combination of cortisone with the steroid receptor may form an inactive complex¹⁸.

Our experiments do not show whether combination of the steroid with the receptor is followed by translocation of this complex to the nucleus. However, the subsequent genetic expression does occur in lung tissue and this involves transcription as well as translation, for inhibitors of RNA as well as of protein biosynthesis block the steroid effect. The disparity of chemical structure and site of action suggests that these inhibitors exert their effect in this system by an action on the RNA/protein synthetic machinery. Interestingly, actinomycin D blocks the anti-inflammatory effect of steroids *in vivo*²³. (During preparation of this paper, Danon and Assouline²⁴ reported findings similar to some of ours in rat renal papillary tissue.)

Clearly, in perfused lungs, steroids induce the synthesis of a factor which blocks phospholipase A_2 and this factor can be transferred from one perfused lung to another. Presumably, it is a peptide or protein, as its biosynthesis is prevented by cycloheximide. The biosynthesis of this factor may be crucial to the anti-inflammatory effect of steroids. We have observed the close relationship between the anti-phospholipase activity and the anti-inflammatory activity of corticosteroids¹². There is considerable evidence linking the generation of prostaglandins with inflammation, pain and fever (see ref. 25), and several other products of fatty acid oxidation such as 12-hydroxy-5,8,14-eicosatetraenoic acid²⁶ or malondialdehyde²⁷ could also be pro-inflammatory or cytotoxic. Furthermore, slow-reacting substance A may be derived from arachidonic acid^{28,29}. The non-steroid aspirin-like drugs block the generation of cyclooxygenase products^{1,30} but because the substrate fatty acids are rate limiting (see refs 12, 13), the anti-inflammatory steroids can prevent the biosynthesis of a whole cascade of lipid mediators, so that the steroid-induced anti-phospholipase factor could be important in controlling the inflammatory response. If this is so, steroids would not have an anti-phospholipase effect in cells which do not contain steroid receptors.

Our discovery that the action of steroids in this tissue depends on the continued biosynthesis of protein and RNA also means

that these drugs do not exert their effect by 'membrane stabilisation'. We do not yet understand how the steroid-induced factor inhibits the phospholipase and what (if any) other pharmacological actions this factor has. Some phospholipases require 'activation' before catalysis can proceed; in some cases this involves the removal of a peptide protecting the active centre³¹. One effect of the steroid-induced factor could be to mimic the action of this peptide. Whatever the mechanism of action, the discovery that the anti-phospholipase activity of steroids is mediated by a soluble 'second messenger' is likely to contribute to the understanding of the anti-inflammatory action of these drugs.

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Stimulatory effect of vanadate on (Na⁺ + K⁺)-ATPase activity and on ³H-ouabain-binding in a cat heart cell membrane preparation

VANADATE has been reported to inhibit (Na⁺ + K⁺)-ATPase activity in membrane preparations from dog kidney¹ and in red cells². This inhibition, which occurs in the nanomolar concentration range in the presence of high Mg²⁺ concentrations and in the micromolar range at physiological Mg²⁺ concentrations^{2,3} is specific as other ATPase enzymes are not affected in these conditions¹. Therefore vanadate has been suggested to be 'an ideal specific regulator' of (Na⁺ + K⁺)-ATPase¹. We have shown⁴ that vanadate has a distinct positive inotropic effect in cat papillary muscle. It has been claimed that the positive inotropic action of cardiac glycosides, which are specific inhibitors of (Na⁺ + K⁺)-ATPase activity^{5,6}, is associated with the

inhibition of that enzyme. If that is so, vanadate would be expected to inhibit (Na⁺ + K⁺)-ATPase, in a concentration-dependent manner, in parallel with its effect on force of contraction. To test this hypothesis, we measured the effect of vanadate on (Na⁺ + K⁺)-ATPase activities and on ³H-ouabain-binding in identical conditions in a membrane preparation taken from cat hearts that were also used for experiments on contraction. We report here, however, that vanadate may stimulate (Na⁺ + K⁺)-ATPase activity transiently and ³H-ouabain-binding permanently in these conditions.

After the papillary muscles had been dissected from the cat hearts⁴, remaining ventricular tissue was frozen quickly and kept at -60 °C for up to 4 weeks until further use. After thawing, (Na⁺ + K⁺)-ATPase (EC 3.6.1.3.) was prepared from 12 hearts as described by Pitts and Schwartz⁷ but without glycerol treatment. Enzyme activity was determined by the coupled optical assay in an ATP-regenerating system⁸. It was measured as 0.32 U mg⁻¹ membrane protein; 97% of total ATPase activity could be inhibited by ouabain (10⁻⁴ M). One enzyme unit is defined as 1 μmol ATP hydrolysed per min at 37 °C. Protein was determined by the method of Lowry *et al.*⁹.

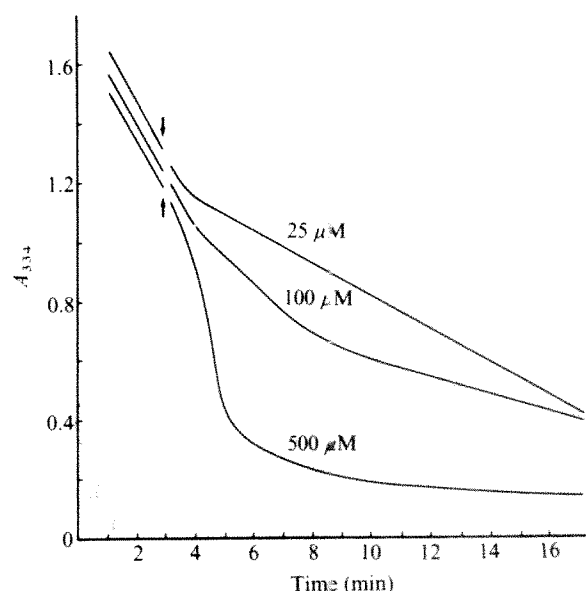


Fig. 1 Stimulatory and inhibitory effect of vanadate on (Na⁺ + K⁺)-ATPase activity. Compounds were dissolved in H₂O daily. Incubation medium was the same as that used for the determination of force of contraction⁴ and contained (mM): NaCl, 136.9; KCl, 5.4; CaCl₂, 1.8; MgCl₂, 1.05; NaH₂PO₄, 0.42; glucose, 5.5 (buffered with NaHCO₃ to pH 7.4); total volume was 2 ml. Enzyme protein (1 mg) was incubated in this medium for 120 min at 37 °C in each test tube with different concentrations of NH₄VO₃ (Merck, Darmstadt). Then 0.2 ml of this mixture was assayed rapidly for ATPase activity in the coupled optical assay⁸, followed by continuous recording photometrically for 20 min at least. The time of addition of NH₄VO₃ is marked by arrows.

The effects of NH₄VO₃ on (Na⁺ + K⁺)-ATPase activity were measured as described in the legend to Fig. 1. A typical photometric recording is shown in Fig. 1. Enzyme activity is followed continuously by the degradation of NADH at 334 nm. The disappearance of NADH is related stoichiometrically to the hydrolysis of ATP in the coupled optical assay⁸. Soon after addition, NH₄VO₃ in a final concentration of 25 μM inhibited (Na⁺ + K⁺)-ATPase about 65%. Higher concentrations initially inhibited the enzyme appreciably less (100 μM) or even stimulated it above control values for several minutes. We refer to this as 'transient initial stimulation'. For instance, 500 μM stimulated the enzyme 180% for about 3 min (Fig. 2). But after about 5 min it caused progressive inhibition. This rather strange effect

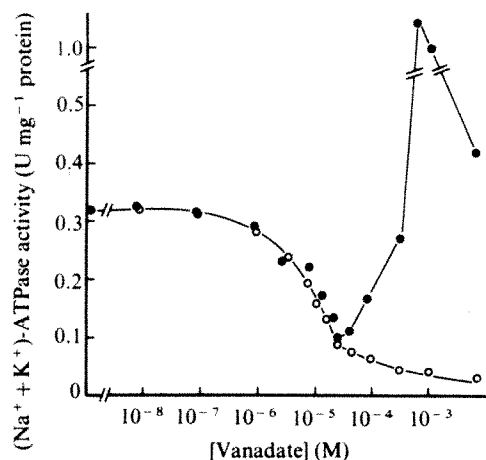


Fig. 2 Concentration-dependent effect of vanadate on $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ activity. Cat heart ventricular cell membranes (1 mg protein) were preincubated for 120 min in medium, as described in the legend to Fig. 1, to which was added increasingly large amounts of NH_4VO_3 , total volume 2 ml each. After 120 min, 0.2 ml of this medium containing enzyme protein was assayed rapidly for ATPase activity in the coupled optical assay with an ATP-regenerating system⁸ at the same vanadate concentrations as during preincubation. ●, The highest initial ATPase activity, measured usually after 3 min (Fig. 1); ○, The lowest activity measured within 20 min. Vanadate concentrations above 30 μM cause an initial transient stimulation, followed by inhibition. Similar results were obtained in three other experiments.

was not due to the action of NH_4VO_3 on the enzymatic reaction in the coupled optical assay (phosphoenolpyruvate, pyruvate kinase, lactic dehydrogenase, NADH) because the incubation medium alone (without $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ -containing cell membranes), or with heat-denatured cardiac cell membranes, did not respond in this way to the addition of NH_4VO_3 in the respective concentrations.

In a second set of experiments cat heart ventricular membranes were preincubated for 120 min in the same incubation medium used for the determination of force of contraction in the cat papillary muscles⁴ (as described above), with and without increasingly large concentrations of NH_4VO_3 (0.005–2.500 μM) at 37 °C. After 120 min an aliquot of membranes was assayed for $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ activity by the coupled optical assay⁸ (Fig. 2). The results reveal a concentration-dependent inhibition of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ activity up to 25 μM NH_4VO_3 , half-maximal at 5 μM . An initial transient stimulation was seen at the same concentrations as in the absence of preincubation, starting at 50 μM and becoming maximal at 500–1,000 μM . Further experiments showed that preincubation had no effect unless ATP was present, thus suggesting that vanadate is bound

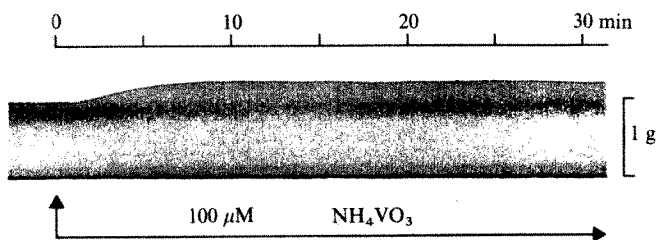


Fig. 3 Time course of the positive inotropic effect of 100 μM NH_4VO_3 in an electrically driven cat papillary muscle, mounted for the recording of isometric contractions, as described previously⁴. NH_4VO_3 was added at zero time and was present throughout the experiment. Frequency of stimulation, 0.2 Hz; temperature, 35 °C, extracellular Ca^{2+} concentration, 1.8 mM; preparation diameter 0.8 mm.

by $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ only if it is enzymatically active. This agrees well with the demonstration by Cantley *et al.*³ that vanadate exerts its effect at the inner side of the erythrocyte cell membrane, where the enzymatically active site is said to be located⁶.

$(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ activity was stimulated again by 50 μM NH_4VO_3 , reaching maximal stimulation at 500–1,000 μM . Previous experiments have demonstrated the positive inotropic effect in the same concentration range⁴. Thus, there was a correlation between the transient stimulatory effect of vanadate on $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ activity and the positive inotropic effect when both experiments (that is with papillary muscles and with membrane preparation) involved tissue from the same heart assayed in identical media. Such a correlation did not apply to the inhibitory effect of vanadate.

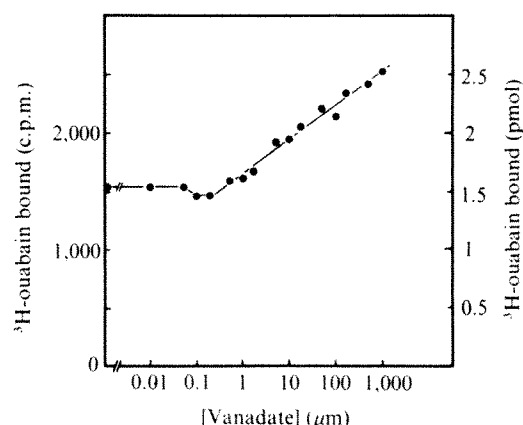


Fig. 4 Effect of vanadate on ^3H -ouabain-binding to cat cardiac cell membranes. Cat heart ventricular cell membranes (0.17 mg protein) were incubated for 120 min in medium, as described in the legend to Fig. 1, to which was added 3 mM ATP (Boehringer, Mannheim), 6 p mol ^3H -ouabain (specific activity 14.4 Ci mmol⁻¹, lot no. 500-183, New England Nuclear, Dreichenheim) and the indicated concentrations of NH_4VO_3 . ^3H -ouabain bound to the membranes was determined after rapid filtration (Whatman GF/C glassfibre filters). The radioactivity on the filters was determined by liquid scintillation counting¹⁰. Unspecific binding of ^3H -ouabain to the membranes was determined after addition of unlabelled 10^{-4} M ouabain. It was always less than 3% of total ^3H -ouabain-binding and was subtracted from the amount of total bound radioactivity. Similar results were obtained in two other experiments.

The reasons for the apparent discrepancy between our results and reports that vanadate exclusively inhibits $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ from various sources may be either the use of the phosphate determination method^{1,2,11} (which does not facilitate continuous monitoring of the hydrolysis of ATP) or the determination of enzyme activity by the coupled optical assay after equilibrium had been established ('after... the time curve became linear')³. It is possible that the initial transient stimulation of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ activity by vanadate has been overlooked by other investigators. This effect was not unique to the cat heart enzyme, however. Similar results were obtained with $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ preparations from horse and human hearts (not shown). Furthermore, in accordance with previous reports³, the concentrations of vanadate needed to inhibit the enzyme were much lower in the presence of high concentrations of Mg^{2+} (10 or 20 mM). NH_4Cl alone did not affect ATPase activity in the conditions used. Further addition of 20 mM NH_4Cl caused no measurable change in the effects of vanadate on enzyme activity. Results were the same with NH_4VO_3 or Na_3VO_4 , which had the same positive inotropic action.

The biological significance of the transient stimulatory and steady-state inhibitory effects of vanadate on $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ activity is not clear. Transient stimulation occurred at

the same concentration range as did the inotropic effects⁴. Both began rather rapidly (1–2 min) after the application of NH_4VO_3 , but the former lasted only for a few minutes, whereas the latter remained virtually stable for at least 30 min, which was the longest time investigated (Fig. 3). Perhaps the stimulatory effect triggers the inotropic effect. Of course both the stimulation and the inhibition of the enzyme might be an artefact due to the disruption of the cardiac cell membrane. However, the transient stimulatory effect of vanadate concentrations that also increase the force of contraction might be related to the reported stimulation of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ by very low but positive inotropic concentrations of cardiac glycosides^{6,12,13}. That effect is controversial⁶, however, although recent studies have suggested stimulation of the active transmembranous transport of cations after addition of low but positive inotropic concentrations of cardiac glycosides^{14–16}.

It is well established that the enzyme protein containing $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ activity spans the cell membrane and specifically binds cardiac glycosides with high affinity on the outside of the cell membrane⁶. Cardiac glycosides supposedly exert their positive inotropic effect after binding to the enzyme^{6,17}. Thus, it seemed reasonable to examine the effect of vanadate on specific ^3H -ouabain-binding to $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ of the cat ventricular cell membrane preparation in the same conditions. Figure 4 shows that vanadate at concentrations greater than $0.5 \mu\text{M}$ stimulates ^3H -ouabain-binding in a concentration-dependent manner. Further experiments (not shown) demonstrated that this effect was due exclusively to an increased affinity of the binding sites for ouabain as the number of binding sites stayed constant. Cantley *et al.*^{3,18} have shown, however, that vanadate does not interact with ouabain-binding sites but rather with the ATP-binding sites of the enzyme at the inner side of the cell membrane. Thus the increased binding of ouabain to $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ seems to be an indirect effect of vanadate in certain conditions.

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Thyrotropin-releasing hormone: central site of action in antagonism of pentobarbital narcosis

THYROTROPIN-RELEASING HORMONE (TRH) is capable of antagonising pentobarbital narcosis, and this effect is independent of its hypophysiotropic function^{1,2}. The potency of intraventricular injections^{2,3} and the localisation of TRH in nerve terminals from both hypothalamic and extrahypothalamic sites^{4,5}, suggest that TRH may function as a neuromodulator in the central nervous system (CNS)⁶. Although TRH antagonism of pentobarbital has been behaviourally and electroencephalographically^{7,8} characterised, the underlying neuroanatomical substrate remains unknown. In an attempt to identify the central substrate mediating this effect, we have used intracerebral microinjection of TRH into specific brain loci. Brain sites were chosen to represent large neuroanatomical regions—as cholinergic activation has been hypothesised to mediate pentobarbital antagonism by TRH, structures belonging to the cholinergic activating system⁹ were examined, and we also examined medially located brain regions that Wei *et al.*¹⁰ found to be most active at inducing TRH-mediated shaking. While a number of sites were found responsive at 500 ng of TRH, the septal region proved exceptionally sensitive by responding significantly at only 5.0 ng TRH.

Male Sprague-Dawley rats (230–330 g at the time of surgery) were individually housed under a 12-h light-dark cycle. The animal was allowed at least 7 days to recover, following surgery and a minimum of 5 days between administrations of drug. Using pentobarbital anaesthesia (50 mg per kg body weight intraperitoneally), each animal was bilaterally implanted with guide cannulas (26 gauge) in 2 or 19 brain loci (four cannulas per animal). Implantations were made to a depth of 1 mm above designated injection sites, and, where applicable angled to avoid ventricular penetration. Injections were made with a 33-gauge needle connected to a $1\text{-}\mu\text{l}$ Hamilton syringe by polyethylene tubing (PE-10). A small air bubble was introduced into the tubing to give a visible indication of fluid movement. Total injection volumes were $0.5 \mu\text{l}$ per hemisphere administered over a 30-s period, and 10 s were allowed to elapse before removal of the needle. Test animals were injected intraperitoneally with 40 mg per kg pentobarbital. At 20 min after loss of righting reflex, TRH (Abbott) dissolved in 0.9% saline, or an equal volume of saline alone, was bilaterally injected. After intracranial injection, the animal was placed on its side, and return of righting reflex recorded when the animal had righted itself three times in 60 s. Cannula location was verified histologically. Before intracardiac perfusion with 10% formalin,

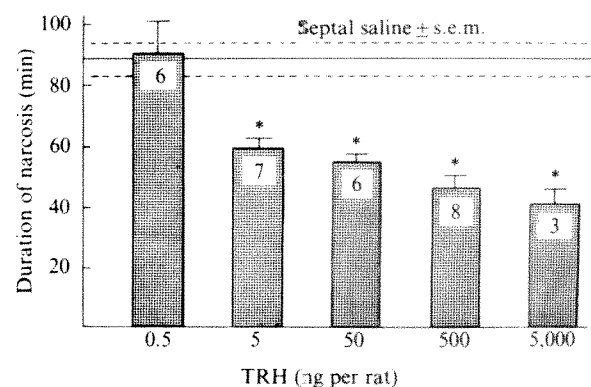


Fig. 1 Dose-response curve for intraseptal TRH antagonism of pentobarbital narcosis. the number of animals tested at each dose is shown in each column. * $P < 0.001$ compared with septal saline, two-tailed Student *t*-test.

Table 1 Effect of intracerebral TRH on the duration of pentobarbital narcosis measured from loss of righting reflex

Brain region	Dose (μ g)	Stereotaxic coordinates (mm) [†]	Narcosis duration [‡] (min)
		anterior lateral vertical	
Saline in septum [‡]	—	7.4 0.3 1.5	87.9 $\pm$ 5.1 (14)
Somatosensory cortex	5.0	8.6 1.5 5.6	103.8 $\pm$ 10.6 (5)
Lateral hypothalamus	5.0	6.8 3.0 -2.0	95.7 $\pm$ 15.3 (6)
Amygdala	5.0	6.0 4.8 -2.1	90.5 $\pm$ 11.0 (6)
Caudate	5.0	7.8 3.0 1.0	79.5 $\pm$ 8.0 (6)
Cerebellum	5.0	-5.3 0.5 -1.0	78.6 $\pm$ 5.8 (8)
Lateral preoptic area	5.0	7.6 2.0 -1.9	75.5 $\pm$ 5.8 (6)
Parafascicular nuc.	5.0	3.6 1.3 -0.3	65.7 $\pm$ 5.0 (7)*
Parafascicular nuc.	0.5		71.4 $\pm$ 7.5 (5)
Dorsal hippocampus	5.0	3.2 2.8 2.0	63.5 $\pm$ 5.1 (6)*
Dorsal hippocampus	0.5		77.3 $\pm$ 3.0 (6)
Mesencephalic reticular nuc.	0.5	1.8 2.0 -2.2	69.7 $\pm$ 2.9 (7)
Interpeduncular nuc.	0.5	1.6 0.3 -4.8	63.1 $\pm$ 4.7 (7)*
Medial hypothalamus	0.5	5.0 0.5 -3.0	59.9 $\pm$ 1.6 (8)**
Accumbens	0.5	9.2 1.5 0.0	56.7 $\pm$ 3.2 (7)**
Substantia nigra	0.5	1.8 2.0 -4.0	56.6 $\pm$ 4.2 (7)**
Thalamic PVG	0.5	3.6 0.3 -2.1	55.6 $\pm$ 4.0 (7)**
Locus coeruleus	0.5	-1.8 0.6 -3.0	55.6 $\pm$ 3.3 (5)*
Locus coeruleus	0.01		75.8 $\pm$ 2.3 (5)
Mesencephalic PVG	0.5	0.8 0.5 -0.7	53.0 $\pm$ 2.9 (8)**
Mesencephalic PVG	0.01		68.4 $\pm$ 4.7 (7)
Medial preoptic area	0.5	7.9 0.5 -1.7	50.7 $\pm$ 5.0 (7)**
Medial preoptic area	0.01		65.8 $\pm$ 5.8 (5)
Medial thalamus	0.5	5.2 0.7 0.0	48.9 $\pm$ 2.7 (8)**
Medial thalamus	0.01		67.8 $\pm$ 4.3 (6)
Septum	0.5	7.4 0.3 1.5	45.9 $\pm$ 3.4 (8)**
Septum	0.01		59.6 $\pm$ 2.6 (7)**

[†] The coordinates are from the atlas of Pellegrino and Cushman²³ and are relative to the interaural line with the upper incisor bar elevated to 5 mm.

[‡] Duration of septal saline narcosis is not statistically different from that of rats receiving pentobarbital alone ($\bar{x}$ = 99.5 $\pm$ 6.9 min, N = 10, P > 0.1). All significance values are with respect to septal saline, determined with a two-tailed Student t -test.

[§] Mean $\pm$ s.e.m.; no. of determinations shown in parentheses.

* P < 0.01; ** P < 0.001

small lesion was placed 1 mm below each guide cannula by passing 1 mA of anodal current for 3 s. Brains were frozen, 50- μ m sections made at the lesion site, and sections stained with cresyl violet.

Doses of TRH for each site were chosen on the basis of preliminary data obtained using whole-brain doses of 500 ng. Sites not demonstrating a significant decrement in the duration of narcosis at 500 ng, as compared with animals receiving only pretreatment with 40 mg per kg pentobarbital, were further examined with 5- μ g doses of TRH. Even with 5 μ g of TRH several areas proved to be insensitive, including the amygdala, cerebellum, somatosensory cortex, caudate, lateral hypothalamus, and lateral preoptic area (P > 0.01) (Table 1). In contrast, the hippocampus and parafascicular nucleus did demonstrate TRH sensitivity to 5- μ g doses. However, considering the magnitude of the dosage and the proximity of these sites to the ventricular system and/or sites demonstrating TRH sensitivity at 500 ng, the possibility that TRH may be diffusing from the injection site to more sensitive areas should be considered. That many areas remained insensitive to TRH, even at a dose of 5 μ g, is in contrast to the results of Carino *et al.*¹¹ who found both the cortex and cerebellum of the rabbit to be sensitive to a dose of 1 μ g. A possible explanation for the differences observed might be that rabbits and rats respond differently. Alternatively, Carino *et al.* used injection volumes of

2 μ l, and diffusion of TRH to active sites could also be an explanation.

As in the investigation of TRH-induced shaking by Wei *et al.*¹⁰, we found that the more medially located brain regions were most sensitive to TRH antagonism of pentobarbital narcosis. The moderate sensitivity observed in the present study with the relatively lateral nucleus accumbens and substantia nigra is an exception. The most sensitive area was the septal region. The dose-response curve for TRH antagonism of pentobarbital narcosis in the septum demonstrates that significant antagonism occurred with a dose as low as 5.0 ng (Fig. 1). While several sites responded significantly at 500 ng TRH, when the five most sensitive areas, including the septum, medial preoptic area, mesencephalic periventricular grey (PVG), locus coeruleus, and medial thalamus, were examined at a dose of 10 ng, only the septum differed significantly from septal saline injections (P < 0.01). This finding indicates at least a twofold higher sensitivity of the septum over the next most sensitive areas and implicates it as the most important site of action in TRH antagonism of pentobarbital narcosis. This evidence of marked sensitivity is supported by numerous data, including immunohistochemical¹² and chromatographic¹³ identification of TRH in the septal region, the demonstration of calcium-dependent release of immunoreactive TRH from septal synaptosomes in the presence of elevated potassium concentrations¹⁴, and the demonstration by Winokur and Beckman¹⁵ that septal neurones respond to iontophoretic TRH. Furthermore, Burt (ref. 16 and personal communication) has found that the greatest concentration of high-affinity TRH-binding sites in calf and sheep extrahypothalamic brain tissue was located in the septum and nucleus accumbens.

TRH has often been associated with modulating cholinergic transmission. This is based on investigations indicating that anti-muscarinic compounds can effectively antagonise TRH-induced arousal of pentobarbital-narcotised animals¹, and studies by Yarbrough¹⁷ demonstrating TRH potentiation in the excitatory response of cortical neurones to iontophoretic acetylcholine (ACh). Moreover, it has long been known that pentobarbital increases cortical and hippocampal ACh levels¹⁸, and Schmidt¹⁹ has shown that TRH will reverse this effect of pentobarbital on ACh levels in both the hippocampus and cortex. The importance of the septum in central cholinergic pathways is demonstrated by the observation that this region is heavily stained for acetylcholinesterase⁹. In addition, there are many hippocampal afferents from the medial septum; and various lines of evidence²⁰ support the hypothesis that the septohippocampal pathway is cholinergic. Further, it has been demonstrated that electrical stimulation of the septum results in a marked increase in ACh output from both the hippocampus²¹ and cortex²². Therefore, in view of the present findings, activation of the septum by TRH is a possible mechanism mediating the decrease in hippocampal and cortical ACh levels reported by Schmidt in the pentobarbital-pretreated rat.

Thus the septum is highly sensitive to TRH when compared with other brain regions, but with systemic administration it is likely that TRH acts in part through less sensitive brain sites.

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Corticotrophin releasing factor may be modulated vasopressin

CORTICOTROPHIN RELEASING FACTOR (CRF) was the first of the postulated hypothalamic factors controlling the release of hormones from the pituitary gland to be demonstrated biologically¹. However, it is extraordinary that after 24 years of research, techniques which have established the identity of the hypothalamic peptides involved in the release of thyrotrophin, the gonadotrophins and growth hormone, have been unsuccessful when applied to the search for a factor responsible for

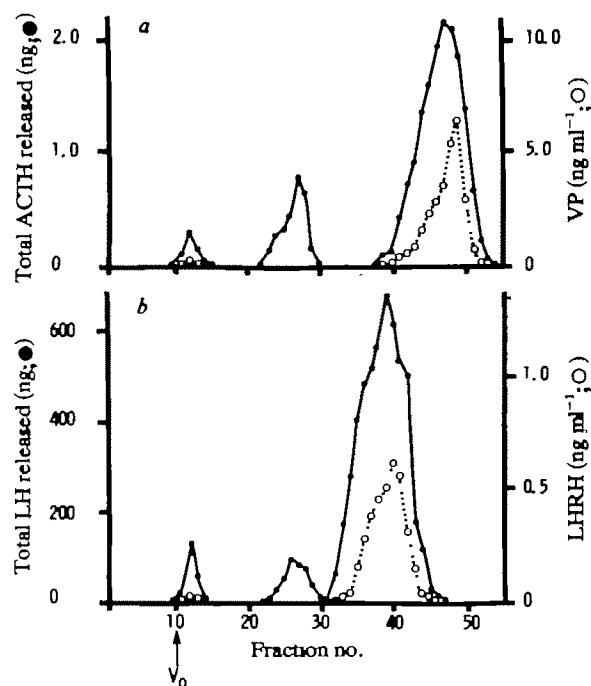


Fig. 1 Chromatography of rat SME on BioGel P2 (200-400 mesh). Twenty rat stalk-median eminences (4 mg tissue wet weight per SME) were homogenised in 1.5 ml 10 mM HCl. After addition of NaCl (9 mg ml⁻¹) and centrifugation (3,000 g) the supernatant was applied to a column (1.5 × 50 cm) of BioGel P2 (200-400 mesh) equilibrated with 10 mM HCl containing NaCl (9 mg ml⁻¹) and ascorbic acid (1 mg ml⁻¹) at 4 °C. The column was eluted with the same buffer and hourly fractions (2.5 ml) were collected. Each fraction was neutralised with 25 µl 1.1 M NaHCO₃ and was diluted 1.5 in the appropriate assay buffer. *a*, ●, CRF bioactivity, as measured by output of immunoreactive ACTH from the cell column; ○, AVP immunoreactivity (ng ml⁻¹ in 1:5 dilution). *b*, ●, LHRH bioactivity as measured by output of immunoreactive LH from the cell column; ○, LHRH immunoreactivity (ng ml⁻¹ in 1:5 dilution). No significant amounts of ACTH or LH were detected in any of the fractions. V₀, void volume of column.

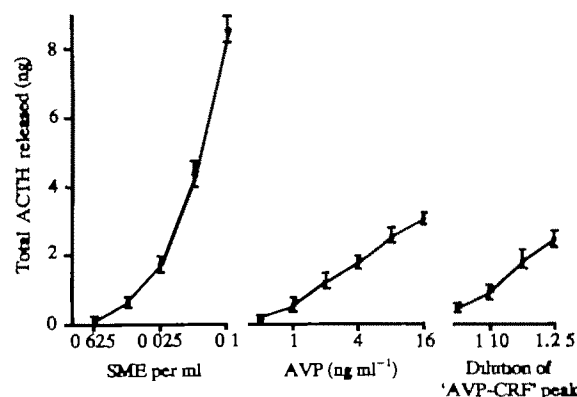


Fig. 2 Comparison of the CRF bioactivity of crude SME (one SME per ml: 1 ml contains neutralised acid extract prepared from the rat stalk-median eminence), synthetic AVP, and the 'AVP-CRF' peak shown in Fig. 1*a* (pooled fractions 43-51). These results were obtained from a chromatogram run in 10 mM HCl containing NaCl (9 mg ml⁻¹) without ascorbic acid. Doses were given randomly in triplicate and results represent mean ± s.e.m.

adrenocorticotrophin (ACTH) release^{2,3}. Vasopressin invariably releases ACTH from the anterior pituitary, but its partial agonistic properties *in vitro*⁴⁻⁶, its low potency *in vivo*⁷ and the isolation of active CRF fractions with little or no vasopressin activity^{2,3,8,9} have led to its rejection as a physiological CRF^{10,11}. However, recent immunological and immunocytochemical findings have led to renewed interest in the role of vasopressin in ACTH release. High concentrations of immunoreactive vasopressin have been found in portal blood and a direct vasopressin-neurophysin containing axonal pathway to the hypophyseal portal system, which seems to be under the influence of the adrenal cortex, has been identified^{12,13}. Using a sensitive releasing factor bioassay system, the perfused isolated anterior pituitary cell column¹⁴, and various immunological techniques, we have presented evidence to support the idea that vasopressin plays a major part in control of ACTH release⁶, and we now report the existence of hypothalamic factors with weak, labile CRF activity which potentiate the CRF activity of vasopressin to give it full agonistic properties indistinguishable from crude extracts of rat stalk median eminence (SME). Thus we propose that CRF is vasopressin modulated by other hypothalamic factor(s) released into the hypothalamo-hypophyseal portal system.

We reported previously that the immunological characteristics of the vasopressin-like material in rat SME were identical to synthetic arginine vasopressin (AVP) although the AVP content could never account for more than 30% of the biological activity of the extract⁶. The luteinising hormone releasing hormone (LHRH) bioactivity of SME, however, was completely in accord with its immunoreactive LHRH content. Surprisingly, when we attempted to remove the 'interfering' AVP from SME by preincubation with AVP antisera, the CRF activity was also specifically and totally quenched⁶.

The CRF biological activity of a crude extract of SME was fractionated by gel filtration (Fig. 1). The first small peak at the void volume (V₀) contained negligible CRF activity (Fig. 1*a*). The material in the second peak (fractions 22-28) appeared to be nonspecific (N₀) in the bioassay as it also released luteinising hormone (LH) (Fig. 1*b*). The major peak of CRF activity (fractions 39-53) eluted in the expected position for AVP and contained the peak of AVP immunoreactivity (Fig. 1*a*). The major peak of LHRH bioactivity coincided with the immunoreactive peak of LHRH (Fig. 1*b*). The log-dose response characteristics of the material in the 'AVP-CRF' peak (fractions 43-51) were indistinguishable from those of synthetic AVP, each was a partial agonist in the CRF bioassay when compared with SME (Fig. 2). Using three vasopressin antisera of differing specificities⁶, the vasopressin immunoreactivity of this peak was characterised as AVP.

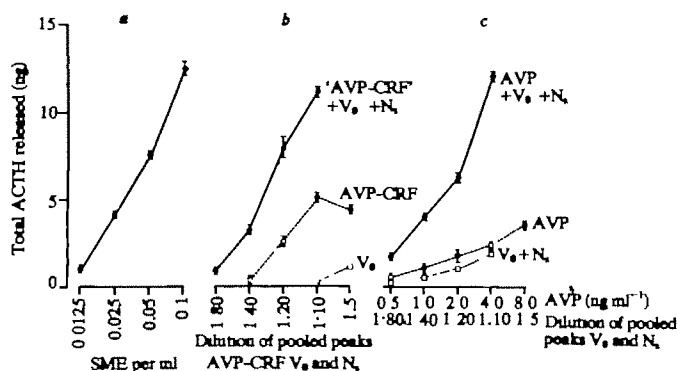


Fig. 3 The synergistic effect of recombination of the CRF peaks in Fig. 1 and synthetic vasopressin. The results were obtained from a chromatogram run in 10 mM HCl containing NaCl (9 mg ml⁻¹) and ascorbic acid (1 mg ml⁻¹) at 4 °C. V₀, N₁ and 'AVP-CRF' are pooled fractions 11–31, 22–28 and 43–51, respectively, in Fig. 1.

Thus the material of the major CRF peak resembles synthetic vasopressin immunologically, chromatographically (on BioGel P2) and biologically (in our CRF bioassay) and the lack of full agonist bioactivity of 'AVP-CRF' suggests that chromatography has removed some synergistic factors.

The reappearance of biological activity after recombination of all the fractions after gel filtration of SME has been reported previously¹⁵, but in a recent review, Saffran and Schally³ reported that this work could not be repeated. Initially we failed to observe any potentiation when AVP was recombined with fractions from other areas of the chromatogram. However, when the chromatographic column was equilibrated and developed with eluant containing the antioxidant ascorbic acid, we consistently observed potentiation of the CRF bioactivity after recombination of the 'AVP-CRF' peak with other areas of the chromatogram. This potentiation was also observed with synthetic AVP. Figure 3b shows that the combined 'AVP-CRF', V₀ and N₁ fractions had bioassay log dose-response characteristics similar to those of crude SME. Equivalent quantities of synthetic AVP added to a mixture of V₀ and N₁ also gave a curve similar to that for SME (Fig. 3c). There was some slight synergism between V₀ and N₁ but this activity was unstable after storage for short periods at -20 °C. As crude extracts of normal SME are stable to storage and heat treatment⁶, it seems that AVP has a stabilising influence on the CRF activity of SME.

Studies with Brattleboro rats¹⁵, which are vasopressin deficient, have shown significantly reduced CRF bioactivity in their SME (20% of normal) which is unstable to storage at -20 °C, thus resembling the activity of V₀ and N₁ peaks of normal SME. Chromatography of Brattleboro SME yielded V₀ and N₁ peaks similar to those shown in Fig. 1 but the 'AVP-CRF' peak, with respect to both CRF bioactivity and AVP immunoactivity, was absent. The Brattleboro LHRH chromatogram was identical in all aspects to that for normal SME¹⁶.

We conclude that the CRF bioactivity of a crude stalk median eminence extract is due to a multi-factor system. Vasopressin is the major component whose potency is modulated by synergistic factors which have weak inherent CRF bioactivity. They lose most of their bioactivity and all of their ability to potentiate vasopressin when separated from it under non-reducing conditions as indicated by the negligible synergism of the components of the chromatogram when ascorbic acid is omitted. In view of the apparent compound nature of CRF bioactivity and the instability of some of its components, it is not surprising that the search for a pure peptide, distinct from vasopressin, with potent CRF activity has been unsuccessful.

This multi factor hypothesis is an attractive one because of the variety of stress situations (physiological, pathogenic, psychological), in addition to circadian rhythm, in which ACTH is secreted. Thus, the weak agonistic properties of vasopressin could be modulated by a specific factor or permutation of factors

in different situations. The factors, in turn, could be involved in the simultaneous release of growth hormone and/or prolactin, which are also released during stress.

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Effect of electroconvulsive shock on monoaminergic receptor binding sites in rat brain

CHRONIC administration of either tricyclic antidepressant drugs or monoamine oxidase inhibitors, which are effective in the treatment of endogenous depression, has been shown to decrease the sensitivity of noradrenaline (NA)-stimulated adenylate cyclase^{1–5} and to decrease the apparent density of β -adrenergic receptor binding sites in rat brain^{6–10}. This alteration in receptor mechanisms by antidepressant drugs seems to be selective, as neither α -adrenergic nor serotonergic receptor binding in rat cortex is altered by the tricyclic drug desipramine⁷. It is therefore of interest to know whether other forms of antidepressant intervention alter receptor mechanisms in the brain and if so, whether they display a time course and selectivity similar to that of antidepressant drugs. Here, we have investigated the effects of a single and of multiple electroconvulsive shocks (ECS) on rat brain monoaminergic receptor binding sites and report that the density of β -adrenergic receptors is decreased.

Electroconvulsive shock (150 mA, 200 ms) was administered to male Sprague-Dawley rats through saline-moistened corneal electrodes. Controls were handled in the same manner except that no current was passed. Shocks were administered for 1 day and for 7 consecutive days. All shocked rats experienced generalised tonic-clonic seizures. Rats were decapitated 24 h after their last shock and the cortex and corpus striatum were dissected from the brains, frozen on dry ice and stored at -85 °C until assayed. The kinetic properties of α -adrenergic, β -adrenergic and serotonergic receptor binding sites were measured in the cortex and those of dopaminergic binding sites were measured in the striatum. The cortices were suspended in 19 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 23 °C) containing 0.1% ascorbic acid. The striata were suspended in 39 volumes of ice-cold buffer containing 50 mM Tris-HCl, 2 mM CaCl₂, 5 mM KCl, 120 mM NaCl, 1 mM MgCl₂, 0.1% ascorbic acid and 10 μ M pargyline (pH 7.7 at 25 °C). The tissues were disrupted using a Brinkmann polytron (setting 6, 30 s), and the homogenates centrifuged at 48,000g for 10 min. The pellets were rehomogenised and centrifuged as before. The cortical pellets were resuspended in 19 volumes of buffer and the striatal pellets in 39 volumes of buffer (based on original weight).

Aliquots of tissue equivalent to 10 mg of cortex and to 5 mg of striatum were assayed.

Binding of ^3H -*l*-dihydroalprenolol (^3H -DHA) to β -adrenergic receptors was carried out at 23 °C for 15 min in 0.5 ml 50 mM Tris-HCl buffer (pH 8.1 at 23 °C) containing 0.1% ascorbic acid^{11,12}. Assays were carried out in sextuplicate with half the tubes containing 20 μM propranolol to assess nonspecific binding. The α -adrenergic receptor binding sites were assayed using ^3H -dihydroergocryptine (^3H -DHE) and ^3H -WB-4101 as the binding ligands¹³⁻¹⁶. Dihydroergocryptine is a mixed agonist-antagonist at α -adrenergic receptors and is thought to bind to both an agonist and an antagonist α -adrenergic receptor site in our assay conditions^{13,14}. The antagonist WB-4101 seems to bind only to the α -adrenergic antagonist site¹⁶. Incubations were carried out in sextuplicate at 25 °C for 45 min with ^3H -DHE and for 15 min with ^3H -WB-4101 in 2 ml 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) containing 0.1% ascorbic acid. Nonspecific binding was determined by adding 100 μM *l*-NA to half the tubes. The serotonergic binding site was assayed using ^3H -serotonin (^3H -5-HT) as the binding ligand¹⁷. Tissues were preincubated for 10 min at 37 °C in 2 ml 50 mM Tris-HCl buffer (pH 7.1 at 37 °C) containing 0.1% ascorbic acid, 4 mM CaCl_2 and 10 μM pargyline, an inhibitor of monoamine oxidase. After preincubation, the tubes were returned to ice and ^3H -5-HT was added in sextuplicate. Half the tubes also contained 20 μM nonradioactive 5-HT to assess nonspecific binding. Incubations were carried out for 10 min at 37 °C. The dopaminergic binding site in the striatum was assayed in quadruplicate, using ^3H -spiroperidol (^3H -spiro) as the binding ligand^{18,19}. Tissues were preincubated for 5 min at 37 °C and returned to ice. To assess nonspecific binding, 10 μM (+)butaclamol was added to half the tubes. Incubations were carried out for 20 min at 37 °C in 0.5 ml of the same buffer used to homogenise the tissue. All incubations were terminated by adding 4 ml of ice-cold buffer to each tube and rapidly filtering the samples under reduced pressure through Whatman GF/B glass fibre filters. The tubes and filters were then rapidly rinsed four times with 4-ml aliquots of cold buffer. Filters were transferred to counting vials containing scintillation fluid and counted in a Searle mark III scintillation counter at an efficiency of

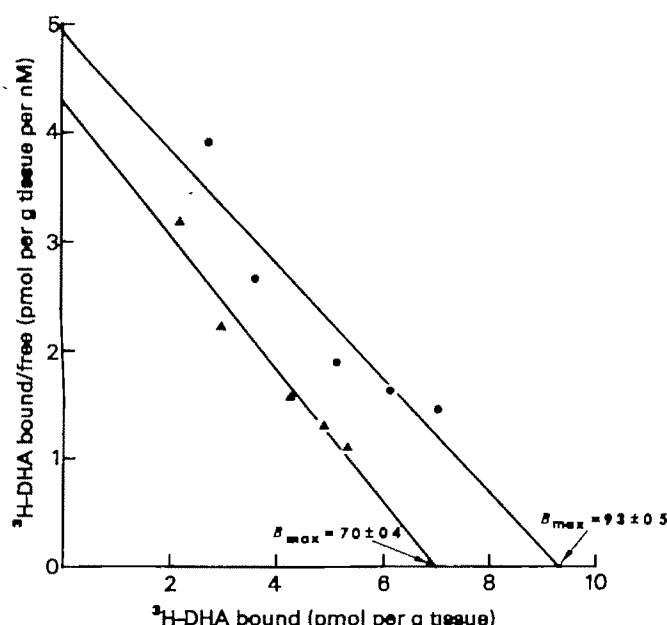


Fig. 1 Scatchard plot of specific ^3H -*l*-dihydroalprenolol binding to cortical fractions from control (●) and ECS-treated (▲) rats. The concentrations of ^3H -DHA used varied from 0.5 to 5.0 nM. This experiment is representative of results obtained from three separate experiments. Data points are the means from 2 control and 2 ECS-treated rats, each assayed in triplicate. Slopes and intercepts of the lines were determined by the method of least squares.

Table 1 Maximum specific binding and dissociation constants of monoamine receptor ligands in brain fractions from control and ECS-treated rats

Region	Treatment	Maximum binding (pmol per g tissue)	Dissociation constant (nM)
Cortex	^3H -DHA Control	10.9 ± 0.9	2.7 ± 0.5
	^3H -DHA ECS	7.8 ± 0.6*	2.1 ± 0.2
	^3H -DHE Control	28.1 ± 1.6	1.9 ± 0.3
	^3H -DHE ECS	27.0 ± 0.7	1.9 ± 0.1
	^3H -WB-4101 Control	7.8 ± 0.5	0.28 ± 0.01
	^3H -WB-4101 ECS	7.9 ± 0.4	0.24 ± 0.02
Corpus striatum	^3H -5-HT Control	15.8 ± 2.0	8.5 ± 1.4
	^3H -5-HT ECS	14.7 ± 1.4	7.1 ± 1.1
	^3H -Spro Control	24.0 ± 0.7	0.33 ± 0.07
	^3H -Spro ECS	23.3 ± 0.7	0.37 ± 0.08

Maximum specific binding and dissociation constants were determined from least-squares fit to Scatchard plots of the data. The results are the mean ± s.e.m. from 5–8 rats. Statistical analyses were carried out by grouped Student's *t*-test.

* $P < 0.02$.

40–42%. In these conditions, specific binding, defined as the difference between counts bound to tissue in the presence and absence of saturating concentrations of nonradioactive ligand, was between 60 and 90% depending on the assay and the concentration of the ^3H -ligand.

There was no apparent alteration in the α -adrenergic, β -adrenergic or serotonergic receptor binding characteristics after a single ECS. However, Scatchard analysis²⁰ of ^3H -DHA binding to cortical membranes indicated that 7 days of ECS reduced the number of binding sites by approximately 25% compared with the handled controls (Fig. 1). There was no change in the apparent affinity of the binding sites for ^3H -DHA. In contrast to the changes observed in β -adrenergic binding, 7 days of ECS produced no apparent alterations of α -adrenergic or serotonergic binding in the cortex nor of dopaminergic binding in the corpus striatum (Table 1).

As the animals were not anaesthetised, it is possible that a nonspecific stress response contributed to the reduction in the β -adrenergic receptor seen following chronic ECS. To ascertain whether another form of stressful situation would decrease ^3H -DHA binding, we investigated the effects of electric foot shock on β -adrenergic binding. Foot shock was applied by placing the rat in a 28 × 20 × 15 cm test chamber containing a grid floor connected to a constant current shock source with a shock scrambler (BRS Foringer shock generator scrambler model SGS-001). The rats received foot shocks of 2 mA intensity and 1.0 s duration at a variable time (VT) schedule with a mean interval of 15 s. The rats were shocked daily for 10 min for 7 consecutive days. Control rats were treated similarly except that no current was delivered to the chamber grid. Rats were decapitated 24 h after their last session in the test chamber. Cortical β -adrenergic receptor binding of 0.7 nM ^3H -DHA in tissue from control ($n = 6$) and foot shock-treated ($n = 6$) rats was found to be 1.76 ± 0.18 and 1.64 ± 0.19 pmol per g tissue, respectively, and 7.28 ± 0.26 and 7.13 ± 0.23 pmol per g using 4.5 nM ^3H -DHA. This absence of reduction in specific ^3H -DHA binding with foot shock suggests that nonspecific stress alone was not a major factor in reducing the density of β -adrenergic receptors in the brains of rats subjected to chronic ECS.

Electroconvulsive shock has been shown to affect several biochemical parameters of the central noradrenergic system. It increases the activity of tyrosine hydroxylase,²¹ endogenous NA concentration²² and turnover^{22,23}, and the release of NA²², and decreases the high affinity uptake of NA into synaptosomes²⁴. Through these effects, ECS might be expected to increase the availability of NA at the synapse²² and could initiate a neurochemical adaptation at the receptor level. Sulser *et al.* have

reported that administration of ECS to rats for 8 days reduces the activity of the NA-stimulated cyclic AMP-generating system in limbic forebrain^{2,3}.

The development of neurotransmitter receptor subsensitivity following exposure to agonist compounds or drugs that potentiate the action of existing transmitters has been reported previously^{6-10,26-30}. We report here another example of the development of receptor subsensitivity, the decreased density of β -adrenergic receptors in rat brain cortex following chronic ECS. It is particularly interesting that chronic ECS results in a decrease in cortical β -adrenergic receptor density similar in magnitude and selectivity to that following chronic administration of desipramine⁷. In both cases the time course of reduction of β -adrenergic receptor density closely parallels the reported decrease in activity of the NA-stimulated cyclic AMP-generating system^{2,3}.

These results indicate that the effects on neurotransmission at the β -adrenergic synapse produced by chronic administration of tricyclic antidepressant drugs or ECS are opposite to the effects produced by acute administration of these treatments. Thus, although both tricyclic antidepressants and ECS increase the amount of NA available to interact with receptors in the synapse, the reduction in the number of β -adrenergic receptors after chronic treatment would be expected to result in a decrease in the transmitted message. The reduction in activity of the NA-stimulated cyclic AMP-generating system following chronic administration of either tricyclic antidepressants or ECS¹⁻⁴ seems to support this.

The selectivity of the response of neurotransmitter receptors to chronic ECS reported here agrees closely with that reported for desipramine⁷ and suggests that an adaptation of central β -adrenergic receptors may be important for the therapeutic effects of antidepressant treatment.

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Bio-selective membrane electrode using tissue slices

MEMBRANE electrodes based on living bacterial cells¹ as biocatalysts have been shown to be useful sensors for amino acids and other biomolecules. We report here that we have now developed a highly effective membrane electrode for glutamine using slices of porcine kidney tissue as the biocatalytic layer. This tissue-based electrode yields excellent selectivity and sensitivity while having the additional advantages of simplicity and low cost. Further, the tissue-based electrode shows a much longer useful lifetime than comparable electrodes prepared with the isolated porcine enzyme.

The electrode assembly (Fig. 1) was prepared by immobilising a thin (~0.05 mm) slice of porcine kidney at the surface of an ammonia gas sensing membrane electrode (Orion model 95-10) by means of a dialysis membrane or a mesh of monofilament nylon. Pork kidneys were obtained from freshly killed animals and stored at 4 °C until use. In separate experiments the kidney slices showed high glutaminase activity as indicated by their ability to metabolise glutamine rapidly to products including ammonia. Moreover, the enzyme activity was retained within the intact tissue over extended time periods. This finding is consistent with the view that the glutaminase enzyme is situated in the kidney cell mitochondria².

Evaluations of the potentiometric response of the kidney slice electrode were made at 26 °C in 0.1 M phosphate buffer, pH 7.8, containing 0.02% sodium azide as preservative. Between experiments, the electrodes were stored in the buffer at room temperature. Figure 2 shows a typical calibration curve of the electrode for L-glutamine. The slope, linear range and detection

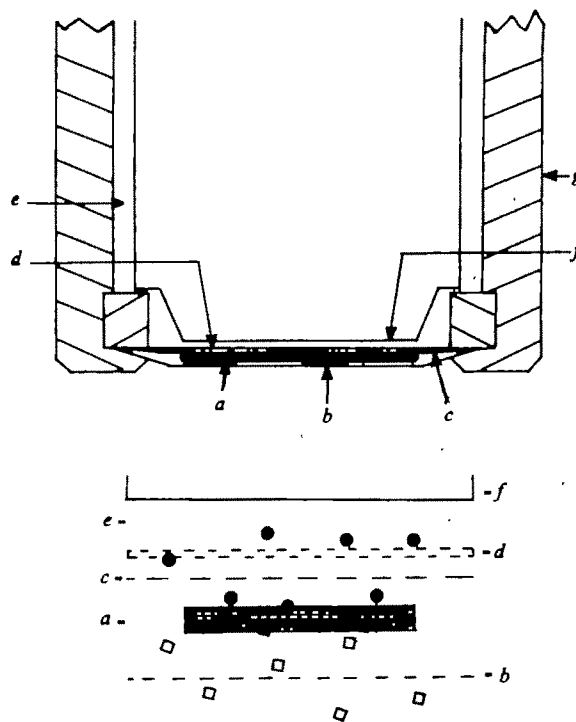


Fig. 1. Top, schematic diagram of the kidney tissue-based membrane electrode for glutamine; a, the slice of porcine kidney tissue; b, a monofilament nylon mesh; c, the protective dialysis membrane; d, the gas-permeable membrane; e, the internal electrolyte solution; f, the pH sensing glass membrane; and g, the plastic electrode body. (Components d-g represent the Orion model 95-10 ammonia gas sensing electrode.) Bottom, expanded view of the electrode sensing tip showing components and phases. □, Glutamine; ●, ammonia; identifying letters as above.

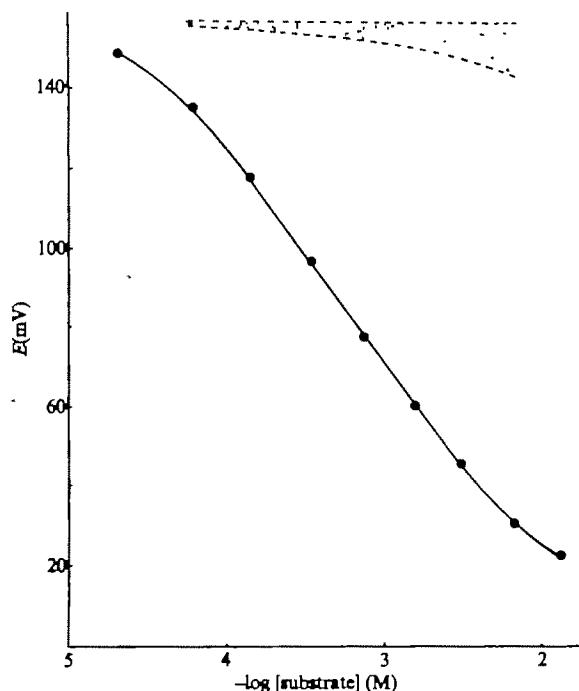


Fig. 2. Potentiometric response and selectivity of the kidney-based electrode in 0.1 M phosphate buffer, pH 7.8, with 0.02% sodium azide preservative at 26 °C. Solid line shows the electrode response as a function of glutamine concentration over the range 2×10^{-2} to 5×10^{-5} M. Dotted region shows the maximum response to the 13 possible interference compounds tested.

limit remained unchanged over at least 28 d of electrode use. Typically, the electrode attained a steady potential value within 5–7 min of each addition of glutamine to the test solution.

The kidney tissue electrode yielded negligible response to compounds causing possible interference such as urea, L-alanine, L-arginine, L-histidine, L-valine, L-serine, L-glutamic acid, L-asparagine, L-aspartic acid, D-alanine, D-aspartic acid, glycine or creatinine. This high degree of potentiometric selectivity compares favourably with that of conventional electrodes³.

Parallel electrode experiments were also carried out using the isolated porcine kidney glutaminase enzyme (Sigma, grade Vi), known to be thermally unstable⁴. Small volumes (~15 μ l) of enzyme suspension were immobilised at the surface of the ammonia gas sensing electrode by means of a dialysis membrane, and electrode response to L-glutamine was evaluated in the buffer medium. Freshly prepared enzyme electrodes yielded moderate potentiometric response (44 mV per 10-fold change in substrate concentration) but lost more than 50% of their activity within 2 d and showed negligible response after 4 d at room temperature. Thus, it is clear that the kidney tissue electrode is a very substantial improvement, in terms of lifetime and response, over the conventional enzyme electrode. In view of continuing advances³ in the development of potentiometric membrane electrodes, other tissue-based electrodes of fundamental and practical significance can be expected.

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Elevation of protein synthesis is a complex response to fertilisation

PROTEIN SYNTHESIS in unfertilised eggs of the sea urchin proceeds at a low rate. However, fertilisation triggers a series of reactions beginning with changes in intracellular cation concentrations within a few seconds of sperm entry and extending to a cascade of more complex events¹. As these events are sequential in time, each might depend on the preceding event, or there might be a parallel series of independent pathways. The latter hypothesis was shown to be correct by Epel *et al.*², who demonstrated that artificial activation with ammonia failed to initiate events such as sodium influx and the cortical reactions but did initiate a variety of others including potassium conductance, protein synthesis, DNA synthesis and mRNA polyadenylation.

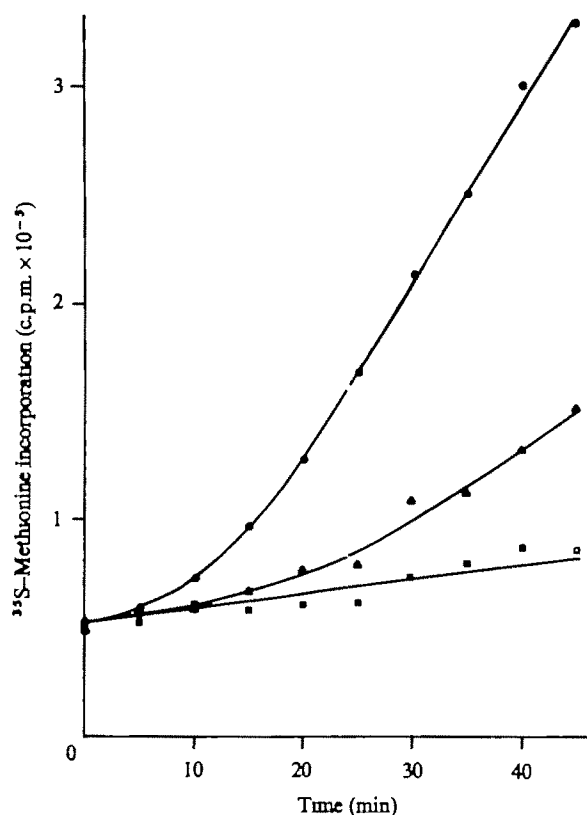


Fig. 1. Elevation of protein synthesis as a function of activation method. Eggs (2×10^5 per ml, total volume 35 ml) were pulsed with $10 \mu\text{Ci ml}^{-1}$ ^{35}S -methionine ($1,130 \text{ Ci mmol}^{-1}$) for 60 min at 17 °C. The sea water contained 50 mg ml^{-1} each of penicillin and streptomycin adjusted to pH 8.1 with NaOH. After incubation, the eggs were washed 3 times by pelleting them at 200 g. The final pellet was resuspended in 15 ml and distributed equally to each of 3 culture vessels at 6.7×10^4 eggs ml^{-1} , final volume 35 ml). At time zero, 2.0 ml of sea water was added to one of the vessels (■), 2.0 ml 200 mM NH_4Cl was added to the second culture (▲) and 2.0 ml of concentrated sperm suspension (1 drop sperm as obtained from the gonads in 5 ml sea water) was quickly squirted into the third culture (●). Taking precautions to avoid cross-contamination, 1.0-ml samples were removed from each culture at 5 min intervals. The samples were squirted directly into an equal volume of ice-cold 20% trichloroacetic acid (TCA) plus 50 mM methionine. The eggs and embryos were pelleted, washed once with the TCA mixture, and heated to 90 °C for 30 min. During heating each tube was vigorously agitated several times. Precipitates were collected on Whatman glass fibre filters (GF/C), washed 4 times with 2 ml 10% TCA plus 25 mM methionine, and once with 95% ethanol, and the disks were then placed in scintillation vials. Protein was digested off the disks in sealed vials by adding 0.5 ml 0.2 M NaOH and heating to 60 °C for 2 h. The hydrolysates were cooled, neutralised with 1 M acetic acid, dissolved in 10 ml Instagel scintillation fluid, and counted.

We have been studying the elevation of protein synthesis in detail. The rate of protein synthesis increases severalfold during the early cleavage stages³ and eventually reaches an absolute rate which is 100 times higher than that in the mature oocyte⁴. Kaumeyer *et al.*⁵ and Jenkins *et al.*⁶ have demonstrated that unfertilised eggs contain 'masked' messenger ribonucleoprotein particles which are not translatable *in vitro* unless modified or deproteinised. Most of the increase in the rate of protein synthesis following fertilisation is due to the recruitment of this oogenic mRNA as a result of unmasking⁷. However, a significant portion of the rise also results from an increase in translational efficiency⁸. As defined by Palmiter⁹, translational efficiency is the number of completed polypeptides released per unit time per message. The change in efficiency following fertilisation of sea urchin eggs is reflected in an increase in the rate of ribosome movement (that is, the time required to complete an average size protein decreases after fertilisation) coupled to a concomitant increase in initiation rate. The net effect on protein synthesis of an increase in number of mRNAs available for translation and an increase in translational efficiency is multiplicative. We present evidence here that these two processes are under separate controls.

As Fig. 1 shows, in *Strongylocentrotus purpuratus* eggs the rise in protein synthesis induced by ammonia lags behind the rise caused by fertilisation. The same response was observed in a different species by Epel *et al.*². They found that even though the

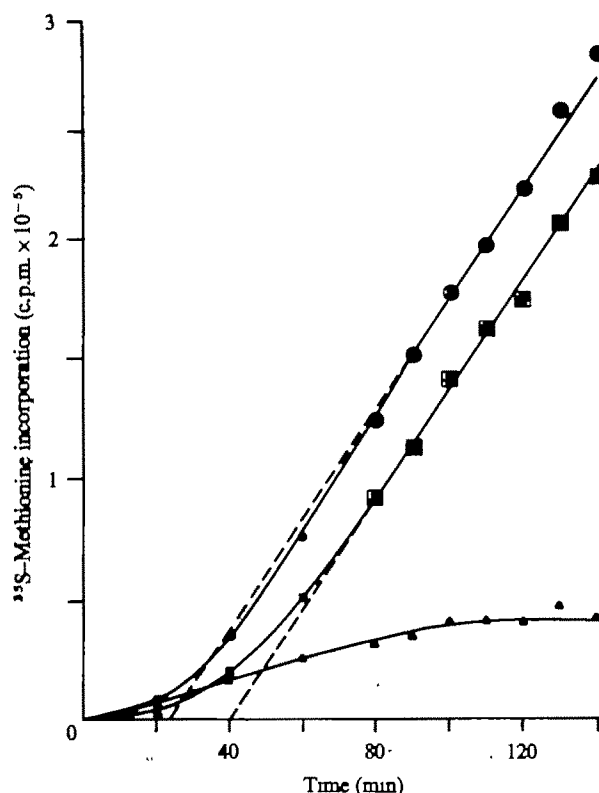


Fig. 2 Determination of ribosome transit time for ammonia-activated, unfertilised eggs. A culture of eggs (32,000 per ml) in sea water containing penicillin and streptomycin (pH adjusted to 8.1 with NaOH) was treated with sufficient 200 mM NH_4Cl to bring the final concentration of NH_4^+ to 10 mM. Five minutes later, ^{35}S -methionine was added ($5 \mu\text{Ci ml}^{-1}$; 900 Ci mmol^{-1}) plus unlabelled methionine to yield a final exogenous amino acid concentration equal to $0.32 \mu\text{g ml}^{-1}$. The addition of isotope was defined as time zero, after which 5.0-ml samples were removed and processed as described elsewhere⁸. Transit times were obtained by multiplying by two the distance between the extrapolated 'total' and 'soluble' lines. All extrapolations were based on those points after the polysome phase had reached steady-state labelling, when the flow of radioactivity into total and soluble proteins is linear⁸. Points used for extrapolation are outlined. ●, Total incorporation; ■, incorporation into soluble proteins; ▲, incorporation into polysomes.

Table 1 Ribosome transit times for ammonia-activated eggs

Experiment	Isotope	Transit time (min)
UF eggs	^3H -Leu	41.6
UF eggs	^{35}S -Met	38.6
		Average = $40.4 \pm 4.0^\dagger$
30 min zygotes	^3H -Leu	14.0
30 min zygotes	^{35}S -Met	19.0
		Average = $18.0 \pm 4.4^\dagger$
NH_3 -activated eggs	^3H -Leu*	33.2
NH_3 -activated eggs	^{35}S -Met*	39.1
		Average = $36.2^\ddagger$

Cultures of unfertilised (UF) eggs and zygotes were handled as described elsewhere⁸. In activation experiments, unfertilised eggs in Millipore-filtered sea water containing $50 \mu\text{g ml}^{-1}$ each of penicillin and streptomycin (pH adjusted to 8.1) were treated with sufficient 200 mM NH_4Cl to bring the final concentration of ammonium ion to 10 mM. Five minutes later, either ^3H -leucine (New England Nuclear, $57.4 \text{ Ci mmol}^{-1}$) or ^{35}S -methionine (Amersham, 900 Ci mmol^{-1}) was added to the culture. The addition of isotope was defined as time zero after which samples were removed and processed as described in Fig. 2.

* The total final exogenous amino acid concentrations, including isotope, in $\mu\text{g ml}^{-1}$ were 0.32 for methionine (32,000 eggs ml^{-1}) and 1.45 for leucine (23,000 eggs ml^{-1}). The final exogenous radioactivity was $5 \mu\text{Ci ml}^{-1}$ in each experiment.

† The averages of 5 determinations for each stage of development ± 1 s.d. The 2.2-fold difference in means yields a $t = 7.48$ for 8 degrees of freedom or $p < 0.001$, indicating that the difference is significant at the 99% confidence level in a standard t test⁸.

‡ The average transit time for ammonia-activated eggs is within 1 s.d. of the unfertilised egg. A χ^2 test¹² for group variance indicates that this value is probably a subset of the parent group unfertilised egg value, but not of the parent group zygote value.

rise in protein synthesis induced by ammonia lagged behind the rise caused by fertilisation, the same level of recruitment of ribosomes (and thus of mRNA) into polysomes was attained in both ammonia-activated and fertilised eggs. These observations suggested to us that ammonia activation may cause unmasking of mRNA but not stimulate a change in translational efficiency.

To test the hypothesis that ammonia-activated eggs recruit mRNA but fail to increase the rate of ribosome movement, we measured the average transit time in ammonia-activated eggs using the method developed for time-course incorporation into sea urchin eggs and embryos as shown in Fig. 2. The results are summarised in Table 1. We found that ammonia-activated eggs require as much time as unfertilised eggs to complete an average size polypeptide. This is about twice as long as the time required by zygotes. Thus, the increase in protein synthesis caused by ammonia treatment is the result only of an increase in the amount of available mRNA in the absence of an increase in the rate of ribosome movement. Therefore, the two events responsible for the rise in protein synthesis following fertilisation of the *S. purpuratus* egg must be independent of one another.

The normal acceleration of synthetic events following fertilisation seem to result from a transient elevation in intracellular pH caused by a massive influx of sodium ions coupled to a loss of protons¹⁰. Ammonia is believed to function as an activating agent by raising the internal pH of the egg. Weak organic bases enter the unfertilised egg in the uncharged form and then gain protons, thus raising the intracellular pH and mimicking the normal Na^+/H^+ exchange¹¹. This rise in internal pH must stimulate unmasking, but as ammonia does not stimulate a change in the rate of ribosome movement, the increase in translational efficiency in zygotes must depend on an as yet unidentified mechanism.

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Efficiency of protein synthesis after fertilisation of sea urchin eggs

ELUCIDATION of the mechanisms involved in the cytoplasmic control of protein synthesis is essential for an understanding of the initiation of embryogenesis. On fertilisation, echinoid eggs increase their rate of protein synthesis many-fold, independent of any increase in ribosomal or mRNA synthesis¹⁻⁴. Within 2 h of fertilisation the number of ribosomes in polyribosomes increases, about 25-fold without any change in the size distribution of poly ribosomes^{5,6}. In addition, the polyribosome number doubles again by gastrulation. However, the total rate of protein synthesis increases 113-fold from egg to gastrulation⁷. The discrepancy between the number of ribosomes recruited into polyribosomes, an increase of ~50 fold, and the total increase in the rate of protein synthesis, a 113-fold increase, could be explained if there were also an increase in the rate of movement of ribosomes along each message (elongation) and/or an increase in the rate of release of completed peptides (termination). We report here that the translational rate is increased about 2.5-fold after fertilisation in the sea urchin, *Strongylocentrotus purpuratus*. The average transit time (elongation plus termination time for an average-sized peptide) was measured for these eggs and several stages of embryos.

The transit times of eggs and embryos of *S. purpuratus* were measured at 12 °C by modifications of Fan and Penman's time course method^{8,9}. The low temperature used is that of the sea urchin's normal habitat and gives accurately measurable, slow elongation rates. The time course method is independent of variations in the rate of initiation of peptide synthesis, the number of polyribosomes and the specific activity of the amino acid pools. The method compares the ³H-amino acid incorporation into total peptides (incomplete nascent peptides still covalently bound to tRNA on the polyribosomes plus completed, released peptides) and the incorporation into released peptides determined after removal of the polyribosomes by centrifugation. One transit time after the amino acid pools have equilibrated, the rates of incorporation into total peptides and released peptides should be equal and graphically form two parallel lines separated by one-half the transit time⁸. In our experiments these lines, shown in Fig. 1 as open circles and solid circles respectively, did not become parallel but diverged with time. Presumably, this occurs because newly synthesised histones and other basic proteins attach to large deoxyribonucleoprotein or ribonucleoprotein particles¹³⁻¹⁵ and are removed during centrifugation. Analysis of the polyribosomal pellets for peptidyl-tRNA and peptides by DEAE-cellulose chromatography^{9,12} did show the presence of 15-55% released peptides. When the counts in the released peptide fraction

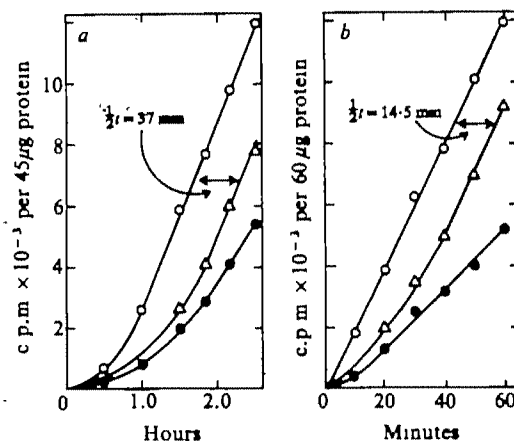


Fig. 1 Representative determinations of the average mRNA transit time in *S. purpuratus* unfertilised eggs (a) and 2-4-cell embryos (b). The time course methods of Fan and Penman⁸ and Palmiter⁹ were modified for sea urchins to determine elongation rates. Eggs and embryos were cultured in rotating tubes at 12 °C with ³H-leucine at various specific activities and at various culture densities as specified in Table 1. Samples of 1 ml were removed at the times indicated and added to 40 ml of -2 °C buffer I (0.2 M KCl, 0.05 M triethanolamine, 0.01 M MgOAc₂, pH 7.8) to stop synthesis and preserve polyribosome structures. The eggs and embryos were collected by centrifugation at 500g_{max} and washed by an additional 40 ml of buffer I; 1 ml of buffer I containing 2-3 mg ml⁻¹ purified yeast 4S RNA to compete for ribonuclease activity, 0.5% Triton X-100 and 0.5% Na deoxycholate was added to each sample. The eggs or embryos were homogenised either by rapid passage through a no. 20 G needle fitted with 22-μm Nitex Bolting or with a dounce homogeniser. The fertilisation membrane of the embryos had been softened by exposure to 50 mM 3-amino-1,2,4-triazole during fertilisation¹⁰. The homogenates were centrifuged in conditions normally used for removal of mitochondria or larger organelles, that is, at 14,000g_{max} for 10 min at 2 °C. The incorporation of ³H-leucine into precipitable peptides was essentially identical in aliquots taken before and after this centrifugation (±7%), indicating complete homogenisation of the eggs and embryos and total solubilisation of membranes, organelles and labelled cellular proteins. Aliquots from the low-speed supernatant, therefore, represented total incorporation (○). To eliminate sampling variations of about 10% the incorporation data were normalised to an average protein concentration after the determination of the total protein content by a modified Lowry procedure¹¹. The low-speed supernatant was centrifuged at 138,000g_{max} for 2 h at 2 °C in a Beckman type 65 rotor to remove polyribosomes. Aliquots of the resulting high-speed supernatant were removed to measure completed proteins released from the polyribosomes (●). All aliquots for counting were precipitated in 7.5% (w/v) trichloroacetic acid containing 0.1 mg ml⁻¹ unlabelled L-leucine, and collected on glass fibre disks, digested with N-chlorosuccinimide (Amersham) and counted in a toluene-based scintillant. They were not heated because it is not necessary to hydrolyse the ³H-leucyl-tRNA which occurs in both the aliquots of total incorporation and the released peptides fraction, and because heating selectively removes small peptidyl-tRNAs from the total incorporation. Peptides and peptidyl-tRNA in the S-138 pellet were separated on DEAE-cellulose^{9,12} as described in Fig. 2. Counts from the proportion of the pellet that eluted as peptides were added to those from the high-speed supernatant for total released peptides (Δ). The horizontal distance between the parallel lines for total incorporation (○) and total released peptides (Δ) represent one-half an average transit time, which was 37 and 14.5 min, respectively, for a and b.

recovered from the polyribosomal pellets were added to those of the released peptides in the high-speed supernatant, parallel lines were obtained and transit times determined (Fig. 1, open circles and triangles). The 2-h centrifugation time for polyribosomes was chosen for removal of peptidyl-tRNA with minimal co-centrifugation of released peptides (Fig. 2).

Table 1 summarises the results obtained by time course measurements such as those in Fig. 1. No variation in stage-specific transit times was observed by varying culture conditions such as the concentrations of amino acids or centrifugation times. This showed that, as predicted, the transit times we measured were independent of the specific activities of the amino acid pools, and that our analysis of the released peptides co-sedimenting with polyribosomes was valid and accurate. At 12 °C the transit time is about 69 min per average length peptide chain in unfertilised eggs, which is about 2.5-fold longer than for embryos (Table 1). The transit times were 20-24 min for pre-cleavage embryos, 29 min for embryos undergoing 2nd cleavage during the experiment, and 28-32 min for embryos in the 4th cleavage. Therefore, the translational rate increased as early

Table 1 Average mRNA transit time in *S. purpuratus*

Stage	Transit time (min at 12 °C)	μM leucine†	mCi ^3H per μmol leucine	Culture density‡
Unfertilised eggs	66	0.52	24.1	13,800
	64–69*	0.93	13.4	32,100
	72–78	1.36	12.3	21,400
	66–70	2.42	8.3	57,100
Average	69			
Fertilised eggs (60 min, 12 °C)	20–24	199.0	0.10	30,900
Embryos (2–4 cells)	29*	49.8	0.25	13,100
Embryos (16–32 cells)	29	82.9	0.20	28,600
Embryos (16–32 cells)	28–32	199.0	0.10	28,600

The transit times were determined as in Fig. 1 except that samples marked with an asterisk were centrifuged for 2.5 h. The range of graphical error is given where it is greater than 1 min.

† The initial concentration in seawater. Because the rate of amino acid uptake by eggs is very slow compared with embryos¹⁶, the concentration and radioisotopic activities of amino acids is necessarily very different.

‡ The number of eggs or embryos per ml was calculated assuming 40 ng protein per egg or embryo¹⁷.

after fertilisation as we were able to obtain accurate transit times, and did not vary significantly through these early stages.

How valid is the measurement of the transit times, which depend on the weight average molecular weight (MW) of the peptides being synthesised? Bradhorst¹⁸ identified 400 distinctly resolved peptides synthesised before and 30–60 min after fertilisation of *Lytechinus pictus* eggs and found that at least 90% of the spots co-electrophoresed and had similar relative intensities. He found similar results for *S. purpuratus* eggs and zygotes and concluded that there was a quantitative rather than a qualitative regulation of the translation of stored mRNA after fertilisation. Other evidence which supports this conclusion is the similar sedimentation of newly synthesised peptides from eggs and embryos of *L. pictus* in SDS-urea sucrose gradients¹⁹, a similar average MW distribution of *S. purpuratus* nascent chains on SDS-polyacrylamide gels²⁰, and a similar size distribution of *S. purpuratus* polyribosomes²⁰. Therefore, the increased translational rate we observed after fertilisation is not due to a decrease in the weight average peptide length.

At first glance, our measured transit times of about 29 min at 12 °C for embryos seem long compared with those of mammalian systems measured at 36 °C and summarised in Table 2. However, Craig^{21,22}, in a very thorough and careful manner, has shown that the transit times increase dramatically (7–50-fold) at low temperatures (Table 2). Mouse L-cells synthesising an average peptide chain length of 85,000 daltons have an average transit time of 22 and 13 min at 10 °C and 15 °C, respectively. Reticulocytes synthesising α and β -globin (MW = 17,000) have an average transit time of 20.7 and 6.4 min at 10 °C and 15 °C, respectively. Therefore, the transit times observed for *S. purpuratus* embryos are not unusually long compared with other systems at low temperatures, especially considering tissue variations such as those in Table 2 for hamster ovary cells and chick oviduct at 35–36 °C. However, the transit time of 69 min for unfertilised *S. purpuratus* eggs is unusually long, presumably reflecting the quiescent state of the eggs and their need to conserve materials for later development.

In a paper published after submission of our manuscript, Brandis and Raff²⁰ similarly report long transit times in *S. purpuratus* eggs at 16.5 °C and show a 2–3-fold acceleration following fertilisation. Our observations at 12 °C are in close quantitative agreement if the Q_{10} for elongation is 3 in sea urchins. Changes in elongation rates have also been observed, by Palmiter⁹, in chick oviducts. Stimulation of the oviduct with oestrogen caused a 1.5-fold increase in the elongation rate compared with unstimulated oviduct.

There are several possible reasons for the failure of others to detect the difference between the translational efficiency of eggs and of embryos in sea urchins. For example, Humphreys¹⁹ measured the incorporation of ^{14}C -amino acids into peptides

after only 10 and 20 min of labelling at 18 °C. This was probably too short to achieve equilibrated labelling of the nascent peptides on the polyribosomes, especially for eggs in which he observed an 8-min lag before labelling of the total cellular peptides became linear. Both linear kinetics and complete labelling of nascent peptides are necessary for accurate determinations of transit times. Furthermore, Craig has recently demonstrated the temperature dependence of transit times in mammalian systems^{21,22}; this makes the 1–2-min transit times determined by Humphreys¹⁹ for eggs and embryos at 18 °C seem unreasonably rapid.

Felicetti *et al.*²³ found that, within minutes of fertilisation of *Paracentrotus lividus* eggs, there was a threefold increase in the activity of T1 elongation factor plus a 1.5-fold increase in the activity of T2 elongation factor. The rapid activation of these factors suggests mobilisation of pre-existing proteins. Whether the activity of elongation factors increases only to keep pace with increasing numbers of polyribosomes or whether they are also responsible for increased rates of translation has not been known, but it is now apparent that some of the factors contribute to an increase in translational efficiency.

As the polyribosome size in embryos is the same as that in eggs²⁰, initiation factors must also be available to keep pace with both the increased translational efficiency and the increased recruitment of ribosomes into polyribosomes. Whether these initiation factors pre-exist in an active form, are mobilised to an active form at fertilisation, or are newly synthesised, is not known. However, in the mid-blastula stage the supply of initiation factors does not keep pace with the demand, as about 50% of the mRNAs are not fully loaded with ribosomes²⁴.

What is the relationship of our results to previous studies on the activation of protein synthesis at fertilisation? We propose that the increased rate of elongation after fertilisation will account for observed differences between the increased numbers of ribosomes in polyribosomes^{5,6} and the changes in the absolute rate of protein synthesis⁷. The observed recruitment of ribosomes and mRNA into polyribosomes after fertilisation could occur by a release of mRNAs from inhibited, preformed messenger ribonucleoprotein particles^{4,14} and/or by an increase in the rate with which ribosomes attach to mRNA and initiate protein synthesis^{25–28}. Humphreys⁵ accurately determined the per cent of ribosomes in polyribosomes in *L. pictus* by measuring the ribosomal RNA content of these fractions. He found an average of 0.75% of the ribosomes in polyribosomes in unfertilised eggs and about 20% as polyribosomes in 2–6-h (18 °C)

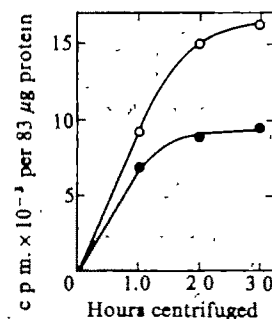


Fig. 2 Determination of optimum centrifugation times for the polyribosome fraction. The low-speed supernatant from eggs labelled with ^3H -leucine was centrifuged at 138,000 $\times g_{\text{max}}$ at 2 °C for 1, 2 and 3 h to pellet polyribosomes. The ribonucleoprotein pellets thus obtained were subjected to DEAE-cellulose chromatography to separate released peptides and peptidyl-tRNA^{9,12}. The polyribosomal pellets were dissolved overnight at 0 °C in 0.5 ml of 3 M LiCl, 6 M ultra pure urea (Schwarz/Mann), 50 mM NaOAc and 5 mM dithiothreitol, pH 5.2, and stored at –80 °C. The solubilised pellet was diluted 30-fold with 8 M ultra pure urea at pH 5.4 containing 6 mM β -mercaptoethanol and applied at room temperature to a 0.5 $\times$ 1 cm DEAE-cellulose column pre-equilibrated with buffer II (0.1 M NaOAc, 8 M ultra pure urea, 6 mM β -mercaptoethanol, pH 5.6). Peptides were eluted with buffer II and peptidyl-tRNA was eluted with buffer III (0.75 M NaOAc, 8 M ultra pure urea, 6 mM β -mercaptoethanol, pH 5.6), and buffer IV (1 M KCl, 0.1 M Tris-HCl, 8 M ultra pure urea, 6 mM β -mercaptoethanol, pH 7.4). The total incorporation associated with the polyribosomal pellet (O) and the fraction occurring as ^3H -peptidyl-tRNA (●) are shown. At 2 h peptidyl-tRNA in the pellet reaches a maximum.

Table 2 Temperature dependence of transit times

Tissues	Transit time (min)			MW*	Ref.
	10 °C	15 °C	35–36 °C		
Mouse L-cells	22.0	13.0	2.0	85,000	21
Reticulocyte cells	20.7	6.4	0.42	17,000	22
Hamster ovary cells			2.0	85,000	8
Chick oviduct			3–4	38,000	9

* Weight average MW.

embryos, a total increase of 27-fold. By measuring the absorption of polyribosomes and monoribosomes at 260 nm, Infante and Nemer⁶ found the percentage of ribosomes as polyribosomes to be 5% in unfertilised eggs, 25% in early cleavage stages, 50% in blastulae and 40% in gastrulae of *S. purpuratus*, a stimulation of 8–10-fold. The largest discrepancy between these data and those for *L. pictus* is for the unfertilised egg. How much of this difference is due to species variation and how much to methods is not known. If, however, we assume that Humphrey's more accurate method establishes the lower limit for polyribosomes in unfertilised eggs at slightly less than 1%, the maximum increase in polyribosomes in *S. purpuratus* by gastrula stage would be 50-fold. In the absence of better data we will use this value for comparison with changes in the total rates of protein synthesis.

Absolute rates of protein synthesis are difficult to obtain because of the 25-fold slower uptake of amino acids by eggs compared with embryos¹⁶ which results in a large difference in the specific activities of their amino acid pools. Regier and Kafatos⁷, however, measured the specific radioactivity of precursor aminoacyl-tRNA pools to obtain accurate data. They found a 113-fold stimulation in the absolute rate of protein synthesis in *S. purpuratus* at the gastrula stage compared with unfertilised eggs. Although this value must theoretically include the 50-fold increase in ribosomes as polyribosomes by the gastrula stage, it is more than twice the latter. As newly synthesised ribosomes are less than 10% of the total ribosome population by gastrulation and as there is also some degradation of old ribosomes²⁹, increased numbers of ribosomes cannot account for any significant increase in rates of protein synthesis. The difference between the 113-fold stimulation in the absolute rate of protein synthesis and the 50-fold increase in polyribosomes can be accounted for by the increased efficiency of translation we report here³⁰.

The unmasking of maternal messenger RNA and/or the increased availability of ribosomal subunits to initiate protein synthesis result in a very large stimulation of protein synthesis after fertilisation, as shown by Humphreys³. However, the additional 2.5-fold increase in the rates of elongation can no longer be ignored as contributing to the observed activation of these eggs and the probable doubling of the growth rate of these embryos.

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2-5A synthetase: assay, distribution and variation with growth or hormone status

PROTEIN SYNTHESIS in cell-free systems from interferon-treated cells and rabbit reticulocyte lysates is exceptionally sensitive to inhibition by double-stranded RNA (dsRNA)^{1,2}. Two distinct mechanisms seem to be involved: (1) inactivation of initiation factor eIF2 by dsRNA-mediated phosphorylation and (2) degradation of mRNA by one or more nucleases which are activated by sub-nanomolar levels of an unusual oligonucleotide, pppA2'p5'A2'p5'A (refs 3–10). The enzyme responsible for synthesising pppA2'p5'A2'p5'A requires dsRNA for activity, binds to poly(I) · poly(C)-Sepharose, and can be used conveniently in this insoluble form to synthesise pppA2'p5'A2'p5'A and related oligomers^{4,11}, collectively referred to as 2-5A. The discovery of relatively high levels of 2-5A synthetase in rabbit reticulocyte lysates¹² raised the possibility that the 2-5A system might be found in other cells which have not been treated with interferon and might, therefore, have a wider significance. We have developed a more convenient assay for the synthetase based on its activity when bound to poly(I) · poly(C)-paper rather than poly(I) · poly(C)-Sepharose and show here that this enzyme is distributed widely in mammalian cells. Moreover, different cells or tissues have widely differing levels of this enzyme and in the case of chick oviducts there is a substantial increase in synthetase level after withdrawal from stimulation by oestrogen.

The 2-5A synthetase from interferon-treated mouse L cells or rabbit reticulocyte lysates was equally stable and active when bound to poly(I) · poly(C)-paper or poly(I) · poly(C)-Sepharose. However, the paper has a number of advantages, especially reproducibility of preparation, stability to storage and convenience in use, particularly with multiple small volumes. There is little or no loss of poly(I) · poly(C) from the paper during prolonged incubation (>10 d) with the bound enzyme, allowing the same paper to be re-used several times. The paper-bound enzyme retains full activity on storage for at least 6 weeks at –70 °C.

One square centimetre of poly(I) · poly(C)-paper was at least five times more than enough to bind all the 2-5A synthetase available in a 200-μl extract from reticulocytes or interferon-treated L cells. There was a linear relationship between the amount of extract used in the assay and the amount of 2-5A synthesised. When different amounts of cell sap from interferon-treated cells were mixed with sap from control cells before adsorption of the synthetase to poly(I) · poly(C)-paper, the yield of 2-5A after incubation was proportional to the amount of interferon cell sap used. The levels of ATP used were not limiting. Optimum conditions for synthesis of 2-5A were identical with the chick oviduct, reticulocyte or interferon enzyme bound to poly(I) · poly(C)-paper. Therefore, these conditions

were used throughout the survey of synthetase levels in different cells and tissues described in Table 1.

2-5A synthetase was detected readily in all the mammalian extracts tested, but the amount of enzyme activity varied more than 1,000-fold (Table 1). It was lowest in MRC5 cells in which there was little change in level with passage number, with the possible exception of a significant decrease at passage 55 just before senescence at passage 57. There was slightly more enzyme in pig lymphocytes, with or without stimulation by phytohaemagglutinin. Significantly higher levels were obtained in mouse erythroleukaemia (Friend) and in human lymphoblastoid (Namalva) cells. The fact that the different Friend cell lines F4N, F4N+2 and F4N+ (ref. 13), have different levels is

Table 1 2-5A synthetase levels in a variety of cells and tissues

	Source	Specific activity (pmol 2-5A per A ₂₆₀ per h)
1	Interferon-treated mouse L cells (splitter culture)	600
	Control cells	1
2	Interferon-treated human diploid fibroblasts (ICRF23)	600
	Control cells	10
3	Rabbit reticulocytes	300*
4	Chick oviduct (see Table 2)	5-200
5	†Guinea pig mammary gland:	
	pregnant	70
	lactating	5-50
	2 day-withdrawn	60-80
	4 day-withdrawn	100
6	†Mouse erythroleukaemia (Friend) cells:	
	F4N	10
	F4N+	4
	F4N+2	115
7	†Human lymphoblastoid cells (Namalva)	50
8	Mouse L cells (monolayer) subconfluent to 8-day confluent	0.6-6
9	†Green monkey kidney cells (Vero): subconfluent to 2-day confluent	0.1-1
10	Human diploid fibroblasts (MRC5): passage 37, subconfluent to 6-day confluent	<0.1-0.3
11	†Pig lymphocytes	1.5*
	Phytohaemagglutinin-stimulated	3*
12	† <i>Xenopus laevis</i> :	
	liver	<0.04
	pooled blood cells	<0.04
13	† <i>Drosophila melanogaster</i> :	
	20-h embryos	<0.04
	3rd instar larvae	<0.04
	adult flies	<0.04

Extracts (post-mitochondrial supernatant fractions) were prepared at 0-4°C from packed cells or finely minced tissue which had been frozen at -70°C as described previously⁹ except that the homogenisation buffer contained 0.5% NP40 where indicated (†). (Control experiments showed 0.5% NP40 to be without effect on the assay of the synthetase.) The absorbance of the extract at 260 nm (A₂₆₀) was determined after dilution with 0.4 M NaOH. The synthetase was bound to poly(I)·poly(C)-paper by incubating 100-200 µl of extract with 1 cm² of paper for 1 h at 25°C. The paper was washed thoroughly with 90 mM KCl, 10 mM HEPES, pH 7.5, 1.5 mM magnesium acetate, 7 mM 2-mercaptoethanol, 20% glycerol (v/v). It was incubated for 30 min at 25°C with 1 ml of the same buffer containing 50 mM KCl, and then for 18 h at 30°C with 10-200 µl of this buffer containing 3 mM ATP and 8.5 mM magnesium acetate. The yield of 2-5A was assayed by determining the dilution required for 50% inhibition of protein synthesis in an L-cell-free system programmed with encephalomyocarditis virus RNA, in comparison with a standard preparation of 2-5A assayed in parallel.^{11,15} Specific activities are based on the amount of 2-5A produced per h, in AMP equivalents. Poly(I)·poly(C)-paper (10-20 µg poly(I)·poly(C) per cm²) was prepared by a modification of the method of Alwine *et al.*¹⁶. To a 20×10 cm sheet of diambenzylxymethyl-paper were added 5.0 ml of a mixture of 0.5 ml poly(I)·poly(C) (10 mg ml⁻¹), 0.9 ml of 25 mM sodium phosphate buffer, pH 6.0 and 3.6 ml dimethyl sulphoxide. After incubation overnight at room temperature, the following mixture was added to allow the denatured RNA to rehybridise: 3 ml bovine serum albumin (2 mg ml⁻¹, pretreated with 0.1% diethylpyrocarbonate for 1 h at room temperature), 1.5 ml 10% SDS, 25 ml 2×SSC (300 mM NaCl, 30 mM trisodium citrate), 30 ml formamide, 8.5 µl poly C (5 mg ml⁻¹). After 24 h at room temperature with gentle shaking, the paper was washed thoroughly 3 times with 2×SSC, blotted dry and stored at -70°C. Interferon treatment of mouse L cells was as described previously²; treatment of the human diploid fibroblasts (ICRF23) was for 24 h at 37°C with 10⁵ units ml⁻¹.

* The A₂₆₀ of these extracts was not determined and was assumed to be 100 for the calculation of specific activity. Values of 20 to 160 were determined for the remainder of the samples.

interesting but its significance is not known. More interestingly, the level of enzyme in chick oviducts varied with the time of withdrawal from stimulation by oestrogen (see below and Table 2). A preliminary search for a similar phenomenon in mammary glands from lactating guinea pigs, which involute on removal of the young, has yielded equivocal results so far (R. K. Craig, G.R.S., R.E.B. and I.M.K., unpublished). Finally, in a total of six experiments, enzyme levels were compared in subconfluent and confluent monolayers of L, Vero and MRC5 cells. The level of enzyme in confluent cells increased very gradually for up to 8 d.

In the experiments with chick oviducts, despite some individual variations (see Table 2 legend), the same trend was seen in multiple assays of extracts from five different sets of oviducts. It seems reasonable to conclude, therefore, that the assayable enzyme remains relatively low in the first 24 h after hormone withdrawal and increases gradually thereafter, to reach final levels about 10-fold higher, approaching those in reticulocytes or interferon-treated cells (Table 1). Control experiments indicated that the differences observed with extracts made as described in Table 2 probably reflect changes in the level of 2-5A synthetase rather than changes in the levels of an antagonist or degradative enzyme. For example, a dominant inhibitor was not detected in mixing experiments. In addition, synthesis of 2-5A was linear for up to 40 h with paper-bound enzyme from oviducts early (1 h) or late (10 d) after withdrawal from hormone, and added 2-5A was equally stable in either case.

The inhibitor of protein synthesis generated by incubation of the enzyme bound to poly(I)·poly(C)-paper was not characterised in most of the experiments shown in Table 1. However, the product from chick oviducts was radioactively labelled¹¹ and characterised as 2-5A by thin-layer chromatography, analysis on DEAE-cellulose in the presence of 7M urea^{4,7}, and high pressure liquid chromatography. It also had the same specific biological activity as mouse or rabbit reticulocyte 2-5A in inhibiting cell-free protein synthesis.

These results demonstrate the wide distribution and variation in level of detectable 2-5A synthetase activity in a variety of cells and tissues. Small differences in activity are probably not significant with the biological assay used, but it seems improbable that the large differences observed in Tables 1 and 2 are trivial. The relationship between the increase in 2-5A synthetase and the decrease in the proportion of tubular gland cells which accompanies long-term withdrawal of the oviduct from oestrogen stimulation is not known, but the enhanced level of synthetase suggests a possible involvement of the 2-5A-dependent nuclease in the regression of this tissue. A role for the 2-5A system in the rapid degradation of ovalbumin mRNA that quickly follows withdrawal of oestrogen (ref. 14 and W.J.D. and R.T.S., unpublished) is also possible, despite the apparently low levels of 2-5A synthetase at this time (Table 2). Note that here we are assaying only one component of the 2-5A system and we have no means of knowing what levels would be rate limiting in different conditions in the intact cells. Moreover, we cannot yet be certain that pre-activated 2-5A synthetase already bound to daRNA (or alternative activator) might not be sequestered and unavailable to the assay used, that is, here we may be assaying potentially active rather than total, or active, enzyme. 2-5A has a short half life in chick and L-cell-free systems, but saturates the activatable nuclease at very low concentrations, below 10 nM (ref. 9, B.R.G. Williams and I.M.K., unpublished data). Accordingly, to understand the operation of the 2-5A system fully it will also be necessary to determine the nature and level of the (daRNA) activator of the synthetase, whether the level of 2-5A sensitive nuclease varies, what intracellular levels of 2-5A are achieved and how these levels are maintained by a balance of synthesis and degradation in different tissues in different conditions. This last point will require the development of a convenient quantitative assay for 2-5A capable of detecting physiological concentrations (< nanomolar) in extracts of cells. Meanwhile, despite the uncertainties outlined above in the

Table 2 Effect of withdrawal of oestrogen stimulation or 2-5A synthetase activity in chick oviducts

Hormonal status	Specific activity (pmol 2-5A per A ₂₆₀ per h)	
	Expt 1	Expt 2
Fully stimulated	—	12
1 h-withdrawn	6	—
3 h-withdrawn	16	—
6 h-withdrawn	13	—
12 h-withdrawn	17	—
18 h-withdrawn	17	—
1 d-withdrawn	—	12
2 d-withdrawn	—	15
3 d-withdrawn	—	62
5 d-withdrawn	—	63
7 d-withdrawn	—	70
10 d-withdrawn	134	130

Extracts prepared without NP40 from chick oviducts pre-frozen at -70°C were assayed for 2-5A synthetase by the method given in Table 1, scaled down 10-fold. Oestrogen stimulation of 5 to 7 day-old chicks and withdrawal after 14 days were accomplished by subcutaneous implantation and removal of diethylstilbestrol-impregnated silastic pellets (W.J.D. and R.T.S., unpublished). There was some variability in the values obtained in different experiments: for example, 6 different 10 day-withdrawn oviducts yielded values of 85, 88, 120, 130, 134 and 200. The only extensive variation was obtained with five fully stimulated oviducts, which gave values of 1.5, 5.5, 12, 15 and 290. The reason for this variability is not known, although the one very high value might reflect an elevated interferon level due to an undiagnosed virus infection.

Interpretation of the levels of synthetase detected, the very different levels observed, whether they reflect total or only potentially active enzyme, are of interest and indicate a wider significance for the 2-5A system than simply in the antiviral action of interferon. Taken together, the function of 2-5A in activating a nuclease which degrades mRNA, the widespread occurrence of 2-5A synthetase, the high levels of this enzyme in highly differentiated cells where mRNA degradation may have played a part in preparing the cell for specialised function¹² and the increase in the level of synthetase in some situations strongly suggest that the 2-5A system may have an important regulatory function in processes such as maintenance of cell stasis, tissue regression and differentiation.

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'Freezing' of Ca-regulated conformation of reconstituted thin filament of skeletal muscle by glutaraldehyde

REGULATION of skeletal muscle contraction by calcium ions is mediated by the troponin-tropomyosin system located in the groove of actin double strands¹. Acting with tropomyosin, troponin depresses the ability of the actin filament to interact with the myosin molecule in the presence of ATP. Some structural changes in the thin filament associated with this depression have been detected by physical techniques²⁻⁸. When the actin filament is cross-linked with glutaraldehyde, it retains the ability to activate myosin ATPase⁹, and troponin and tropomyosin can no longer exert their regulatory effect on these cross-linked or 'frozen' actin filaments¹⁰. Using glutaraldehyde as cross-linking agent we have now been able to 'freeze' the activated (presence of calcium) and depressed (absence) states of the actin filament induced by the troponin-tropomyosin system: this suggests that the structural changes detected using physical methods are essential for regulation by calcium.

Reconstituted thin filament, composed of 0.25 mg ml⁻¹ troponin, 0.22 mg ml⁻¹ tropomyosin and 1 mg ml⁻¹ actin from rabbit back muscle, was incubated with 0.1% glutaraldehyde (Sigma) in a solution containing 0.1 M KCl, 3 mM MgCl₂ and 50 mM HEPES (pH 7.2) for 7 min at 25 °C in the presence or absence of calcium ions. The cross-linking reaction was terminated by adding KSCN to a final concentration of 50 mM, and the resulting solution containing cross-linked thin filaments was dialysed against 0.1 M KCl containing 3 mM MgCl₂ and 20 mM Tris-HCl (pH 7.6).

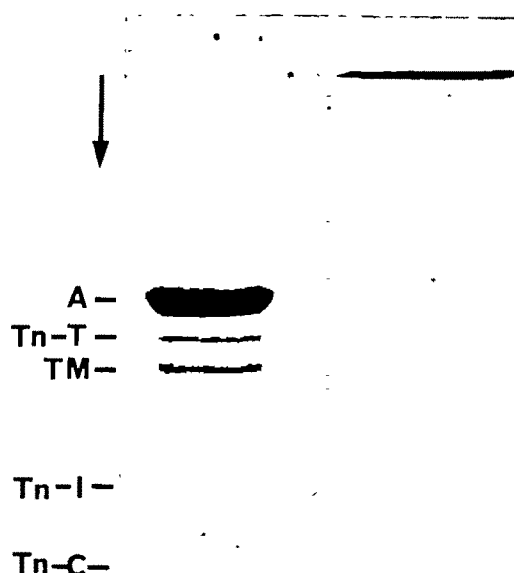


Fig. 1 SDS-polyacrylamide gel electrophoresis¹⁵ of the reconstituted thin filament before (a) and after (b) cross-linking with glutaraldehyde.

Table 1 Myosin ATPase activities activated by cross-linked thin filament

Type of thin filament	ATPase ($\mu\text{mol Pi per min per mg}$)	
	$10^{-7} \text{ M Ca}^{2+}$	$1.3 \times 10^{-5} \text{ M Ca}^{2+}$
Control	0.025	0.112
Cross-linked in the absence of Ca^{2+}	0.042	0.039
Cross-linked in the presence of Ca^{2+}	0.145	0.150

ATPase was assayed at 25°C in a solution containing 0.2 mg ml^{-1} myosin, 0.2 mg ml^{-1} thin filament, 0.1 M KCl , 3 mM MgCl_2 , $20 \text{ mM Tris-maleate}$ ($\text{pH } 6.8$) and 1 mM ATP . Ca^{2+} concentration shown corresponds to the presence of 0.1 mM EGTA alone (10^{-7} M) or the presence of 0.1 mM EGTA containing 0.095 mM CaCl_2 ($1.3 \times 10^{-5} \text{ M}$). ATPase activity was determined by the method of Youngburg and Youngburg¹⁶.

On SDS gels, most proteins of the cross-linked thin filament did not enter the acrylamide matrix, indicating that a considerable degree of intermolecular cross-linking had occurred (Fig. 1). However, under the electron microscope no differences in filamentous features could be seen between the thin filament before and after cross-linking.

As shown in Table 1, the reconstituted thin filament cross-linked in the presence of the calcium ion (that is, in the activated state) did not show the depression of actin-activated myosin ATPase even in the absence of the calcium ion, whereas the filament cross-linked in the absence of the calcium ion (in the depressed state) showed only a low level of activation of myosin ATPase even in the presence of calcium ion. In each case, the ATPase activity was not influenced by the calcium ion.

Thus cross-linking with glutaraldehyde can 'freeze' the structure of the thin filament in a specified functional state. Glutaraldehyde may therefore be useful as an aid in the structural analysis of the thin filament⁸. When dimethylsuberimide dihydrochloride was used as cross-linking agent in place of glutaraldehyde, extensive cross-linking of the thin filament was observed but the interaction of the resulting thin filament with myosin was still regulated by calcium ions.

The method described here will be of wide application, and has already provided evidence to show that gizzard muscle regulation¹¹ is actin-linked, as has been asserted by Ebashi *et al.*¹²⁻¹⁴.

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Movement of myosin heads during a heart beat

X-RAY diffraction studies of heart muscle undergoing cyclic contractions have shown that the myosin heads are transferred from the vicinity of the thick filaments to that of the thin filaments as the muscle passes from the diastolic (relaxed) to the systolic (contracted) state^{1,2}. We have now followed the time course of this transfer using a fast X-ray diffraction technique and found that the transfer occurs almost in parallel with tension development.

A papillary muscle was isolated from the right ventricle of a canine heart and cross-perfused with the arterial blood of a donor dog³. A branch of the arterial tubing was used to drip warm blood on to the surface of the muscle to keep the temperature of the muscle at about 37°C . The muscle was held isometrically at the optimal length (L_{max}) and was stimulated once a second with a square pulse of 5-ms duration (for details see ref. 4). The muscle was connected to a tension transducer (Shinko, UL) and mounted vertically in a low-angle camera of the mirror-monochromator type⁵. The X-ray source was a rotating-anode generator (Rigaku FR) operated at 50 kV with a tube current of 70 mA (the size of the focal spot was $1 \times 0.1 \text{ mm}$). The equatorial diffraction pattern from the muscle was recorded with a position-sensitive counter of the triangular-cathode type⁶ at a specimen-to-counter distance of 70 cm. The output of the counter was fed to a memory circuit⁷⁻⁹ which registered separately the signals from 16 different phases during the cardiac cycle. To obtain reasonable photon statistics for each phase the cardiac cycle was repeated 4,000-10,000 times.

The muscle produced a peak tension of $483 \pm 38 \text{ g cm}^{-2}$ at a time $250 \pm 8 \text{ ms}$ after the beginning of the stimulus (mean $\pm$ s.e.m., number of muscles = 5). The diffraction pattern showed the 1, 0 and 1, 1 equatorial reflections from the hexagonal array of the myofilaments (Fig. 1). We measured the integrated intensities of these reflections and calculated the intensity ratio ($I_{1,0}/I_{1,1}$). On contraction the 1, 0 intensity decreased and the 1, 1 intensity increased, so that the ratio $I_{1,0}/I_{1,1}$ decreased.

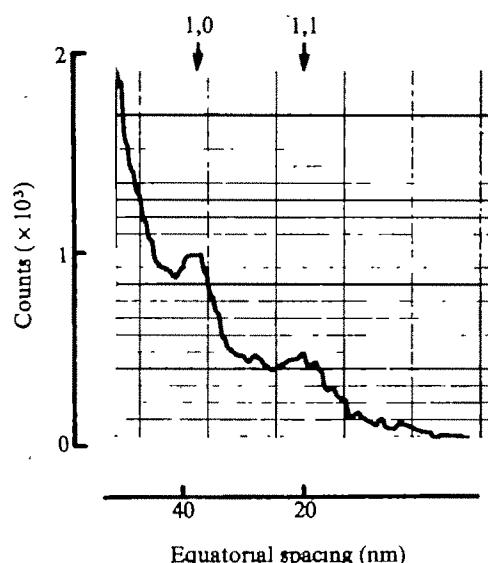


Fig. 1 Equatorial intensity distribution (right half only) of the X rays scattered by a canine papillary muscle in a 62.5-ms period during the diastolic phase. Thin papillary muscles ($0.65\text{--}1.2 \text{ mm}$ thick) were chosen in order to avoid high background scatter. The muscles were capable of contracting more than 20,000 times with little decline of peak tension⁴. Approximately 4,800 contractions were needed for recording the intensity distribution. The intensities of the 1, 0 and 1, 1 reflections from the hexagonal myofilament lattice were obtained by measuring the area under the peaks; the background level under each peak was drawn in by eye.

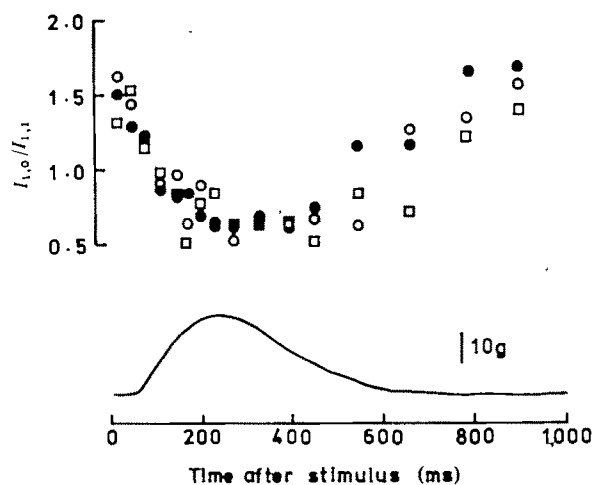


Fig. 2 Changes in the equatorial intensity ratio ($I_{L,0}/I_{L,1}$) in a papillary muscle during the cardiac cycle. The 1-s cycle was divided into 16 phases, and the ratio was measured for each phase; the first 250 ms of the cycle was divided into eight 31.25-ms phases, the following 250 ms into four 62.5-ms phases, and the last 500 ms into four 125-ms phases. Note that, as the contraction was cyclic, the last period of measurement (875–1,000 ms after the beginning of the stimulus) was followed immediately by the first period (0–31.25 ms after the beginning of the stimulus). The same set of diffraction patterns was measured by three different people who made their own estimation of the background level (see legend to Fig. 1); symbols (○, ●, □) represent the measurements by different people. The tension record was obtained by averaging 10,000 contractions with a multichannel analyser⁹. The cross-sectional area of the muscle at its middle was 0.045 cm².

Figure 2 shows that the time course of the change in intensity ratio was an approximate mirror image of the tension curve; the ratio reached the minimum value of 0.74 ± 0.06 ($n = 5$) at approximately the same time as the peak tension.

The observed decrease in the intensity ratio was caused partly by radial transfer of myosin heads from the thick to the thin filaments and also by slight shortening of sarcomeres; although the muscle was held isometrically, the sarcomeres in the middle of the muscle became about 10% shorter at the peak of contraction². Figure 3 shows the result of an experiment in which we assessed the contribution of the sarcomere shortening. First we obtained an intensity-ratio time-course with a muscle length of L_{max} (solid line). Then we repeated the measurements (dotted line) on the same muscle with a muscle length of $0.9L_{max}$ so that the sarcomeres were already 10% shorter in the diastolic phase (and therefore about 20% shorter at the peak of contraction). If the sarcomere length had stayed constant (at the length which was attained at the peak of contraction for the first set of measurements) throughout the cardiac cycle, the intensity ratio would have changed in the following manner: (1) the ratio would have corresponded to a point on the dotted line at the beginning of the cycle, and moved toward the solid line as the tension developed; (2) the ratio would have reached the solid line at the peak of tension; the minimum ratio at a constant sarcomere length would have been found near the minimum of the solid line; and (3) the ratio would have moved toward the dotted line during the falling phase of tension, reaching the dotted line when the tension reached the steady diastolic level. Such an imaginary curve would represent more correctly the radial transfer of myosin heads; a decrease in the ratio at a constant sarcomere length represents an increase in the number of heads in the vicinity of the thin filaments^{10,11}. Our results suggest that the number reaches a maximum approximately at the time of peak tension, at ~250 ms after the stimulus at 37°C. This is much slower than in skeletal muscle in which the number reaches a maximum within 70 ms after the stimulus at 0°C and even earlier at higher temperature^{9,12} (the peak tension in skeletal muscle occurs 150–160 ms after stimulus at 0°C). The slow

transfer of myosin heads in heart muscle may be the basis of the slow development of the active state in heart muscle¹³.

The maximum intensity ratio obtained during the diastolic phase was 1.72 ± 0.09 ($n = 5$) at L_{max} , and smaller at $0.9L_{max}$ (Fig. 3). It is known that a quiescent heart muscle at $0.9L_{max}$ gives an intensity ratio of 3.07 (ref. 1) which is considerably greater than the maximum ratios obtained in the present study. This indicates that, during the cardiac cycle, the myosin heads as a whole never go into the 'quiescent' state; there is always a considerable number of heads remaining in the vicinity of the thin filaments during the diastolic phase. However, these myosin heads are unlikely to be producing significant force during the diastolic phase as a previous study² has shown that a change in the number of 'remaining' myosin heads is not accompanied by a change in the diastolic tension.

It is of interest to know how the behaviour of myosin heads during the cardiac cycle is affected by the physiological means used to change the size and the time course of tension development. We studied the effect on the intensity ratio of repetitive stimulation with paired pulses; this type of stimulation increases the peak tension and shortens the time from stimulus to the peak tension¹⁴. A muscle was first stimulated once a second with single pulses. Then the same muscle was stimulated every second with a pair of pulses to cause stronger contractions. The interval between the pair of pulses was set at 270 ms to produce maximal potentiation. The time course of the intensity ratio for the two patterns of stimulation are compared in Fig. 4. When stimuli were changed from single to paired pulses, the muscle had to be stretched slightly in order to re-adjust its length of L_{max} ; the internal shortening of sarcomeres was enhanced by the increased tension. This re-adjustment allowed us to compare the minimum intensity ratios, which were obtained at the time of peak tension both with single and paired pulses, at approximately the same sarcomere length. The minimum ratio was smaller with paired pulses than with single pulses, suggesting that paired-pulse stimulation increases the number of myosin heads found in the vicinity of the thin filaments at the peak of contraction. This is likely to be the basis of the tension increase caused by paired pulses. The intensity ratios in the diastolic

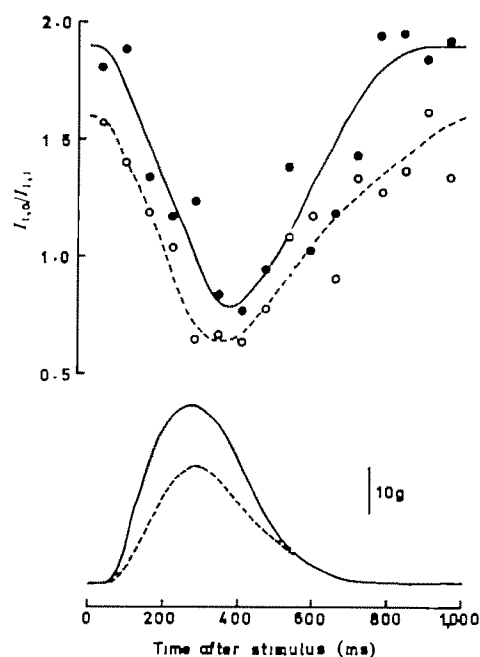


Fig. 3 The intensity ratios obtained from the same muscle at different muscle lengths, L_{max} (filled circles and solid line) and $0.9L_{max}$ (open circles and dotted line). The 1-s cardiac cycle was divided into 16 phases of 62.5-ms duration. The tension records are averages of all the contractions needed for recording diffraction patterns at L_{max} and $0.9L_{max}$. The cross-sectional area of the muscle at L_{max} was 0.084 cm².

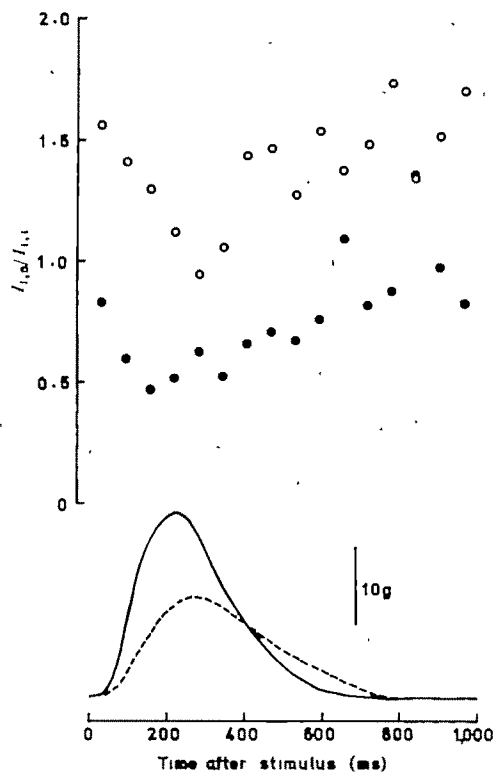


Fig. 4 The intensity ratios obtained from the same muscle contracting at the same frequency (1 s^{-1}) but with different types of stimuli; single pulses (○) and paired pulses (●). The interval between the pair of pulses (270 ms) was in the range where maximal potentiation was obtained. The tension records are averages of all the contractions needed for recording diffraction patterns with single pulses (dotted line) and paired pulses (solid line). The cross-sectional area of the muscle was 0.023 cm^2 .

phase were also smaller with paired pulses than with single pulses. This decrease in the ratio was not attributable to the re-adjustment of muscle length as stretching a muscle would be expected to increase the ratio (Fig. 3). Therefore, our observation suggests that the number of myosin heads remaining in the vicinity of the thin filaments during the diastolic phase is also increased by paired-pulse stimulation.

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Tn554—a site-specific repressor-controlled transposon in *Staphylococcus aureus*

PREVIOUS studies have defined a specific element in *Staphylococcus aureus* containing determinants of inducible erythromycin resistance and spectinomycin resistance. This element has many plasmid-like properties^{1,2} but is not associated with detectable extra-chromosomal DNA². The resistances are similar in mechanism to those ordinarily associated with plasmids^{3,4} (J. Davies, personal communication); the element can be transferred by transduction² or transformation to a *rec*⁻ recipient with the same efficiency as to a *rec*⁺, and the two determinants show essentially 100% linkage in genetic transfer. Consequently, this linkage group has previously been referred to as a 'pseudoplasmid' (ref. 2). On the basis of the results presented here, we suggest that this pseudoplasmid is actually the prototype of a new class of transposon, which functions by means of a highly efficient, repressor-controlled, site-specific integration-excision mechanism. This transposon, designated Tn554 (EmSp), can be most easily envisaged as a prophage-like element that lacks replicative autonomy and other vegetative phage functions.

Table 1 lists the *S. aureus* strains used. Transduction and transformation were carried out by standard methods^{5,6} whole-cell and plasmid DNA were isolated and analysed by standard methods^{6,7}.

Initial transformation mapping results indicated that Tn554 is weakly (co-transformation frequency of $\leq 1\%$) but definitely linked to the chromosomal *tetr* (tetracycline-minocycline resistance) locus previously mapped by Pattee and Neveln⁶. Concurrently, mapping of a series of chromosomal insertions of a different transposon, Tn551 (ref. 8), suggested that one of these, $\Omega 34$, was located at a site very near to that of Tn554 (ref. 9). Accordingly, we carried out a series of transductions to test for linkage between the two, and found that Tn554 and $\Omega 34$ are indeed very closely linked (Table 2). Somewhat inconveniently, Tn551, first found on plasmid p1258 (ref. 8), also encodes erythromycin resistance (in this case, constitutive). Its properties are similar to those of the various *Escherichia coli* transposons¹⁰: it translocates with a frequency of about 10^{-4} per donor genome and inserts at many different chromosomal and

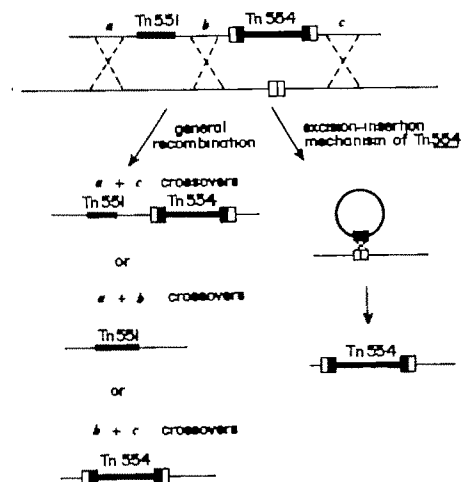


Fig. 1. Possible mechanisms for Tn554 integration. Thin lines represent chromosomal sequences; hatched bar represents the $\Omega 34$ insertion of Tn551; solid bar represents Tn554; open blocks represent the putative attachment site of Tn554; dashed lines represent possible homologous crossovers, resulting in the insertion of the entire region ($a + c$), of Tn551 only ($a + b$), or of Tn554 only ($b + c$). In addition, Tn554 can evidently excise and insert independently; this is presumably its primary mode of transfer to a *rec* recipient.

Table 1 Staphylococcal strains and plasmids

Plasmids		Genotype*	Derivation
Plasmid no.			
pRN1107		pI524 <i>repA2</i> Δ 137 [<i>bla asa asi</i>]	pI524 <i>blaI1 repA2</i>
pRN4166		Ω 171 [pRN1107::Tn554 <i>ermI1 spc-1</i>]	pRN1107
Strains	Host strain		Derivation
stock no.		Chromosomal markers	
RN27	8325-3 (80 α)		RN25, 80 α lysogen
RN496	8325-4	Ω 5 [chr::Tn551]	
RN1030	8325-4 (ϕ 11)	<i>recA1</i>	
RN2573	8325 (80 α)	Ω 34 [chr::Tn551]	
RN2784	8325-4	Ω 34 [chr::Tn551], [chr::Tn554 <i>ermA4</i>]	Ω 34 $\rightarrow$ RN2867
RN2863	8325-4	chr::Tn554 <i>ermI1 spc-1</i>	
RN2870	8325-4 (ϕ 11)	chr Tn554 <i>ermA5</i>	Tn554 <i>ermA5</i> $\rightarrow$ RN451
RN2896	8325-3 (80 α)	chr Tn554 <i>ermA5</i>	Tn554 <i>ermA5</i> $\rightarrow$ RN27
RN3078 to RN3082	8325-4 (ϕ 11)	<i>recA1</i> [chr::Tn554 <i>ermA5</i>]	Tn554 <i>ermA5</i> $\rightarrow$ RN1030

* Key: Ω , insertion; Δ , deletion; $\rightarrow$, transfer

plasmid sites. Although its transposition is *rec*-independent, it cannot be transduced at a detectable frequency from a chromosomal site to a *rec*⁻ recipient². This is presumably merely a question of multiplied probabilities; a transposition frequency of 10^{-4} coupled with a transduction frequency of 10^{-6} – 10^{-7} results in a combined probability of $<10^{-10}$, which is well below the limits of detectability in this system.

Transduction with RN2784, carrying Ω 34 and an Em^r mutant of Tn554, as donor, revealed 50% linkage between Tn554 and Ω 34 when selection was for the Tn551 Em^r determinant, but only 10% when selection was for the Tn554 Sp^r determinant. This asymmetry could either be due to transductional polarity (involving the specificity of transducing fragment ends)¹¹, or to specific excision–insertion of Tn554, which inevitably disrupts the linkage with the Ω 34 locus. Two lines of evidence support the latter interpretation. In a similar experiment carried out by transformation, the same asymmetry was obtained; and in a transductional cross using the same donor but a *rec*⁻ recipient, no co-transduction of Tn554 and Ω 34 was observed; all of 315 Sp^r transductants were Em^r and no Em^r transductants were obtained. Further, in these experiments, the entire Tn554 *ermA4* element was acquired; all of five Em^rSp^r transductants tested reverted to Em^r (inducible) at the same frequency as the original Tn554 *ermA4*.

On the basis of these results, it seemed possible that specific excision–insertion of Tn554 was responsible for its transfer to the *rec*⁻ recipient. Two other possibilities were considered: (1) that it was maintained as a plasmid in the *rec*⁻ strain, and (2) that it carried the *rec*⁺ allele, enabling it to recombine into the chromosome. These were ruled out by a failure to demonstrate plasmid DNA in two different *rec*⁻ transductants and by a demonstration that these were unable to accept the chromosomal *tna* marker and were, therefore, still *rec*⁻. Because transfer of Tn554 to a *rec*⁺ recipient could involve either homologous recombination or specific insertion, whereas transfer to a *rec*⁻ could involve only the latter, the site specificity of the putative insertion process was tested with several independent *rec*⁻ transductants. Tn554 was mapped in the *rec*⁻ by scoring for transductional displacement of Tn551 in *rec*⁺ recipients containing Ω 34 in comparison with Ω 5, a Tn551 insertion unlinked to Tn554 by transduction. Five different *rec*⁻ Tn554 strains each gave about 8% displacement of Ω 34 but no detectable displacement of Ω 5, consistent with insertion of Tn554 in the *rec*⁻ chromosome at the same location as in the *rec*⁺ (Table 2).

The high frequency of excision and insertion required to account for the efficient transfer of Tn554 to the *rec*⁻ strain, and the very great stability of the element once established, suggested that excision, at least, is controlled by a transposon-specific repressor. If so, the high frequency of transfer to the *rec*⁻ would be analogous to zygotic induction, as the recipient would lack repressor; that is, the element would excise from the transducing

fragment on which it was injected by the phage, and then insert by site-specific recombination into the chromosome. A probable mechanism for this sequence of events is that proposed originally by Campbell¹² to account for the excision and insertion of coliphage λ (Fig. 1). The excision step could, of course, occur in the donor during phage maturation, in which case one need not postulate a repressor to account for the results described so far. In any case, as a preliminary test for the presence of a repressor, we compared the transduction frequency of Tn554 *ermI1 spc-1* to recipient strains with and without Tn554 *ermA5* and found that the presence of the Tn554 derivative in the recipient markedly inhibited super-transduction. This inhibition was greater in the *rec*⁻ recipient (about 100-fold) than in the *rec*⁺ (about 10-fold), which is expected regardless of the mechanism of inhibition, as recombinational displacement occurs in the *rec*⁺ but not in the *rec*⁻. Although this result is consistent with the existence of a repressor of excision or of

Table 2 Site specificity of Tn554 insertions in the *S. aureus* chromosome

Donor	Recipient	Selection	Transductant classes (%)*		
			Em ^r Sp ^r	Em ^r Sp ^r	Em ^r Sp ^r
RN2573	RN2896	Em ^r	64.5 (200)	—	35.5 (200)
RN3078	RN2573	Sp ^r	—	7.8 (90)	92.2 (90)
RN3079	RN2573	Sp ^r	—	10.8 (120)	89.2 (120)
RN3080	RN2573	Sp ^r	—	8.7 (160)	91.3 (160)
RN3081	RN2573	Sp ^r	—	6.0 (100)	94.0 (100)
RN3082	RN2573	Sp ^r	—	7.0 (100)	93.0 (100)
RN496	RN2870	Em ^r	0 (100)	—	100.0 (100)
RN3078	RN496	Sp ^r	—	0 (100)	100.0 (100)
RN3079	RN496	Sp ^r	—	0 (100)	100.0 (100)
RN3080	RN496	Sp ^r	—	0 (100)	100.0 (100)
RN3081	RN496	Sp ^r	—	0 (100)	100.0 (100)
RN3082	RN496	Sp ^r	—	0 (100)	100.0 (100)

* Transductants per plaque-forming unit of phage (PFU) were approximately 10^{-6} for Tn551 donors and 10^{-5} to 10^{-6} for Tn554 donors

Figures in parentheses represent numbers of transductants scored for this value. Key. Em^r = erythromycin resistant, Sp^r = spectinomycin resistant; Em^s = erythromycin sensitive; Sp^s = spectinomycin sensitive.

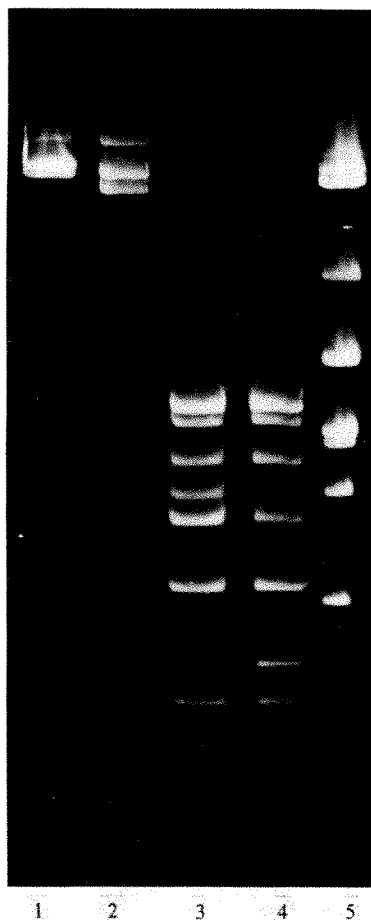


Fig. 2 Agarose (0.7%) gel electrophoresis of plasmids digested with *Bam*HI and *Bgl*II. In each pair, the digest of the plasmid carrying the Tn554 insertion is on the left. Slot 1 = pRN4166 cut with *Bam*HI; slot 2 = pRN1107 cut with *Bam*HI; slot 3 = pRN4166 cut with *Bgl*II; slot 4 = pRN1107 cut with *Bgl*II. Slot 5 contains phage λ *Eco*RI fragments. Note the loss of a 2.6-kilobase pair *Bgl*II fragment (slot 4) and the appearance of two new fragments, one of which co-migrates with a fragment already present, and thus appears as a doublet (slot 3).

excision-insertion, an alternative possibility is direct interference by the resident transposon with the integration of the superinfecting one, through insertional disruption of the putative attachment site (Fig. 1).

To distinguish between these two possibilities, it was necessary to obtain a Tn554 insertion in a secondary site, most conveniently a plasmid. This was initially attempted with a strain carrying a penicillinase plasmid (pI524) plus Tn554 as a donor in a transduction with double selection for the plasmid-linked cadmium resistance (*Cd*^r) and Tn554-linked *Em*^r markers. Although a few doubly resistant transductants were obtained, these were all found to contain the original plasmid and a chromosomal Tn554. In retrospect, this is the expected result if, indeed, there is zygotic induction of Tn554 transposition. As this result suggested that Tn554 could insert into the plasmid, we adopted the approach of transducing Tn554 *ermI1 spc-1* into a *rec*⁻ recipient containing Tn554 *ermA5* in the chromosome (as the source of the putative repressor), and a plasmid, pRN1107, as a secondary target for the transposon. The plasmid used was a pI524 derivative with a thermosensitive replication defect (*Tsr*)¹³, permitting the scoring of plasmid linkage by testing for co-elimination during growth at 43°C, the restrictive temperature. As before, there was a 100-fold reduction of Tn554 *ermI1 spc-1* transduction frequency in these conditions, but in one of 10 *Em*^r transductants obtained, the incoming Tn554 *ermI1 spc-1* was co-eliminated with cadmium resistance during growth at 43°C, and was therefore presumed to have inserted into the plasmid. The apparent plasmid linkage was confirmed by

agarose gel electrophoresis after digestion with restriction endonucleases. These results revealed a 6.2-kilobase insertion in the plasmid carrying Tn554 (Fig. 2).

Having obtained a plasmid insertion of Tn554 *ermI1 spc-1*, we were able to test more directly for the action of a repressor. This was accomplished by further transduction of this plasmid, pRN4166, into two new recipient strains, one carrying a chromosomal Tn554 *ermA5* insertion (RN2896), the other not (RN27). In this experiment, *Cd*^r (plasmid-positive) transductants were tested first for the presence of the Tn554 *ermI1 spc-1* *Em*^r marker and then, by temperature-induced curing, for the linkage of Tn554 *ermI1 spc-1* to the plasmid.

The results of this test indicated that the plasmid and the inserted transposon were co-transduced with a frequency of about 50% (the reason for this is now under investigation); among the co-transductants obtained, the linkage between the plasmid and the transposon was disrupted in all of 10 *Cd*^r*Em*^r clones derived from the recipient lacking a chromosomal Tn554 and was preserved in all of 10 derived from the recipient containing one. Here again, the linkage was evaluated by temperature-induced curing of the plasmid.

This result is consistent with a repressor of Tn554 excision encoded by the transposon itself. If site-specific interference with insertion were responsible for the inhibition of transduction described above, then the chromosomal Tn554 element should not have had any effect on excision of the incoming transposon from the plasmid, only on its subsequent insertion into the chromosome. It is conceivable, however, that excision and insertion are integrated processes, so that if insertion is blocked, excision is automatically blocked also. We consider this unlikely because excision regenerates an intact plasmid; this suggests that Tn554 is excised in the double-stranded configuration.

The simplest mechanism in which double-stranded excision is coupled with restoration of continuity is that proposed by Campbell for the excision-insertion of coliphage λ (ref. 12). In this mechanism, involving the generation of a circular intermediate by a site-specific intramolecular crossover, excision occurs independently of the fate of the excised molecule. If this is, in fact, the correct interpretation for Tn554 transposition, then it is the first example of transposition occurring by the Campbell mechanism. We note that other transposons, particularly Tn9(*Cm*), also show *rec*-independent excision with preservation of the carrier genome, but there is no evidence for a Tn9 repressor, and the excised transposon is nearly always lost rather than reinserted at another site¹⁴.

Figure 1 is a summary of the postulated mechanisms of transfer and insertion of Tn554. Experiments are now in progress with the aim of confirming the existence of a repressor and analysing the excision-insertion process it controls.

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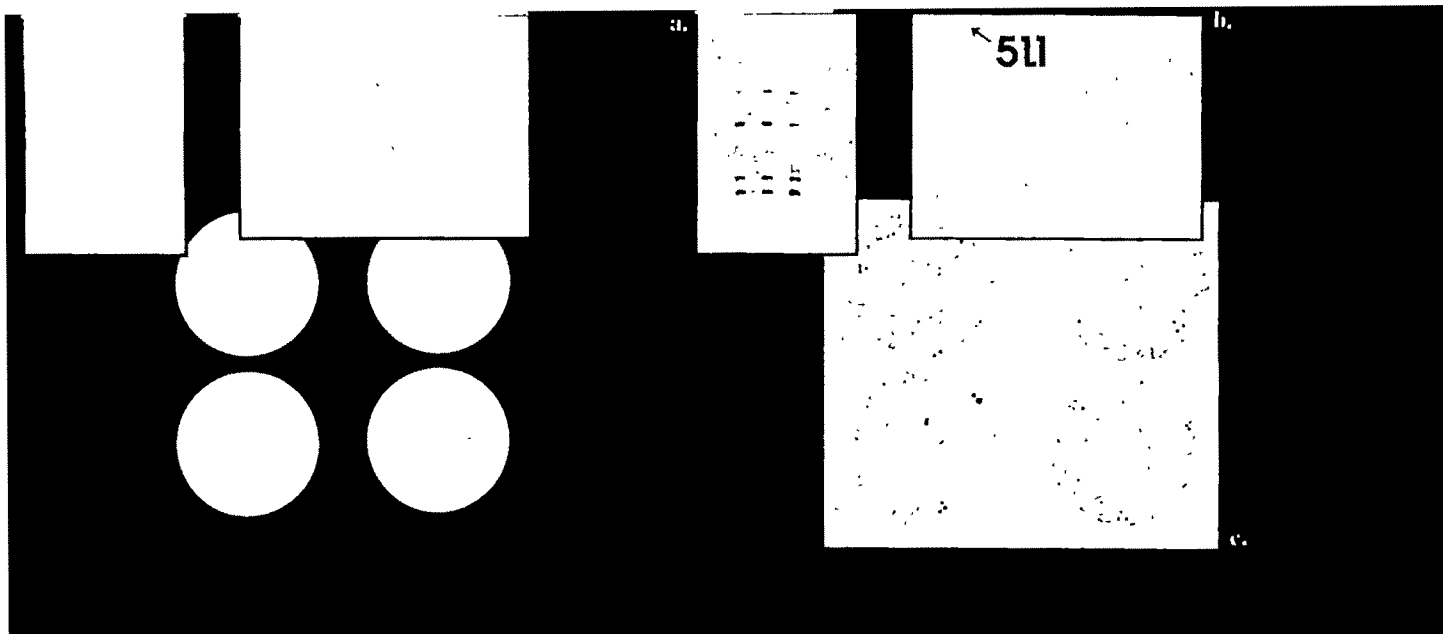
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1) Blattner, F. R. *et al.* 1978 *Science* 202:1279-1284, 2) Smithies, O. *et al.* 1978 *Science* 202:1284-1289

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matters arising

Early Precambrian oxygen

FEW of the arguments presented by Towe¹ seem strong enough to build up a case against the photosynthetic origin of Precambrian oxygen as defended, for example, by Margulis *et al.*². Note especially that he does not discuss the relative importance of hydrogen escape compared with recombination after water photolysis although it seems to be a critical issue. To the uninformed, however, one argument might seem decisive: the requirement of oxygen for chlorophyll-*a* biosynthesis. If this has been proved absolute it would be difficult not to admit the existence of some free oxygen before the formation of that molecule necessary for oxygen releasing photosynthesis.

Towe has cited Bogorad³ whose Fig. 1, which summarises chlorophyll-*a* biosynthesis, shows an oxygen-requiring step in the oxidative decarboxylation of coprophenol. This is, however, a common step to every porphyrin (haem as well as chlorophylls and bacteriochlorophylls) which in 1966 had essentially been studied with animal mitochondria. In this text (p. 492) it is mentioned that oxygen cannot be universally required for that reaction as it suppresses bacteriochlorophyll production in the purple non-sulphur bacterium *Rhodospirillum rubrum*. In a more recent treatment of this subject⁴, Bogorad indicated in his Fig. 2 that an alternative to oxygen exists for that step and gives full reference to the work of Tait⁵ who showed that photosynthetic bacteria have both aerobic and anaerobic coprophenolase activity. Since some blue-green algae are clearly able not only to survive but to sustain abundant growth in anaerobic conditions with photosynthesis based on reduced sulphur like some photosynthetic bacteria⁶⁻⁸, those species must be equally able to decarboxylate coprophenol without oxygen. Towe seems to consider that the anaerobic capacity of blue-green algae favours his hypothesis. I would rather consider that it also agrees with the reverse hypothesis, stating like Brock⁹ that "If the bulk of the atmospheric O₂ is indeed of biogenic origin, the organisms first involved in O₂ formation would of course have developed in an anaerobic environment and would have had to be able to live anaerobically".

The argument that in a reducing atmosphere there would be no selection pressure to tap the immense hydrogen resources of water is unconvincing. What would be the difference between

competition for dwindling reductants whether those are consumed by an oxidising atmosphere or prokaryotic photosynthesisers? Anyway even admitting immense resources of H₂S or other reductants which could be used for bacterial photosynthesis, in the competition between organisms, depletion can be a phenomenon localised to some biotic environments.

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TOWE REPLIES—Demoulin is disturbed primarily by my statement¹ that unless the molecular oxygen requirement of chlorophyll-*a* had been a later modification, the evolution of this pigment in an anaerobic early Precambrian environment would have been unfavorable. The ability of some extant blue-green algae to sustain abundant growth in reducing conditions certainly implies that they can make the necessary decarboxylation step without free oxygen. Whether or not such anaerobic decarboxylation of chlorophyll-*a* took place in early Precambrian ancestral forms having photosystem I only, is unknown. But if the sequence data of Schwartz and Dayhoff² are correctly interpreted the relevance of this point may be moot. After all, if, as they say, the evolutionary development of aerobic respiration preceded that of photosystem II (and hence oxygenic photosynthesis) the oxygen requirements of chlorophyll-*a* biosynthesis would be of limited significance.

On the other hand, the ability of some modern blue-green algae to thrive in reducing environments while endowed with photosystem II but using photosystem I only³⁻⁵ is certainly not, as Demoulin has asserted also in agreement with the reverse hypothesis; namely, that photosystem II evolved in a reducing environment. The algae in question³ may

survive anaerobic conditions using their photosystem II but they can do so only in the absence of suitable reductants, and tolerance for anaerobic conditions is not the same as dependence on such conditions⁶. There are, it is true, other blue-green algae living today that can grow in a reducing environment⁷ but these require photosystem II at all times. Here the exogenous reductants (H₂S) keep the *pO₂* low rather than to act as primary electron donors for photosystem I. Thus it is important to distinguish between not only the different algal metabolisms but also between the effects of an anaerobic environment and those of an anaerobic-reducing environment. The point is that even today in the presence of reducing conditions, water is not the photosynthetic electron donor of choice for those blue-greens able to choose between photosystem I and II³⁻⁵. There is no such choice for the other modern blue-greens⁷ and, of course, no choice was available to any of the early Precambrian ancestors equipped only with photosystem I, energetically unable to take advantage of the hydrogen resource of water regardless of its immensity and ready availability. Thus I emphasised¹ that "only by removal of reduced substances as potential electron donors for photosystem I would there have been significant pressure (to) develop photosystem II".

Certainly, the effectiveness of atmospheric dissociation is important to the early Precambrian environments, and I stated¹ as much, but there are simply too many debatable assumptions involving the solar constant, water vapour mixing ratios, and, especially, hydrogen sources and sinks, implicit in the calculations to make any answer definitive. Photodissociation cannot be ruled out as a likely source of free oxygen in the early Precambrian.

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Effect of prostaglandin E₂ on prostacyclin production by endothelial cells

It has been suggested by Tomasi *et al.*¹ that prostaglandin (PG) E₂ inhibits the synthesis of PGI₂ (prostacyclin) by endothelial cells. This conclusion was based mainly on their observation that the inhibition of platelet aggregation produced by a suspension of isolated hepatic cells (described as "endothelial-rich") was reduced when the hepatic cell suspension was preincubated for 10 min. with 1.5–3 μ M PGE₂. An alternative explanation for this effect, apparently not considered by Tomasi *et al.* is that PGE₂ interferes with the action of PGI₂ rather than its synthesis. We therefore investigated the effects of PGE₂, first on PGI₂ synthesis by aortic endothelial cells, and second, on the inhibition of platelet aggregation caused by exogenous PGI₂.

Production of PGI₂ was measured by radioimmunoassay (RIA) of 6-oxo-PGF_{1 α} (the stable metabolite of PGI₂) in the medium above monolayers of cultured endothelium. In this RIA, 2 μ M PGE₂ showed appreciable cross reactivity, and all samples were therefore treated with 0.3 M KOH in methanol to convert PGE₂ to PGB₂, which showed very little cross reactivity. This treatment did not affect the immunoreactivity of 6-oxo-PGF_{1 α} . The production of 6-oxo-PGF_{1 α} was not reduced by 2 μ M PGE₂ (Table 1); in fact, samples incubated with PGE₂ showed some increase in the amount of immunoreactive 6-oxo-PGF_{1 α} present. Similar increases were, however, measured when 2 μ M PGE₂ was added at the end of the incubation or when 2 μ M PGE₂ was assayed in the same volume of medium alone. Figure 1 shows that as little as 0.1 μ M PGE₂ abolished the inhibition of arachidonic acid-induced platelet aggregation produced by 20 nM PGI₂.

On the basis of these results we conclude that PGE₂, even at the relatively

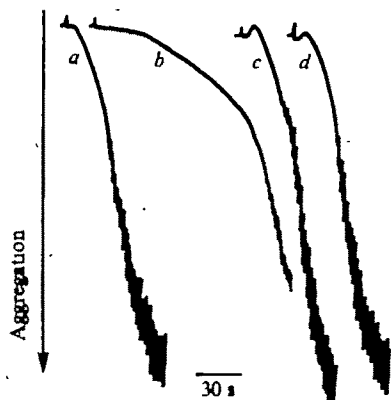


Fig. 1 Effect of PGE₂ on the inhibition of platelet aggregation caused by PGI₂. Platelet aggregation was measured photometrically in 0.1-ml samples of human citrated platelet-rich plasma⁸. Aggregation was induced by 1 mM arachidonic acid alone (a) or 1 min after the addition of 20 nM PGI₂ (b). 0.1 μ M PGE₂ was added 1 min before arachidonic acid either alone (c) or with 20 nM PGI₂ (d).

high concentration of 2 μ M, does not block PGI₂ synthesis by aortic endothelial cells, but that a lower concentration of PGE₂ can overcome the inhibitory effect of PGI₂ on platelets. The action of PGE₂ may result from competition at the platelet receptor binding PGI₂, although this seems unlikely, as we have evidence that PGE₂ and PGE₁ act at different receptors on the platelet², and PGI₂ apparently acts at the same receptor as PGE₁ (ref. 3). Alternatively, this action could be a consequence of the ability of PGE₂ to facilitate platelet aggregation induced by several stimuli, including products of arachidonate metabolism⁴. Although the hypothesis advanced by Tomasi *et al.*¹ may be valid for the hepatic cell fraction they used (although this still remains to be unequivocally established), it does not apply generally to vascular endothelium.

RIA was from Drs F. Dray and H. Sors, and PGI₂ was from Dr P. L. Walton.

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TOMASI ET AL. REPLY—We had considered the possibility that PGE₂ interferes with the PGI₂ receptor, and experiments showing that PGE₂ at the concentrations used (1.5–3.0 μ M) was unable to counteract PGI₂ (2–4 ng ml⁻¹) inhibition in platelet-rich plasma aggregated with sodium arachidonate, were carried out. We have now repeated these experiments with two different sources of PGI₂ with similar findings. However, we were surprised to observe that in the conditions described by Gordon *et al.* (0.1 μ M PGE₂) the results were similar to those reported in their Fig. 1. We agree that this finding cannot be explained by an antagonism of PGE₂ on the PGI₂ receptor. The most likely explanation is that at low doses (0.1 μ M) PGE₂ decreases cyclic AMP levels in platelets¹, counteracting the increase due to PGI₂ (ref. 2) and facilitating aggregation, whereas at higher doses, when PGE₂ has no effect or increases cyclic AMP¹, its action on PGI₂ inhibition is minimal or absent. Thus, in our conditions the effect of PGE₂ must be explained on the basis of a reduction of PGI₂ synthesis. This is supported by experiments showing that our preparations preincubated with PGE₂ and washed twice with buffer were much less effective than controls in inhibiting arachidonate-induced aggregation.

The failure of Gordon *et al.* to observe an effect of PGE₂ on 6-keto-PGF_{1 α} formation may be accounted for: (1) by assuming that their endothelial cells from pig aorta do not contain a PGE₂-sensitive adenylate cyclase (which would be interesting to verify), or (2) by the possibility that PGI₂ is degraded mainly to 6,15-diketo-PGF_{1 α} (ref. 3), which may not be detectable using a 6-keto-PGF_{1 α} antiserum⁴.

We would be surprised if our hypothesis applies generally to vascular endothelium. We consider that the mechanism we have described may operate mainly inside an organ where PGE₂ produced by the major cell population would mediate a control on the endothelial cells lining the organ vasculature⁵.

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Table 1 The effect of PGE₂ on 6-oxo-PGF_{1 α} production

Treatment	6-oxo-PGF _{1α} (pg)
a Endothelial cells alone	66 ± 2
b Endothelial cells + 2 μ M PGE ₂	104 ± 13
c Endothelial cells alone; 2 μ M PGE ₂ added after incubation	95 ± 6
d 2 μ M PGE ₂ alone	22 ± 2

Figures are mean $\pm$ s.e.m. (six observations in each case) and represent pg per 10⁵ cells (or, in d, pg per sample). Porcine aortic endothelial cells⁵ were incubated in serum-free tissue culture medium for 10 min at 37°C either with (b) or without (a) PGE₂, and 6-oxo-PGF_{1 α} was measured by RIA⁶. Controls (c, d) were incubated to estimate the interference of PGE₂ in the RIA.

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reviews

Angels and devils of science

R. H. Pritchard

Recombinant DNA: The Untold Story. By J. Lear. Pp. 280. (Crown: New York, 1978.) \$8.95.

THIS book contains a brief account of the discoveries and discoverers that made DNA splicing exploitable as a method of amplifying genes and creating new hybrids. It also contains a blow by blow account of the rise and fall of the conviction, and the public debate it led to, that the synthesis of hybrids from organisms whose DNAs do not naturally encounter one another is hazardous because it could lead inadvertently to the creation of new pathogens not controllable by established methods.

This hypothesis in its general form was always a highly speculative one unsupported by any evidence. Its validity was determined not by reasoned argument but by shows of hands at mass meetings and *ex cathedra* pronouncements from groups of eminent scientists. The scientific community was led into a headlong rush to impose upon itself the most stringent safety precautions against totally hypothetical hazards. It caused intelligent scientists to engage in acts of public self-immolation and to public announcements of the destruction of valuable but completely harmless strains of microorganisms. Later there was cheating to get around the regulations and sneaking to get round the cheaters. It has cost millions of dollars and millions in other currencies so far to finance containment laboratories and containment bureaucracies. It has been a damaging episode in the relationship between the scientific community and the public, and there must be important lessons to be learned from it. Readers of Lear's book are in danger of learning the wrong lesson. He is not a dispassionate reporter. He has a particular point of view which leads him to a particular interpretation of the events he records. On the other hand, he has researched his material to a considerable depth. His account of events rings true. He writes entertainingly and often draws amusing and recognisable pen portraits of the central characters. His account of the science itself is admirably lucid with few howlers. It is a book well worth reading.

John Lear doesn't question the validity of the Frankenstein-monster hypothesis. Thus the story he tells is a story of the triumph of good over evil, with the devil threatening a comeback towards the end and the final outcome even now undecided. Good is accountability of scientists to society. The devil, on the other hand, believes in unbridled licence for anyone to do whatever experiment he chooses, accountable to no-one but himself. It is a fairy story, of course, but it seems to be one that Lear believes in.

According to Lear a telephone call from one Robert Pollack to a Professor Berg in 1971 sparked off the great debate. Pollack was worried that Berg proposed to propagate the genome of the monkey virus SV40 in *Escherichia coli* by splicing it into a bacterial virus called λ . Berg subsequently abandoned the experiment. Both men later organised a conference on Biohazards in Biological Research which seems to have been devoted primarily to the hazards of handling tumour virus. The conference did not get widespread publicity inside or outside the scientific community. Lear argues that it was because the press was not alerted. Later in discussing events which really did start the ball rolling he argues that the Pollack-Berg message needed time to sink in. There is another possibility. Pollack's concern was based on a reasoned (although not necessarily valid) extrapolation from well-founded observations. SV40 produces tumours in some animals and causes transformation of human cell lines to forms with cancer-like properties. It also forms natural hybrids with adenoviruses which cause respiratory infections. Anyone familiar with these facts, with the frequency with which animal viruses form hybrids naturally, and with the uncertainty surrounding their pathogenicity, would not lightly synthesise large quantities of SV40 DNA in *E. coli* and it would not be unnatural for a virologist to express his worries to a biochemist whose background might not have alerted him to the potential hazards. But these commendable initiatives could not possibly engender an impassioned debate between the angels and devils of the scientific world. Who would act the devil? Scientists are no more mis-

chievous, no less intelligent and no more suicidal than the population at large.

A conference two years later provided a more favourable breeding ground for devils. A new and trivially simple method of gene splicing was announced. Anybody could do it. The particular and rational concern (about the lack of care in handling the DNA of suspected pathogens like animal viruses) began to merge with a general and irrational one (that any new combination of genetic material will produce organisms with unknown, unpredictable, and hence potentially hazardous properties).

Devils would soon point out that to be a hazard to the population at large the novel hybrid would not only need to be pathogenic but have a positive adaptive value. To them the probability that, for example, a random insertion of fruitfly genes into *E. coli* could produce such an organism seemed, to put it simply, rather less than the probability that a random splicing of bits of the computer tape of an automatic knitting machine programmed to produce pullovers into the tape of a machine programmed to produce socks would not only lead to the generation of a wearable garment (the pullox) but one which would capture a significant share of the market. Naive angels (who seemed to believe that a cholera epidemic could be caused by *E. coli* containing a cholera toxin gene, that diarrhoea could be caused by *E. coli* carrying a cellulase gene, and tooth decay by *E. coli* with a tooth decay gene kit from *Streptococcus mutans*) thought the probability was too high to be ignored. Smart angels agreed that the probability was negligible but asserted that it could not be claimed to be zero. And if it wasn't zero, to ignore it proved one to be a devil. Lovable angels argued that a little nonsense was a small price to pay for a safe code of practice for animal viruses. There were also naive lovable angels who were properly concerned about the spread of plasmid-borne drug resistance in bacteria. They wanted to ban the synthesis of bugs resistant to new combinations of antibiotics by gene splicing. When devils pointed out that to solve a problem the first step was to properly diagnose it, naive lovable

angels comforted themselves with the thought that a ban could do no harm, and it might even help just a bit.

Such a confusion of issues, such a mixture of motives, plus a widespread lack of awareness of population biology and ecology among the protagonists, were necessary raw materials for a controversy. But to raise the emotional temperature and politicise the issue required another factor. Pollack's spark needed a torch and it has been suggested to me by many people that the torch was the deep concern amounting to guilt of many United States scientists with the misuse of scientific knowledge—a concern that was born out of Los Alamos and came to a head for biologists when defoliants were used in an unpopular war. Lear's book offers support for this view at many points and a Roy Curtiss III, who emerges as its hero, is a good exemplar of this factor. He circulates one thousand copies of a sixteen-page signed testimony of self-criticism, admitting his innocent guilt and the frightening consequences of his work, pledging himself to be good in the future, and demanding much more severe constraints than were being mooted at the time. His stature in Lear's eyes is further enhanced by the revelation that he went to the library some years later and discovered that his work wasn't as frightening as he had thought. Thus he not only earns his role as a repository of the conscience of the scientific community but demonstrates his honesty and humility as well.

The bandwagon of public accountability came to a euphoric climax at Asilomar. A panel of assorted angels had been working hard on a code of practice which was accepted, after being modified a bit, because no one else had a better one. For the first time the scale of the hazards of genetic engineering were divined and graded and the revelations confirmed by a show of hands (allowing lesser mortals to publicly affirm their concern). For the first time the public could see that scientists believed that the theoretical hazards were more dangerous than real ones like, say, cholera or typhoid fever.

Before Asilomar public displays of concern could be indulged in at no cost. After Asilomar the bill was presented in the form of a blanket of controls out of all proportion to any reasonable assessment of hazards. Scientists handling totally harmless organisms found themselves embraced by a code of practice which assumed that they were dangerous pathogens. With minds thus concentrated there was growing pressure against the enshrinement of the code in legislation. Some people just went ahead and

ignored it. To Lear this lobbying and cheating proves his thesis that there are enemies of the people who will use any means fair or foul in the pursuit of unbridled scientific licence even to the point of danger to others. He has found his devils and in his enthusiasm to expose them his objectivity fails him. He even discovers a convention among scientists, which is flouted by his devils of course, but also by himself when it suits his purpose. This is the convention, new to me, that it is improper to cite unpublished work.

I should like to be able to record that those scientists who were collectively most influential in setting in motion the so-called debate on the hazards of genetic manipulation had, after due reflection, collectively used their influence to counterbalance the effect of their earlier statements. They have not done so and consequently Lear's thesis is bound to retain some credibility.

This book occasionally alludes to events abroad. The British Parliament reacted with alacrity to the 1974 petition and the Working Party it established completed its work with similar speed. We get a pat on the back for this but I fear that what it

demonstrates is the action of reflexes trained not to be caught with pants down in an unusually conservative society. Unfortunately, the same reflexes make any relaxation of the code of practice here much more difficult than in the more open and adventurous United States. Not yet for us the return to reason represented by the recent statement of the Director of the US National Institutes of Health that the burden of proof is shifting towards those who would restrict recombinant DNA research.

This episode should help to educate the public about science and scientists. The realisation that eminence does not necessarily equal wisdom, that expertise in one area can equal ignorance in another, that scientists are no more objective than anyone else, is healthy enough. The conclusion I draw from this is that on matters of policy we should base our decisions on the quality of arguments and not upon the status of those who expound them, as we have learned to do outside science. If this precept had been followed, Lear would have no story to tell. □

R. H. Pritchard is Professor and Head of the Department of Genetics at the University of Leicester, UK.

Enigmas of filterable, invisible entities

David Baltimore

An Introduction to the History of Virology. By A. P. Waterson and Lise Wilkinson. Pp. 237. (Cambridge University Press: Cambridge and London, UK, 1978.) £12.

TODAY virology is a well defined discipline: it is the study of infective, intracellular parasites that lack the capability to synthesise their own proteins. This definition distinguishes viruses from other microbes and encompasses entities with sufficient similarities to provide the unity needed for defining a field of biology. But even up to the 1930s the essential nature of viruses was so obscure that the basic unity of their life-style was not apparent. All that was known about viruses before the advent of molecular biology was that they were small enough to pass through filters that trapped bacteria, they were invisible in the light microscope and they would not grow in the absence of living cells. And, of course, they caused a range of nasty diseases in humans,

other animals and plants; finding out more about them was therefore imperative.

The bacterial viruses, a group of viruses that were generally thought to be curiosities, generated the clues that brought the modern understanding of viruses. They provided the research materials from which much of molecular biology developed; and once the nature of genes and how they function was known, viruses became understandable creatures. The history of virology is thus a history of ideas as well as a history of discoveries. At first the recognition that certain diseases were caused by tiny, intracellular parasites was a triumph but the later realisation that these agents share the property of being stripped down to the bare essentials of replication was needed to provide a conceptual unity to virology.

There are so many viruses and the road to their understanding has been so complex that to write a history of virology would be an enormous undertaking. Waterson and Wilkinson were right to start with *An Introduction to the History of Virology*, in which they could define the outlines of the history. They did this with a strong emphasis on the conceptual problems.

The early part of their short book takes us back to late nineteenth century when Ivanovski and Beijerinck independently realised that what we now call tobacco

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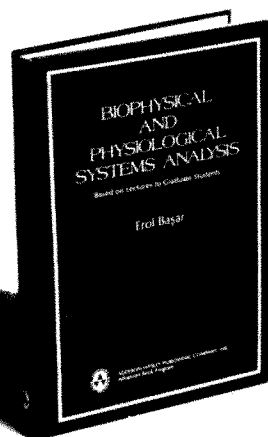
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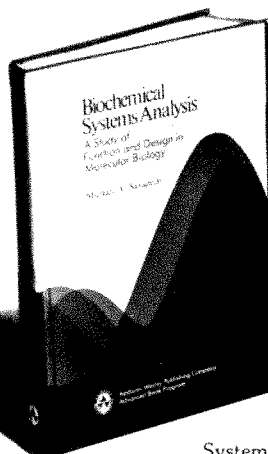
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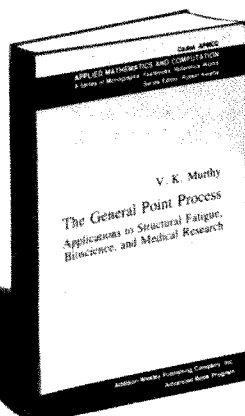
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mosaic virus was so very much smaller than a bacterium that it ought to be considered a separate form of life. One must stand in awe of the pioneers of virology who, working with crude filters and imprecise assays, were able to bring to light a new aspect of the living world. In Beijerinck's earliest publications he originated the modern concept of a virus; the remarkable insight implied by his ability to make such a conceptual leap stands as one of the great intellectual achievements of biology. But, living up to their title, the authors only give us a passing taste of Beijerinck's work. Even a brief discussion of the origins of virology should tell us more about how Beijerinck derived his conclusions.

Another recent short book, Sally Smith Hughes' *The Virus: A History of the Concept* (Heinemann: London, 1977) provides a deeper look at Beijerinck's work. Hughes points to a remarkably simple experiment as the source of Beijerinck's concepts; he put infected plant sap on an agar surface and showed that the infective principle would diffuse through the agar. From this experiment he concluded that the virus is "dissolved and not corpuscular" leading to his concept of tobacco mosaic disease as a "contagium vivum fluidum". Beijerinck saw viruses in chemical terms, possibly as a partial result of his friendship with van't Hoff. This may have been the genesis of his pronouncement in 1898 that:

"... it is to some extent an explanation to consider that the contagium in order to reproduce itself must be incorporated into the living protoplasm of the cell into whose multiplication it is so to speak passively included."

After describing how Loeffler and Frosch opened animal virology with their discovery of the agent of foot and mouth disease virus, Waterson and Wilkinson take us through the discovery of a number of other animal viruses. They follow with discussions of vaccines and tissue culture. They emphasise the difficulty of understanding the nature of viruses without an appreciation of the molecular events underlying genetic phenomena and they show how bacterial viruses provided crucial experimental material for the development of molecular biology. In turn, they show how the development of molecular biology led to an understanding of the nature of viruses. After chapters on plant and insect viruses and on "eruptive fevers" they turn to tumour viruses, a subject that cries out for a more extensive history documenting the shifts of belief about the role of viruses in experimental and human cancer.

For someone like me, who entered virology in 1960 when the essential nature of viruses as intracellular parasites was commonly accepted, it is fascinating to see how the early virologists wrestled

with the enigmas of filterable, invisible entities that would not grow in the absence of living cells. Although this book is often a sketchy introduction and sometimes becomes a catalogue of events, it is a very valuable probe into a central aspect of the history of biology and medicine. Among its useful aspects is a collection of short biographies of

many of the important contributors to unravelling the puzzles presented by viral diseases. It also contains a list of general references and a useful bibliography of the original early literature. □

David Baltimore is an American Cancer Society Professor of Microbiology at the Massachusetts Institute of Technology, Cambridge, Massachusetts.

Plant hormones

Phytohormones and Related Compounds: A Comprehensive Treatise. Vol 1: The Biochemistry of Phytohormones and Related Compounds. Edited by D. S. Letham, P. B. Goodwin and T. J. V. Higgins. Pp.641. (Elsevier/North-Holland/Biomedical: Amsterdam, Oxford and New York, 1978.) \$99.50; Dfl.224.

To be invited to review a book of this nature is a mixed blessing. It is, of course, a privilege to own a copy of the book; on the other hand, wading through 640 worthy but inexpressibly turgid pages in order to pronounce on its value to a prospective reader would be a thoroughly dismal task. I have not, therefore, read this book; I have sampled it. As it is questionable that books like this are ever meant to be read, then my approach is probably representative of any likely customer.

The book is sub-titled *A Comprehensive Treatise*, and this first volume undoubtedly lives up to that claim, although it does seem to be rather out of date. In a survey of the substantial reference lists given at the end of each chapter, I only encountered a handful of 1977 references, and these were principally those of the authors. Even 1976 seemed to be a rather rare date, suggesting that most of the preparation of the book was completed in mid-1976, with perhaps some special references added at the proof stage. Consequently, those looking for up-to-date reviews would be better advised to use *Annual Reviews*, or one of the other periodicals. As a solid compilation of the factual information relating to plant hormones, on the other hand, this book can have no equal.

The first chapter—which I did read—is a thought-provoking, partly historical, account of some of the more provocative general questions concerning plant hormones and their study. It is the only part of the book in which the conceptual content matches the factual content, and is thereby especially welcome. The density of fact, and its repetitive corroboration, is so high in the rest of the book as to make it difficult to digest. Apart from the first

chapter, one would be unlikely to open this book unless one had a specific technical point in mind. On the other hand, it would be invaluable for that purpose.

This first part of the treatise covers the biochemistry of hormones, and does so in the traditional manner, by considering each group of hormones separately. Chapters 2–6 cover auxins, gibberellins, cytokinins, ethylene and abscisic acid in a more or less uniform format, dealing with isolation, identification, chemistry, assays, structure-activity relationships, biosynthesis, metabolism, transport, and the effects of various stimuli on hormone levels. These chapters seem to me to be excellent contributions, and it is extremely valuable to have all this information in one source. Following these chapters is an extremely useful compilation of data on naturally occurring plant growth regulators other than the principal hormones.

The remaining chapters (over 200 pages) are concerned with the effects of hormones on cell structure and metabolism in general, and on DNA replication, transcription, translation, and on post-translational control points. I found the chapter on ultrastructural effects of the hormones particularly rewarding, as it emphasises the syndromic nature of hormone action in higher plants, and illustrates the naivety of assuming that cell-free studies will tell all. These chapters are all written by Drs T. J. V. Higgins and J. V. Jacobsen and represent a considerable, and as far as I could tell, highly critical *tour-de-force*.

My impression, therefore, is of an excellent, though very viscous, treatise which should find a place on every plant physiologist's shelf. My absolute certainty that it will not do so, however, comes from the horrendous price. At \$99.50, or £50, this book—remember it is yet only half a book—will not only be out of the reach of individual purchasers, it will also be expensive for most college or university libraries. What purpose can be in putting together a book which will only be available to a select few?

H.

H. Smith is Professor of Botany, University of Leicester, UK.

Biological effects of ultraviolet radiation

UV-A: Biological Effects of Ultraviolet Radiation, with Emphasis on Human Responses to Longwave Ultraviolet. By J. A. Parrish, R. A. Anderson, F. Urbach and D. Pitts. Pp.262. (Plenum: New York and London, 1978.) £15.75.

It has long been known that sunlight has both beneficial and deleterious effects on man. Many of these effects are caused by the ultraviolet region of the solar spectrum, which has somewhat arbitrarily been divided into three sections—UV-C (wavelength 200–290 nm), UV-B (290–320 nm) and UV-A (320–400 nm). The biological responses of cells, of skin and of the eye to UV-C and UV-B have been well studied. On the other hand relatively little is known about the responses to UV-A, because on a dose-for-dose basis it is much less potent than the lower wavelength regions of the spectrum. The authors of *UV-A* stress that it is nevertheless of growing importance to assess the biological effects of UV-A.

Although it is of relatively low potency, the incidence of UV-A reaching the Earth from the Sun is very much higher than that of UV-B and UV-C. Also, UV-A is finding increasing use both in industry and in medicine, in the latter case particularly in conjunction with photosensitising drugs. Three problems in studying responses to UV-A crop up in almost every chapter: the inadequate dosimetry in most cases; the difficulty in obtaining sources of sufficiently high intensity; and the possibility that the effects observed may in fact be due to contamination of the UV-A with trace amounts of the much more potent UV-B. The authors have therefore wisely not confined themselves solely to UV-A but in each chapter they have discussed effects of ultraviolet radiation of different wavelengths and used this as a background against which to consider the (usually rather scanty) work with UV-A.

The book is very comprehensive in the range of subjects covered, commencing with light sources, filters and detectors, and proceeding with optical properties of the eye and skin—the parts of the body most affected by ultraviolet light. There follow chapters on the biological effects of ultraviolet light at various levels, starting with molecular studies on cells, and continuing with effects on the skin (erythema, tanning, skin ageing and skin cancer) and the eye (keratitis and cataracts).

chapter on the uses of UV-A involving human exposure, and one on safety and protection. The former is chiefly concerned with photochemotherapy using psoralens and UV-A (PUVA) for the treatment of various skin ailments such as psoriasis and vitiligo. PUVA treatment is one of the few instances in which it is quite certain that the biological response is indeed mediated by UV-A rather than by shorter wavelength light. A balanced account is presented of the undoubted benefits of this therapeutic regime and the risk against which this must be offset, namely that in the long term the therapy itself may induce skin cancer.

A most impressive feature of this book is the way in which each topic is introduced with a succinct and lucid description of the system under study, so that the non-specialist reader is provided with the necessary basic information to understand what is subsequently discussed. This applies to

aspects of physics (for example, excited states), biochemistry (DNA repair), physiology (structure of the skin and eye) and medicine (description of psoriasis). The authors have therefore been remarkably successful in producing a book which should be comprehensible to anyone interested in the subject matter, whether he be physicist, biologist or clinician. As so little seems to be really known about the biological effects of UV-A at present, it is questionable whether the subject merits a whole book. On the other hand it contains much useful information on related topics. It will therefore be a handy reference book for any photobiologist and it should certainly be compulsory reading for anyone working with UV-A.

A. R. Lehmann

A. R. Lehmann is a member of the scientific staff of the MRC Cell Mutation Unit, University of Sussex, Brighton, UK.

Experimental phycology

Physiological and Biochemical Methods. Handbook of Phycological Methods. Edited by J. A. Hellebust and J. S. Craigie. Pp. 512. (Cambridge University Press: London, New York and Melbourne, 1978.) £18.

ALGAE serve as experimental tools for physiologists, biochemists, molecular biologists, organic chemists and so on, but the experimental techniques which these workers use are scattered throughout the literature. In 1967 the Phycological Society of America had the good sense to promote the publication of a source book on methods used in experimental phycology, written by phycologists. This one book never appeared; instead four were eventually commissioned, and although progress in getting these off the ground was slow initially (the first, on culture methods and growth measurements, appeared in 1973), the second, on physiological and biochemical methods, has been worth waiting for.

This is a "how to do it" book which should find a place on the shelves of any experimental phycologist. Not that I think it will remain on the shelves for long—it will be in constant use by graduate students, teachers, and others interested in algae. Its great advantage is that within the one cover there is such a mine of information. There are 46 chapters, by 46 authors (not necessarily one each), arranged in

seven major sections: isolation of organelles and membranes (how to isolate chloroplasts, microbodies, membranes, and so on); analysis of chemical constituents (pigments, protein, glycerolipids and fatty acids, β -1,3 glucans, alginic acid, ATP, and so on); enzyme assays (nitrate reductase, ribulose 1,5-bisphosphate carboxylase, aldolases, and so on); measurement of physiological and biochemical processes (uptake of organic substrates, photosynthetic rates, nitrogenase activity, protein synthesis, and so on); nutrient uptake (nitrate, phosphate, and so on); ion content and transport; and use of inhibitors (a most useful chapter).

Each methods chapter is factual, with the sources of algal material (most workers on micro-algae still seem to use strains of *Chlamydomonas*, *Chlorella* or *Euglena*), equipment and chemicals (sometimes even the catalogue numbers) clearly given. There is usually a short discussion and some key references (few later than 1975). There is a useful appendix to the book which lists the addresses of many chemical and equipment suppliers and the suppliers of algal cultures (a notable exception is the American Type Culture Collection of Bacteria, where the blue-green algae are classified as cyanobacteria!)

A possible problem with such a book is trying to ensure that most of the important techniques are included and yet the price is not exorbitant. The book has most of the important ones but there are surprising omissions. One example: the key NH_4^+ -assimilating enzymes, glutamine synthetase and glutamic acid dehydrogenase, are not

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An annual review volume is being produced based on the proceedings of a workshop meeting. The topic of the first of these, published later this year, is *Cellular Oscillators* and it is organised by Michael Berridge and Paul Rapp.

The review volume will be supplied to subscribers of the Journal in 1979 and will also be available for separate sale.

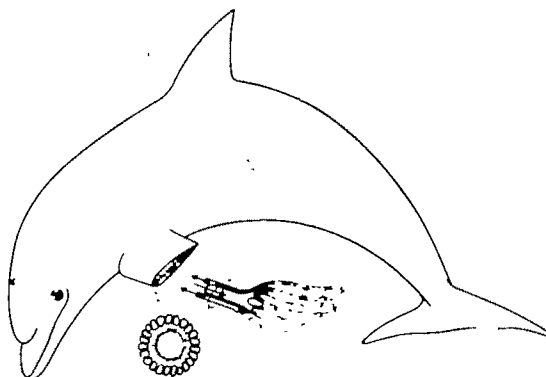
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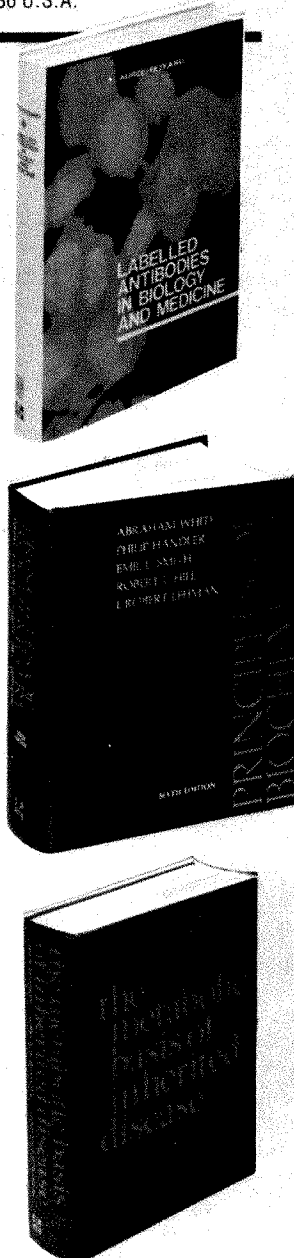
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considered, yet there is room for a chapter on threonine deaminase (an interesting chapter on a rather exotic enzyme). However, the overall balance is good with the more unusual features of algae as plants, for example, the production of alginic acid and fucoidan being adequately dealt with.

The only disappointing part of the book is the plates which are poor; the paper is also thin, but there

is a good stout cover and an adequate index. At £18 for over 500 pages packed with useful information I don't grudge a penny. I recommend it strongly to all with an interest in experimental phycology.

W. D. P. Stewart

W. D. P. Stewart is Professor and Head of the Department of Biological Sciences at the University of Dundee, UK.

Neurochemistry and behaviour

The Chemistry of Human Behaviour. By H. L. Meltzer. Pp. 261. (Nelson-Hall: Chicago, 1978.) \$17.95.

BELIEF in the chemical governance or in the chemical signatures of behaviour has some centuries of history but little current exposition in popular writing. This book, which attempts "a progress report for the informed layman" on "knowledge of brain chemistry and how this might relate to human behaviour" thus had a welcome objective, but neither the expressed objective nor the book's title should be taken literally. Their embodiment is a collection of specialist essays in neuroscience, with a preponderance of neurochemical studies in experimental animals.

The subjects covered are for the most part already well collated or reviewed elsewhere and the text bears only occasional evidence of orientation to the informed layman. Indeed some parts will puzzle the informed chemist: arbitrary variations in bond-length in structural formulae, and giving for glutamine the structure of asparagine. The book's merits lie in giving a largely correct account of several research themes of the past 10 or 20 years, in which chemical methods have been applied in understanding the structure or working of the brain and its influence on behaviour. These themes include malnutrition and brain development; inborn chemical abnormalities; the ion exchanges of nerve impulses; neurotransmitters; the actions of drugs including amphetamines, opiates, lysergic acid diethylamide, and steroid hormones; and chemical correlates of learning and of mental illness. The book's index and Figures are satisfactory, and it carries a 5-page list of books and reviews suggested for further reading. The penultimate chapter on ethical considerations is an excellent and timely exposition of the reasonable researcher's approach to experimentation with animals and human subjects. The chapters on flow of information and on the physical struc-

ture of the brain, though non-chemical, are also apposite.

A book designated to give informed laymen an appreciation of a developing technical subject presents its own specific problems which have been met only partially by Dr Meltzer. His writing in general is crisp, factual and without involved sentences. Some chapters, however, are constructed to attract attention to an extent which, by the chapter's end, is disappointed. Some carry an introductory paragraph only tenuously related to the chapter's main subject; others imply by their title more than current knowledge justifies. Thus "The Chemical Organisation of the Brain" might lead the reasonable layman to expect an exposition of how and why the numerous chemical structures described and illustrated are organised, or organise other components, to yield a brain rather than another organ of the body. Here he will be disappointed and will learn only on the last of the 20 pages involved, that "This has been a brief survey of chemical localisation in the brain . . . intended as an introduction to the diversity of chemical function. . . ."

The most successful expositions of technical knowledge of the brain for general readers have, recently, come from neurophysiology neuroanatomy or psychology. It is significant that the authors concerned with those expositions have not attempted to survey a large proportion of the current research in their subject: rather, they have chosen an intellectually intriguing theme central to their own interests and have been highly selective in the information presented to expound their viewpoints. Their pictures of the brain have included little if any neurochemistry at many points where neurochemistry would have been relevant. Comparably intriguing expositions from a neurochemical viewpoint have yet to be written: despite Dr Meltzer's book, the challenge remains open.

Henry McIlwain

Henry McIlwain is Professor of Biochemistry in the University of London at the Institute of Psychiatry (British Postgraduate Medical Federation), London, UK.

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Sedimentary environments

Sedimentary Environments and Facies. Edited by H. G. Reading. Pp. 557. (Blackwell Scientific: Oxford, UK, 1978.) Hardback £22.50; paperback £13.

THIS book describes modern sedimentary environments and their ancient analogues. For each major facies the editor has arranged for a chapter to be written by an internationally recognised authority on that particular topic. The book commences with an introductory chapter and a chapter on facies by the editor. These are followed by chapters on alluvial, lacustrine and desert deposits by Collinson. Elliott continues with chapters on deltas and clastic shorelines, and these are followed by a chapter by Till on arid shorelines and evaporites. Johnson has contributed a chapter on shallow siliclastic seas (*sic*) and this is followed by one from Sellwood on shallow-water carbonates.

Moving into deeper water Jenkyns and Rupke cover pelagic environments and deep clastic seas respectively. Edwards concludes the spectrum of environments with a contribution on glacial deposits. For a finale Reading and Mitchell have written a chapter relating facies to plate tectonics and the book closes with an epilogue from the editor, a bibliography and a subject index.

As the preceding resumé shows, this work is a veritable *tour de force*. It is well researched, well written and comprehensive, both in the range of environments described and in the global coverage of supporting case-histories. The illustrations all appear to have been redrawn from their original sources so that the book has a pleasing uniform appearance (though this review copy had several grey pages due to an apparent shortage of printers ink).

The emphasis of most of the authors is on the relationship between sedimentary processes and the macroscopic features of their deposits. Thus, the major theme of the book is concerned with the study of lithological sequences and their contained sedimentary structures and fossils.

The emphasis is on macrofacies rather than microfacies. It would have been interesting to have had contributions from this distinguished team of authors on the relationship between sedimentary processes, and the texture and petrophysics of their deposits. This would have widened the appeal of the book for many industrial geologists concerned with the relationship between facies, porosity and permeability.

sections on carbonates, which contain not a single microphotograph.

Similarly it would have been useful to read how the authors see facies analysis in relation to other branches of geology. One of the most stimulating chapters is the one which shows how facies relate to tectonics. It would be interesting to know more about the relationship of facies to stratigraphy (both conventional and seismic), to microfacies and petrophysics (as already mentioned), and to the ways in which facies analysis is applied in the exploration for coal, oil, gas, water and sedimentary ores. This deficiency is particularly apparent when subsurface case-histories are described. It is not sufficiently stressed that these have been interpreted largely from microfacies and wireline logs, as cores are

seldom available for the detailed study of their macroscopic features. Only two figures in the book show the use of wireline logs in subsurface facies analysis. No doubt space was the reason why these topics were omitted, and indeed the editor is to be congratulated on the way so much valuable material has been compressed into 550 pages.

There is no doubt that this scholarly book will be invaluable as a text for senior undergraduate and postgraduate students aiming for an academic career. It will also be an essential source book for all geologists concerned with sedimentary rocks.

R. C. Selley

R. C. Selley is Reader in Petroleum Geology at Imperial College, University of London, UK.

Meteorite reference work

The Nature and Origin of Meteorites. By D. W. Sears. Pp. 187. (Adam Hilger: Bristol, UK, 1978.) £13.50.

THIS is an excellent reference work on meteorites, being comprehensive in scope, yet succinct. The book is divided into six chapters. The first is a historical introduction which makes interesting reading and helps put the succeeding chapters in their proper context. I was glad that "the tortuous and sometimes confusing route by which modern classification schemes have emerged" was dealt with briefly. This is a useful chapter because it indicates how closely advances in physics, chemistry and the Earth sciences have been bound to meteorite research; and this is still true.

Chapter 2 deals with atmospheric flight and impact phenomena, including tektites, which are probably produced in rare, high energy events by fusion or vaporisation of terrestrial materials. Various casehistories of meteorite falls are given. Statistics indicate that, over the Earth as a whole, two or three meteorites arrive per day, yet samples from only about six observed falls per year actually reach the scientist. Next comes 'Classification, mineralogy and petrology'. Here the author, in my opinion rightly, concentrates on the major groups of meteorites, key references to the others being given. The scope of the chapter is, however, wider than the title implies, for estimates of the sizes of meteorite parent-bodies are included. The fourth chapter is on chemical composition and covers the observational data and their theoretical modelling and interpretation. In view of the

sometimes bitter antagonism among those engaged in the field, Sears has done well to present the different cases lucidly, with fair indication of their strengths and pit-falls. Then comes 'Physical properties and processes', including age and isotopic studies. Unfortunately there is no reference to the samarium-neodymium results which have recently made a strong impact on our understanding of the history of terrestrial rocks.

The last chapter is a synthesis of our ideas on the age and origin of meteorites. Because of its extreme brevity, uninitiated readers will have to refer to the preceding text, but most of the necessary cross-references are there.

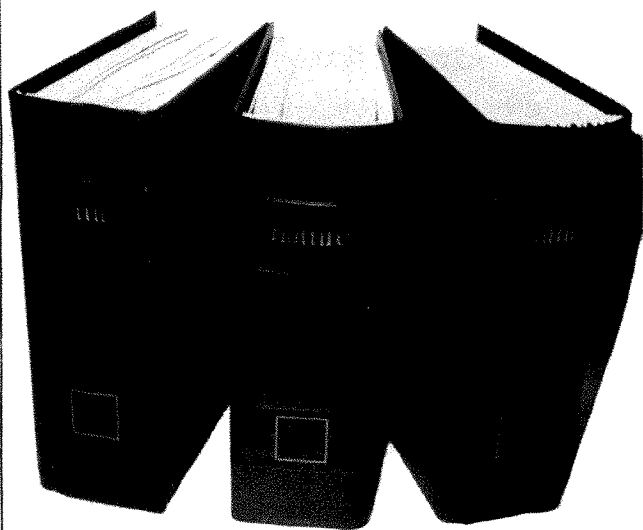
The book is well produced and contains few errors; on p121 the seismic velocity in sandstone is given as 3,000 to 4,000 km per second (it should be m per second), and I am sure that I would enjoy studying a 'cosmogenic tract' (p148). Table 4.1, on meteorite compositions, contains numerous errors and is useless for reference. Both author and publisher are to be congratulated in producing the book so quickly that references up to early 1978 are included, but I wish that titles had not been omitted.

From the points of view of price, accuracy and being up to date, I can recommend this as a good reference text. However, in spite of the statement on the dust-cover, it will be beyond the comprehension of most amateur astronomers and geologists, and is probably more advanced than is necessary for undergraduates. It will be most useful for research students and academics; they will be unable to do better for the price.

Robert Hutchison

R. Hutchison is a Principal Scientific Officer in the Mineralogy Department of the British Museum (Natural History).

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obituary

Alexander Wetmore

ALEXANDER WETMORE, who died in Maryland on 7 December 1978 at the age of 92, was for over 50 years one of the leading figures in world ornithology. Born in Wisconsin on 18 June 1886, he lived all his life in the United States, devoting his professional life to two organisations, first the Biological Survey of the Department of Agriculture (1910–1924) and from 1924 the Smithsonian Institution. From 1945 to 1952 he served as Secretary of the Smithsonian Institution, and he continued to work there after his retirement until a short time before his death.

A good many living and recent ornithologists have had new subspecies, and even the occasional new species of bird named after them; but few have had the distinction of giving their name to a new genus, such as *Wetmore-thraupis*, a new tanager of uncertain affinities discovered in northern Peru in 1963 and named by Lowery & O'Neill in Wetmore's honour 'in recognition of his many outstanding contributions to ornithology, ranging from the classification and systematics of both recent and fossil birds to such diverse facets of the subject as bird migration and pterylosis.' Indeed Wetmore's name has become a household word among ornithologists, for his classification of the birds of the world, forming the basis for the well known 'Wetmore order', has, with modifications necessitated by new knowledge, become the generally accepted arrangement, as adopted in Peters' *Checklist of Birds of the World* and other authoritative compilations.

Dr Wetmore's eminence as a research ornithologist was based on an extraordinary capacity for work, meticulous accuracy, and a very wide range of interests. When he was Secretary of the Smithsonian he customarily arrived at his laboratory at about 6 a.m. and worked steadily on his scientific projects for three or four hours before devoting a full day's work to his duties as Secretary. Up to his late 80s he continued to make regular field trips to Panama, collecting specimens and gathering information for his 4-volume *Birds of the Republic of Panama*, the last volume of which was left unfinished at his death. These qualities, applied to avian palaeontology at a time when the subject was in a state of comparative neglect, quickly made him a leading authority, his con-

tributions providing almost all that was known of the birds from the extensive Oligocene deposits of western North America. Since 1914 he described as new 189 species and subspecies of birds, a record that must be unequalled in the 20th century.

Dr Wetmore was, in a sense, an old-fashioned ornithologist, museum-based, his field work centred round collecting—one who provides the facts that others use. He did not originate new theories, seeming distrustful of the sweeping generalisation, as is often the case with those who are very familiar with the facts and see, more clearly than most, the exceptions and difficulties that need to be taken into account. Advanced though it is in the accumulation of basic facts, ornithology still needs workers of his type; they provide the infrastructure that supports the more exciting new developments.

Alexander Wetmore was a tall man of quietly distinguished presence and great natural modesty, who treated the youngest aspiring ornithologist and the older established with equal courtesy. I never heard any of his American colleagues or friends speak of him other than with respect and affection. He will be remembered by the many British ornithologists who met him at the International Ornithological Congress at Oxford in 1966, still in full vigour and only regretting that he was becoming less efficient in the field as his hearing was beginning to fail.

D. W. SNOW

Sidnie Manton

DR SIDNIE MANTON, FLS, FRS (Mrs J. P. Harding), died peacefully in hospital on 2 January 1979 aged 76. To students of zoology at the end of the last war she was already a legend.

In her first 20 years of professional life she had jointly opened the era of functional morphology with Graham Cannon in their paper on the feeding mechanism of *Hemimysis*; journeyed to Tasmania to study the endemic *Anaspides*; joined the expedition to the Great Barrier Reef and published on black corals; published on the embryology of *Nebalia*; sought-out *Peripatus* in Africa, and published on their feeding, digestion, excretion, sperm trans-

fer, embryology and life-history. A graduate and fellow of Girton, she became college director of studies in natural science, university demonstrator in comparative anatomy and later joined the staff at King's College, London. Her *Manual of Practical Vertebrate Morphology* (1930, with J. T. Saunders) was, and still is (4th edition, 1969, with M. E. Varley) the most respected and reliable guide to vertebrate dissection.

Her election to the Royal Society in 1948 crowned a distinguished career and came as no surprise. No one could have guessed that she was poised to make an even more important contribution to scholarship in the next 25 years. A gap in publication from 1938 to 1945 represents the period her young family and a heavy teaching load at Kings, Birkbeck, Queen Mary and University Colleges were making big demands on her time, but it was then she conceived and began one of the most exciting and ambitious research projects in the history of zoology. The first part of this already classic series on the evolution of arthropod locomotory mechanisms was published in the *Journal of the Linnean Society (Zoology)* in 1950 and the final part (11) appeared in 1973. The series covered 1,000 pages and included almost as many superbly executed drawings gathered into 250 compound text-figures. Functional explanations were given of many arthropod characters previously regarded as curious indications of ancestry.

She was continually in demand here and abroad, to talk about her work. At one Linnean meeting she brought along working models of arthropod legs she had made to help her own understanding; that of the spider was as tall as she, and incorporated the arcuate sclerite, a marvellous engineering device to provide the acute flexure of the femur-patella joint. The nicest device she discovered was surely the rock of the arthropod coxae, lining-up the dorsal hinge joints to translate the depression of proximal podomeres into effective extension of the legs. She was awarded the Linnean Gold Medal in 1963 before half the series was completed.

The locomotion work was certainly conceived and executed in the grand manner, but it was merely a part of

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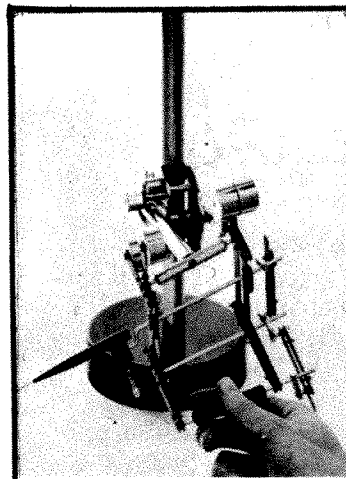
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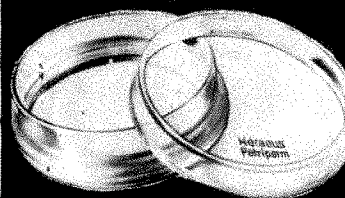
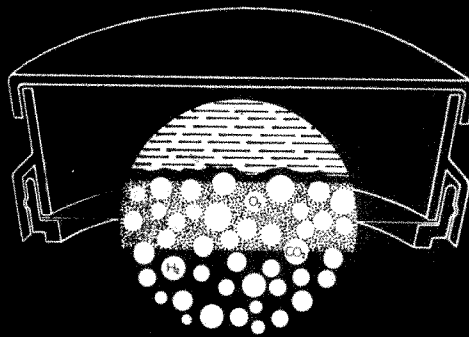
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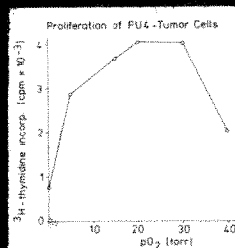
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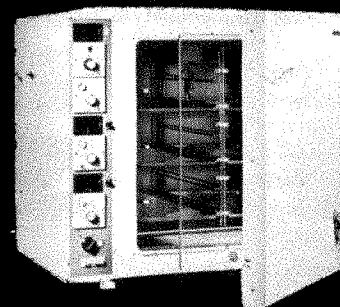
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the greater scheme conceived much earlier, aimed at an understanding of arthropod phylogeny. She had extended and completed a draft of the late O. W. Tiegs on *The Evolution of the Arthropoda* and had discovered that much basic information was just not available. In 1960 she retired from her Readership at Kings and from then on enjoyed the hospitality of the British Museum (Natural History) as Honorary Research Associate and was also a Research Fellow of Queen Mary College. Freed from teaching she felt able to seek the information for herself, beginning with a major examination of the structure and function of the mandible which was published in a wonderfully illustrated paper of over 200 pages between parts 7 and 8 of the locomotion series. She was becoming increasingly impressed by the mutual exclusiveness of the engineering designs between and even within classes. She considered these designs to have evolved in response to different habits which persisted for long periods and that each lineage represented an independent attainment of the arthropod grade of organisation.

About this time she was cruelly afflicted by an arthritic condition, yet she had enjoyed lunch-time tennis on the BM court until she was well over 60. At the Myriapod Congress in Manchester in 1972 she was relatively mobile because of an ingenious mechanical aid for one leg designed and constructed by herself and husband. Looking down a microscope at an exhibit, she drew for me an accurate picture of the cataract which limited her field to a narrow jagged annulus. Cataracts were removed one by one and hip and wrist joints were extensively re-modelled. During this difficult period she wrote *The Arthropoda*, recently published by Oxford University Press, a masterly summary of her 50 years research into their habits, functional morphology and evolution, running to 527 pages and lavishly illustrated by 186 text figures. She corrected the proofs in hospital after one of her operations. During the last two years she had compiled four chapters of a new general text on arthropods, written and presented a paper to a symposium on the evolution of the major arthropod groups at Hull, contributed a review to a book on arthropod phylogeny and undergone a second pelvic joint operation. The strain was too great.

The arthropods were not the only animals in her life. Her home and garden housed a veritable menagerie of birds and mammals which she cared for each day. Over a period of 21 years she developed the long haired Colourpoint cats with such delightful tem-

peraments and exported kittens to all points of the globe. The history of her Mingchui cats is recounted in her book *Colourpoint, Longhair and Himalayan Cats* (1971). This work ran parallel to her arthropod studies and brought her world-wide acclaim from an entirely different public. All this while at the centre of a delightful household, cooking superb meals, a delightful person with a lively conversation and an understanding smile. She will be sorely missed by her husband, Dr J. P. Harding and her two children, Elizabeth and Martyn

J. Gordon Blower

E. R. Creed

EDWIN ROBERT CREED died on 28 November 1978, aged 42, after an illness of three months. His parents were both physiologists, his father being a Fellow of New College, Oxford, also of Winchester. Robert was among that group of boys from Marlborough School who, from the 1920s onwards, became distinguished as biologists.

Like his father, he went to Trinity College, Oxford. After graduating in zoology he remained in the department to carry out research in ecological genetics. It gained for him the PhD Degree, also a Research Fellowship at New College. When this expired in 1970 under a time-limit, he became Lecturer, and later Senior Lecturer, in Zoology at University College, Cardiff, where he was in charge of genetics.

Unlike many scientists and scholars in general, Robert Creed did not find his period of National Service a disastrous waste of time. Among other things, it enabled him to develop his ability in engineering. Indeed, he retained what to some of us seemed an astonishing capacity to interchange and adjust the parts of old motor cars, of which he always had several. Moreover, for the rest of his life he took an active interest in the Territorial Army Volunteer Reserve.

His marriage to Elizabeth Hilton was a source of great happiness. He leaves two daughters, one aged 7 and the other 4 years. He liked to live in fairly large houses plentifully supplied with cats.

He was a good lecturer, but his outstanding educational achievement was as a demonstrator in practical classes. Here he excelled. He would take great care to arrange interesting exhibits and experiments. It was his habit to take away and work up the class results, and he would subsequently explain in what ways they had proved rewarding or unusual.

He was also a successful research worker. In order to investigate the ecological genetics of the butterfly *Maniola jurtina* he once camped with me on an uninhabited island in the Isles of Scilly, and he repeatedly joined our research group studying that insect in the south-west of England. He had a flair for such work, in which his observations were accurate and often revealing; and on these occasions his light-hearted energy was an asset and an encouragement.

His own researches were in particular directed to the polymorphism of the two-spot ladybeetle, *Adalia bipunctata*. This is typically scarlet with a black spot on each wing-cover; but two black forms with, respectively, four and six red spots are also known, all three types being controlled as multiple alleles. Creed showed that the black phases are industrial melanics, the only known instance of that condition in a species protected from predation by a powerfully unpleasant scent and taste: one practising, therefore, the aposematic way of life.

Creed and his colleagues also found that the black phases of *A. bipunctata* are favoured when pollution results from coal of high volatility, not of low volatility as in South Wales; nor does the presence of sulphur dioxide in the atmosphere encourage their spread, as in some moths. Moreover, the two black forms respectively survive better when the fuel is, or is not, subject strongly to caking. This work, described in his published articles, is a valuable contribution to our knowledge of adaptation to industrial conditions.

In spite of his youthful appearance, Robert Creed had a highly developed critical faculty and his comments when the draft of an article was submitted to him were of penetrating value. He was a Conservative in politics, original in research, devoted in friendship. His early death is a tragic loss of one who can ill be spared.

E. B. Ford

Lord Cranbrook

THE death of John David, 4th Earl of Cranbrook on 22 November 1978 deprives many natural history and scientific societies of a skilful adviser and an enthusiastic and knowledgeable colleague.

His multifarious activities in public service and in support of East Anglian naturalists' organisations have been acclaimed elsewhere (*The Times*, 24 November 1978) but it is for his capacities as a scientist of no mean distinction and as a staunch member and officer of national biological societies that we, on behalf of the

Linnean Society of London and of the Mammal Society, would wish him to be remembered. We have no doubt that other societies, such as the Zoological Society of London, would wholeheartedly support this tribute.

Lord Cranbrook's schooling as a naturalist with a world-wide field of interest began in the Far East in association with such pioneers as Kingdon Ward. Thereafter he was deeply committed to the furtherance of field biological studies in Great Britain.

For over 46 years he laboured on behalf of our oldest biological society, the Linnean Society of London, which has always acted as a fruitful meeting ground for amateurs (of which he was an outstanding example) and professionals. He served on its Council for 14 years and was Treasurer for 12 years. His astute business sense, combined with his robust common sense, proved invaluable to the Society during its special appeal for funds in 1969. As a result of this appeal, the Linnean Society was able to rehouse and adequately curate its unique collections of plants and animals on which Linnaeus once worked; a service which was appreciated by scientists throughout the world.

Lord Cranbrook's enthusiasm, energy and eminence among his wide contacts put him in a unique position to foster progress. Nobody appealed to him in vain for advice and it was always given widely, humorously and forcibly.

Compared with the Linnean, the Mammal Society is a relative newcomer with no valuable collections to house and curate. Lord Cranbrook was a founder member in 1954 and, as its assiduous President, guided the Society in its formative years. It was rare for him to miss a council meeting and each year, when he presided over the Annual General Meeting, there was keen speculation as to whether he could cut ten minutes off his previous record for its speedy completion. Nevertheless, there was no skimping and his grasp of essentials, together with his succinct exposition, gave every point due consideration.

As a Trustee of the British Museum (Natural History) between 1963 and 1973, he again provided a valuable link between the naturalists at large and the more circumscribed specialists.

Lord Cranbrook was a valued speaker in the House of Lords: valued especially because of the reliability of his 'homework' and the clarity and force with which he dealt with complex and controversial matters. All naturalists must be grateful for his pilotage during recent years, when he faced increasing disability with great courage, of the Conservation of Wild Creatures and Wild Plants Act 1975.

He contributed numerous publications to scientific journals, of which the most outstanding was his study of a population of noctule bats which came to feed on crickets at a local rubbish dump. Capturing and marking them with the aid of mist-nets was a notable achievement at that time (1965, *Proc. zool. Soc. Lond.* **144**: 1-24).

Lord Cranbrook's cheerful presence and leadership will be sorely missed throughout the ranks of naturalists and biologists.

P. H. Greenwood
H. N. Southern

Jean Clark Dan

JEAN CLARK DAN died of asthma in Tateyama, Japan on 13 November 1978, at age 68. Born and reared in a staunchly Presbyterian family in Westfield, New Jersey, USA, she graduated from Wilson College (1932), and continued study under L. V. Heilbrunn at the University of Pennsylvania and in summer at the Marine Biological Laboratory, Woods Hole. On obtaining her PhD (1936) she married Katsuma Dan who had also received his PhD (1934) with Heilbrunn. They proceeded to Japan via Europe with a stay at the Stazione Zoologica of Naples. Eventually they settled in Tokyo but their early years included long periods at the Misaki Marine Biological Station of Tokyo University.

The Dans produced five children, whose upbringing overlapped World War II. Jean had to move away from home territory but adapted cheerfully, and despite many hardships the whole family managed to survive. Then she undertook multifarious enterprises to surmount postwar adversity: one negotiation utilized army garbage for village rehabilitation while preserving self-respect all around. And she doggedly struggled back to the scientific world.

In 1947 she revisited Woods Hole (to which she would often again return as to another home) where peacetime activities had been re-established. With help from the American Philosophical Society she took a phase contrast microscope back to Japan where, under the wing of Tokyo Metropolitan University, she set up at Misaki a research program on fertilization of marine invertebrates. Publication soon followed. The papers became increasingly important. Her first two 'Studies on the Acrosome' (1952, 1954) demonstrated how the tip of the spermatozoon changes under various conditions, and introduced the term

acrosome reaction. She was a pioneer in examining such material with the electron microscope. Some workers were slow to accept her findings but her mounting evidence, together with that of others, firmly established that the reaction does indeed occur. There followed many other important papers. She explored the sperm lysin problem. Much of her later observation and speculation centered around 'dynamic morphology' (precursors, triggers, etc.) of membranous and other parts of the region she came to call the *acrosomal complex*.

She won grants and fellowships from sources in both countries, received her Japanese PhD, was given the award (1958) of the Zoological Society of Japan, and found herself in international demand for lectures and conferences on spermatology and fertilization. She was on the editorial board of *Development, Growth and Differentiation*.

Jean was appointed Lecturer (1959), then Professor (1973) at Ochanomizu University where she taught and supervised graduate students. Interested in that University's Tateyama Marine Biological Station from its inception, she became Director (1975), and planned to spend much of her time at Tateyama after retiring from the University (1976).

During the war Jean had come to speak fluent Japanese. As Japanese biologists returned to research and writing many sought her help with English usage. For years she devotedly helped transform their texts into English suitable for publication. She translated into English Osada's *Children of the A-Bomb* (1959, with R. Sieben-Morgen), and *Invertebrate Embryology* (1968, eds. M. Kumé and K. Dan). Moreover, she assisted in the English translation of publications of His Majesty, Emperor Hirohito.

The above barely suggests the warm vital Jean Dan. She was a strong swimmer, walked well, liked to gather wild mushrooms or buy a warm sweet-potato from a street vendor. She liked having a dog. Her children enjoyed a whole family of Siamese cats. In her laboratory would be a flower in a beaker, an attractive picture on the wall. She enthusiastically espoused causes as great as world peace, and with equal vigor would propel a specific student into opportunities for advanced study. Jean was seldom at a loss for words; also, her penchant for friendly listening to and advising others knew no barrier in age, sex or nationality, discipline or problem.

She will be missed as a scientist—and as a person.

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FASEB exhibition news

THE Federation of American Societies for Experimental Biology hold their 63rd Annual Meeting, FASEB79, in the Dallas Convention Center between April 1 and April 10. Products of interest to the experimental biologist will be shown at the accompanying exhibition. For more details on products mentioned in this FASEB exhibition news section circle the appropriate numbers on the reader enquiry card bound inside the cover.

Bio-Rad Laboratories

NEW from Bio-Rad is the Quanta-Count II B-12 assay which is sensitive to vitamin B-12 alone, and does not react with its analogues. The test was developed in response to evidence (*New Engl. J. Med.*, 12 October 1978) that most commercial assays for B-12 in serum will give apparently normal values in 10 to 20% of anaemic patients. These discrepancies are attributed to the presence of biologically inactive B-12 analogues in human serum. The analogues bind with non-specific 'R' protein binders contained in most commercial preparations of intrinsic factor, and are measured as true vitamin B-12. Unlike intrinsic factor, which binds only metabolically active B-12, the ubiquitous R proteins cannot distinguish between B-12 and the analogues. In the new system from Bio-Rad the intrinsic factor preparation is treated to eliminate the R protein binding activity. Bio-Rad use the principles of two-site immunoradiometric assay (IRMA) in the new Quantimmune HTSH thyrotropin assay.

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Brinkmann Instruments

FROM Brinkmann Instruments Inc. comes a new plant/laboratory safety device that carefully monitors temperature limits and/or liquid-levels. The Brinkmann Excess Temperature and Liquid-Level Safety Alarm Model R-35 will prevent operation of an instrument above a pre-set critical temperature and prevent low liquid-level operation.



The Brinkman R-35 safety alarm which monitors temperature limits and/or liquid level

It can be used in a variety of instruments (rated at 15 A or less) such as constant temperature circulators, water baths, freezers, ovens and reaction vessels. It is particularly useful for monitoring overnight operations. The desired maximum temperature limit can be easily set at any point between 0° and 350°C. Temperature is monitored through a platinum resistance probe, while liquid-level is monitored through a float switch attached to the platinum probe. If the pre-set temperature limit is reached due to overheating, or, if the liquid-level becomes too low, the R-35 will automatically shut off power to the system and produce a loud, audible warning signal. Power will remain off until the fault has been corrected and a reset button engaged.

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Accurate Chemical & Scientific Corporation

IDENTIFICATION of DNP-amino acids, phenols, lipoxinone, flavonoids, vitamins, antibiotics etc, on thin-layer chromatograms is made simpler by the ready-to-use Cheng-Chin polyamide layer sheets. Cheng-Chin sheets are available from Accurate Chemical & Scientific Corporation who also provide a bibliography listing papers on TLC work on a wide variety of compounds. Reprints of an article by K.-T. Wang and B. Weinstein on the technique of TLC on polyamide layers are also available.

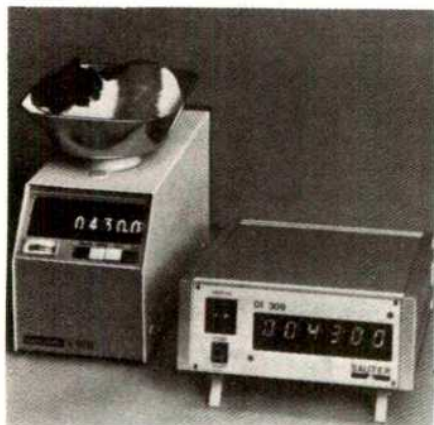
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Sauter

THE K Series of electronic precision balances from August Sauter offer high precision coupled with large capacity. K Series balances all feature two weighing ranges; for example the K 1200 weighs to 120 g with 0.01 g resolution or to 1,200 g with 0.1 g resolution. Results are presented in less than a second on the digital display. The balances are equipped with a tare facility which operates at the press of a key. The analog coarse display gives a continuous reading of the total weight on the balance and both analog and digital outputs can be put through connections to printers or other processors. The problems involved in weighing experimental animals can be minimised by using the DI 309 integrator. The integrator automatically computes the average of a pre-set number of weighings and displays the average on a digital readout. For mice, rats, guinea pigs and for animals as large as rabbits, dogs and monkeys there are balances suitable in the Sauter range. The DI 309, shown here in use with a K 1200 balance, can also

Two radioassays from Bio-Rad. Left, the Quanta-Count II assay for vitamin B-12 and right, the Quantimmune assay for human TSH





The Santer DI 309 integrator and K 1200 balance

be connected to a printer. The Sauter RT Series of balances includes the RT 200 with 200 g capacity and 1 mg resolution and the RT 1200 with 1,200 g capacity and 0.1 g resolution. Both are fitted with a BCD signal so can be used in a data processing application. Sauter produce the MR 401 printing calculator which will display measured weights and also carry out direct calculations on them. The calculator is capable of the four basic arithmetic operations, automatic percentage calculation and storage of intermediate and final totals. **Circle No. 96 on Reader Enquiry Card.**

BDH

AN addition to the BDH range of molecular weight markers is now available. The marker mixture (Product 44247) contains markers of MW values between 2,512 and 16,949. Also new is BDH Product 44168. This new reagent 1-(*m*-nitrobenzyloxy) methylpyridinium-chloride (NBPC), is used in the preparation of aminobenzyloxymethyl paper to facilitate the detection of specific RNA bands (Alwine, J. C. *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 5350-5354, 1977). BDH products are available in the USA from Gallard-Schlesinger Chemicals.

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Buchler

A NEW temperature-regulated water bath with a mating lift assembly has been introduced by Buchler. The Thermo-Lift can be used in a variety of common laboratory procedures requiring the application of heat to a stationary apparatus. Bath temperatures can be maintained at any point in the ambient to 80 °C temperature range as indicated by an integrally mounted dial thermometer. A sturdy scissor-type jack safely raises or lowers the bath throughout the range of 7.5–16.5 ins. Additionally, the bath and lift assembly may be separated to



Buchler's Thermo-Lift waterbath

function as independent units. The large (6×9 ins) base of the lift assembly provides stable support for the bath or any other accessory. Heating is by a 550 W stainless steel immersion heater controlled by a differential expansion thermal sensor. This sensor, with a sensitivity of ± 0.2 °C, maintains the 3-l bath volume within 2 °C of average throughout. Stainless steel and anodised aluminium construction protect the unit from corrosion.

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Bovine IgM (Specific To μ Chain)	Rabbit	64-261
Chicken IgG (H. & L. Chain)	Rabbit	65-196
Human IgG (H. & L. Chain)	Goat	65-207
Rabbit IgG (H. & L. Chain)	Sheep	65-204
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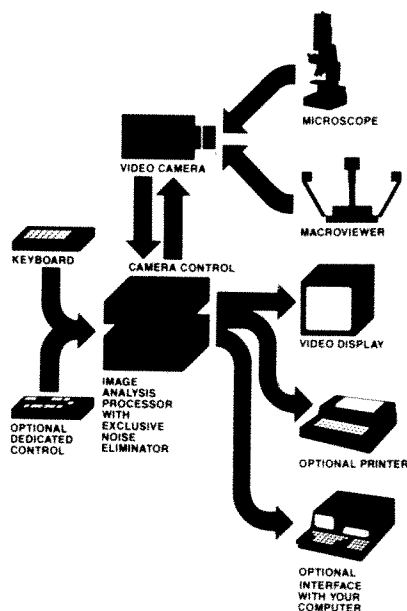
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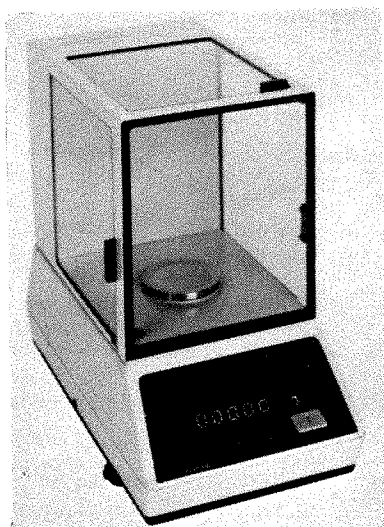
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The C1285 image analysis system from Hamamatsu

system analyses information from images, using a combination of optical, video, and computer hardware to measure, analyse and tabulate height, width, diameter, total area, inner area, outer area, perimeter, per cent area and location of up to several hundred discrete objects in each image. Features such as digital noise reduction, interactive image editing, image storage, and shading correction permit accurate analysis of even difficult images. Operation is as easy as typing on a keyboard or touching a button to request functions or enter operating parameters as the unit requests them. The C1285 is easily interfaced to optical microscopes, macroviewers, film viewers and other optical systems to provide information in a variety of research and quality control applications. An RS232C printer output allows production of hard copy for permanent records.

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Sartorius 1201 MP2-BCD

Cedarlane Laboratories

A BROADLY reactive, strongly cytotoxic mouse anti-Ia^k alloantiserum has been introduced by Cedarlane Laboratories. This antiserum is prepared by immunising A.TH mice with lymphocytes derived from A.TL mice. These two strains are congenic, differing at the I region of the H-2 complex but identical at the K and D regions. The H-2 haplotype of A.TH is K^sI^sD^d and that of A.TL is K^sI^kD^d. The antiserum potentially detects the gene products (Ia antigens) of the entire I^k region. In the presence of a suitable source of complement it is cytotoxic to the expected number of A.TL lymphocytes: spleen 50%; lymph node 40%; thymus 5%; bone marrow 15%. This antiserum can be used to quantitate lymphocytes bearing the appropriate Ia antigens or to remove these cells from a population for functional studies.

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Prochem

At the FASEB show Prochem are exhibiting their comprehensive catalogue of stable isotopes including carbon 12 and 13, nitrogen 15, oxygen 16, 17 and 18, deuterated compounds, multiply labelled compounds, and labelled gases and mixtures. Of special interest is a new line of 99 atom % carbon-13 intermediates and deuterated amino and fatty acids.

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Sartorius

THE new Sartorius fully-automatic electronic analytical top-loading balance, Model 1201 MP2-BCD, offers a weighing range of 30 grams to an accuracy of 0.1mg. This balance does not require dialing-in of weights or arrestment. To operate it, one simply places the sample on the pan and reads the result in just seconds on a large, bright seven-segment digital readout. The Model 1201 MP2-BCD also features: full-range push-button taring, a weighing chamber accessible from three sides and BCD-output permitting interfacing with printers, calculators, etc. For weighing heavier objects the Sartorius 2000 Series of suspended-pan analytical balances offers 0.1 mg readability. The 2003 MP1-BCD Model has a capacity of 166 g and the same features as the 1200 Series. Suitable for use with both 1200 and 2000 Series balances, the A7079 data printer is a low-cost electrostatic printer which allows any number displayed by the digital readout to be printed on tape for a permanent record. At FASEB 79 these Sartorius products are displayed on the Brinkmann Instruments stand.

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New procedure for proteolytic enzyme activity determinations

METHEMO-GLOBIN¹⁴C

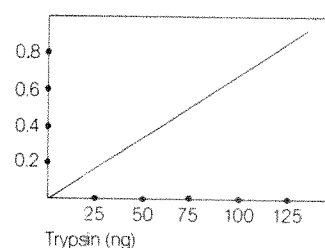
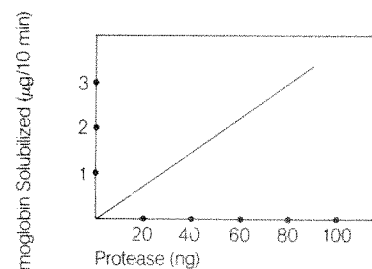
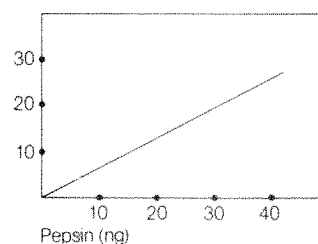
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Aqueous solution in siliconized vial, dry ice.

NEC-728 1 μCi 5 μCi



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Advanced Mechanical Technology

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The OR6-3 biomechanics platform

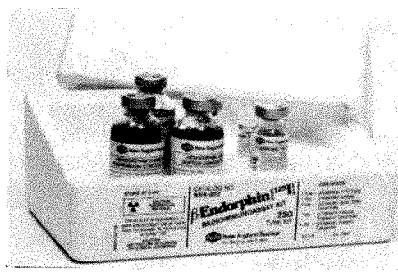
The instrument has many medically oriented applications such as: orthopedics, certain neurological investigations, evaluation of prosthetic devices, athletic performance studies and ballistograms. The platform is characterised by its very high sensitivity, high stiffness and a high natural frequency. Full strain gauge bridges provide operation with a minimum of crosstalk between outputs (less than 3%), the work area is 18.25×20 inches and the unit weighs approximately 60lbs. Maximum suggested excitation level is 10 V per channel. The Model OR6-3 biomechanics platform is available with or without complete instrumentation.

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Refrigeration For Science, Inc.

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NEN's β -endorphin assay

New England Nuclear

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Radiochemical Centre, Amersham

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Sienco Inc.

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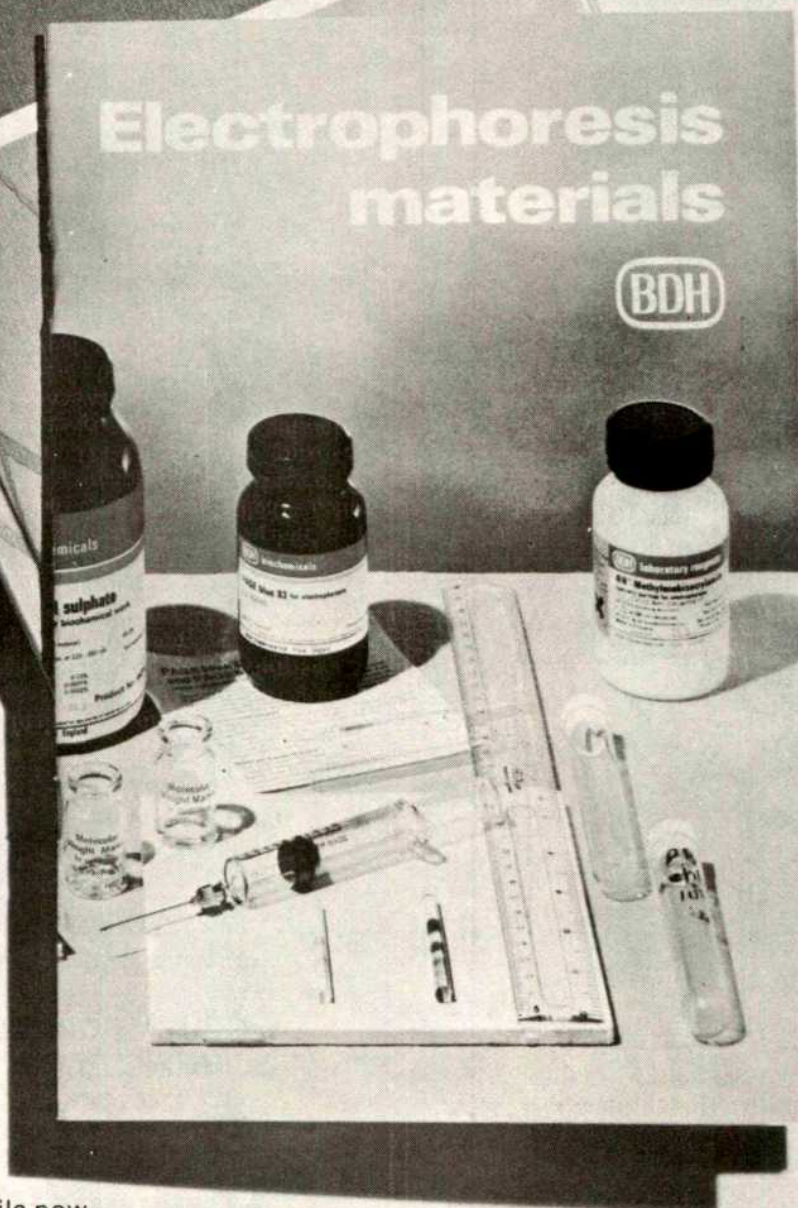
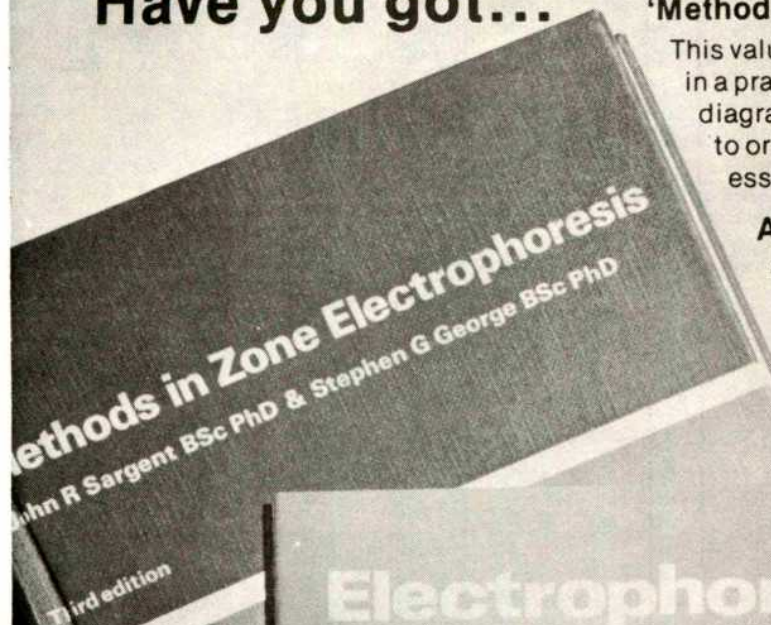
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A dual sample platelet aggregation meter from Sienco

several sample combinations, saves time, and minimises plasma deterioration. An important application for the Sienco aggregation meter is the detection and characterisation of haemostatic disorders associated with haemorrhagic problems (decreased aggregation) and thrombotic tendencies (increased aggregation). The instrument provides excellent aggregation resolution with lipemic plasmas as well as very low platelet count plasmas. In addition to platelet function determinations, the Sienco aggregation meter may also be used to measure plasma coagulation.

Circle No. 85 on Reader Enquiry Card.

Labindustries

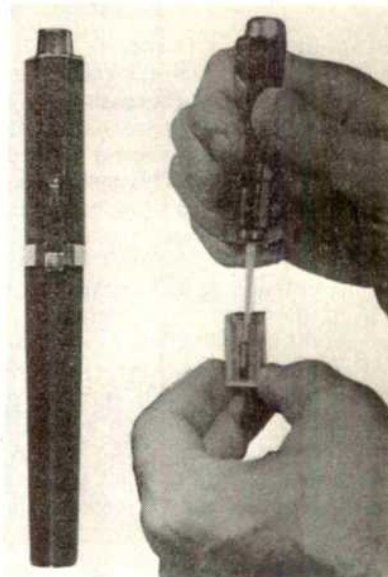
THE first non-glass, positive-displacement micropipettor with disposable, plastic, precision tips that can handle any chemical reagent with extreme accuracy and precision, has been announced by Labindustries. These lightweight L/I micropipettors feature a unique one-handed operation, using inexpensive snap-on snap-off plastic, disposable tips, which are precision volumetric transfer micropipets, for sampling and dispensing of all liquids, including serum. Accuracy and reproducibility range from as low as 0.5% at 1,000 μ l to 1.5% at 5 μ l. Eleven fixed-volume models cover the range from 5 to 1,000 μ l. All are colour-coded for ease of identification. These air-free micropipettors aspirate and discharge the entire aliquot without loss or carry-over, since the pipet is internally wiped clean by its Teflon plastic plunger in each operation. A unique feature of the L/I micropipettor is that its calibration rod and adjustment tool are stored in the handle of the instrument where it cannot be lost. Labindustries supplies its chemically resistant plastic tips bulk packaged in quantities of 100, 250, or 1,000, and also in dual purpose boxes of 250, 500, or 1,000 which serve as both snap-on dispensing and snap-off disposal containers without contact with the operator.

Circle No. 84 on Reader Enquiry Card.

Precision Cells Inc.

THE pen-size battery-driven cuvette stirrer from Precision Cells provides good mixing of solutions for all types of cuvettes down to microcell size. Stirring can be accomplished easily even under the most difficult conditions. The Delrin blade ensures that there is a minimum of cross-contamination, and it is shaped to dispel air bubbles in the solution.

Circle No. 83 on Reader Enquiry Card.



The Precision Cells cuvette stirrer

"Knowledge is of two kinds. We know a subject ourselves, or we know where we can find information upon it."

Samuel Johnson from Boswell, *Life of Johnson* (Spring, 1772)

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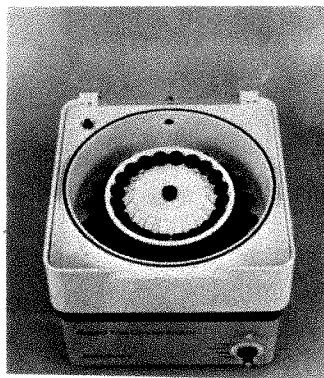
Circle No. 25 on Reader Enquiry Card.

Bio-Molecular Dynamics

A NEGATIVE pressure micro protein dialysis concentrator is now available from Bio-Molecular Dynamics. This self-contained system will simultaneously dialyse and concentrate to predetermined quantitative microlitre volumes . . . completely unattended. Nine models, including autoclavable units, are available providing a selection of predetermined, quantitative final volumes from 50 to 1,000 microlitres. **Circle No. 82 on Reader Enquiry Card.**

Savant Instruments Inc.

In their FASEB exhibit Savant features a new evaporator-concentrator. The Speed-Vac, with magnetic drive isolates the upper vacuum chamber from the lower drive section. This instrument is now widely used for the evaporation and concentration of valuable biological solutions. The instrument eliminates bumping and smearing of the sides of the tubes thus eliminating the necessity for washdowns. The sample is always in a high vacuum environment and at near zero temperature to provide the highest protection against the possible deterioration of the sample. A wide variety of culture tubes and micro-centrifuge tubes may be used with a



The Savant Speed-Vac evaporator-concentrator

variety of available rotors. The Speed-Vac evaporator achieves 100% sample recovery and concentration.

Circle No. 81 on Reader Enquiry Card.

Medical Arts

ANIMAL jackets for biomedical research are available from Alice King Chatham. Animal jackets, suits and restraints alleviate animal stress, resulting in more physiological responses. Suits function as surgical protective devices and provide housing for catheters, electrodes, microinfusion pumps, etc. Made of washable nylon net which can be gas sterilised, all items are semi-custom made and are available in standard sizes for dogs, cats, monkeys, rabbits and rats.

Circle No. 80 on Reader Enquiry Card.



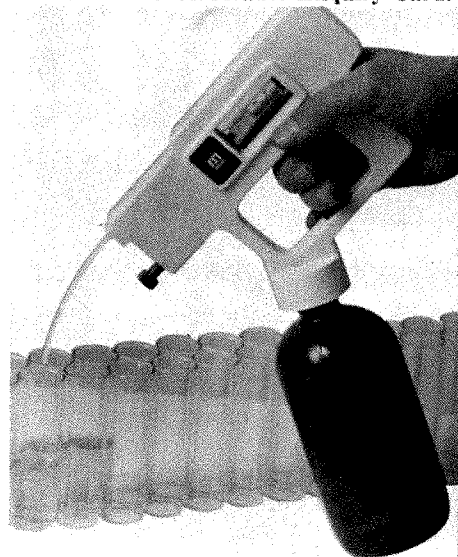
An Alice King Chatham animal jacket

Manostat

THE Pistolpet from Manostat is a pistol-like grip pipetting instrument that can be mounted directly to the most commonly used reagent bottles and provides a quick safe method for dispensing all chemicals. The unit has been designed for most dispensing procedures, simply squeeze to dispense . . . release to refill. The 2-inch short travel trigger contributes to better control while reducing wrist and finger fatigue. Because the unit is completely constructed of Teflon and glass, all chemicals can be handled with this new pipettor. The rugged housing is moulded of high impact plastic to withstand hard use. It is particularly useful for dispensing organic solvents for RIA

and scintillation counting work. Pistolpet is available in two capacity ranges 8.5 ml and 20 ml, both with high reproducibility and are reported accurate to $\pm 0.5\%$ of full scale. Also available is a remote attachment that allows dispensing from one and five gallon containers.

Circle No. 79 on Reader Enquiry Card.



The Manostat Pistolpet in use

BioSciences Information Service

THE Biosis Biosystematic Index of *Abstracts of Entomology* reflects the most recent changes in the major taxonomic categories of viruses and bacteria. Subject headings particularly affected by the expanded biosystematic categories include economic entomology epidemiology, virology, insect pathology and physiology, phytopathology and biological control. These permit the retrieval of complete surveys of a more precise host-microbe taxonomic relationship through currently accepted taxonomic systems of the bacteria and viruses.

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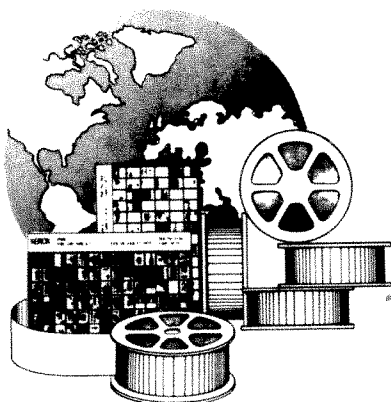
Starter kit for the new Millipore molecular filter

Millipore

THE Millipore immersible-CX molecular separator, a new disposable ultrafilter priced well below disk ultrafilters, provides a simple, rapid means for concentrating and desalting proteins and other macromolecules.

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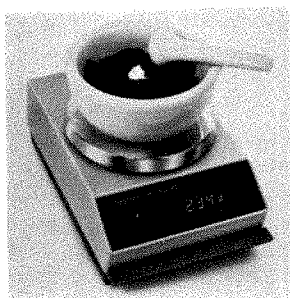
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Newly on the market. For more details about these products circle the appropriate numbers on the reader enquiry card bound inside the cover. These notes are prepared from information provided by the manufacturers.

Electronic balances. Mettler. The new PC range of top loaders from Mettler Instruments is suitable for weighing operations in which both a large weighing range and a high resolution of the measured weight values are needed. It used to take two balances to alternately weigh in light and heavy components, or to weigh small quantities into heavy containers with better than average accuracy. But a single PC balance with Mettler DeltaRange can



A Mettler balance fitted with the Delta Range system

now perform this task much more simply. A 10-times more accurate fine

range—the Mettler DeltaRange—can be recalled anywhere within the overall weighing range as often as needed. Whenever a more accurate readability with one extra decimal place is desired. And this fine range always covers one-tenth of the total weighing range. If the weight of the container exceeds the fine range, the tare weight is automatically indicated with the readability of the coarse range. When the container is tared out, the Mettler DeltaRange is called up at the same time. Weighing now begins at zero with the accuracy of the fine range and continues that way until the limit of the fine range is reached. If the total weight of the various fine weighings exceeds the limit of the fine range, the Mettler DeltaRange can again be recalled by pressing the control bar.

Circle No. 76 on Reader Enquiry Card.

Disposable desiccator plates. Spectrum announce the new disposable blue desiccator plates. They are comprised of a highly porous membrane covering a plate of silica gel. The blue colour slowly changes to a pale pink as the plate absorbs water. The bottom is clear polystyrene, which allows instant visual inspection of drying agent efficacy. It can be regenerated for re-use by heating in an oven, or it can be disposed of.

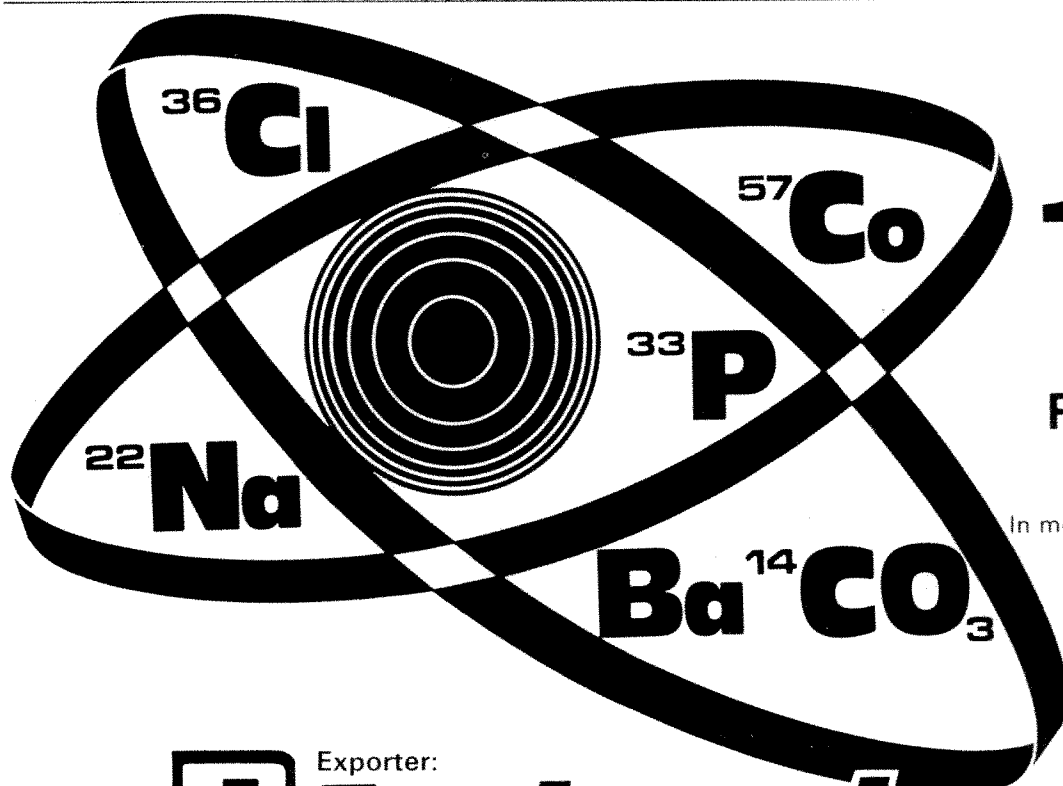
Circle No. 75 on Reader Enquiry Card.

Disposable scoops and spatulas. Spectrum's new disposable scoops are made of heavy duty polyethylene, and can be used for measuring liquids or solids in laboratory functions. They have an easy grip handle and come in a variety of assorted sizes, packaged in sets of 25. The new disposable spatulas are moulded of chemically resistant polypropylene. They are sealed on one end and cut at an angle on the other, for easy access to use in laboratory bottles. They are heat resistant, virtually unbreakable and economically priced, 100 to a package.

Circle No. 74 on Reader Enquiry Card.

Horizontal electrophoresis cell. Bio-Rad Laboratories' Model 1415 horizontal electrophoresis cell is designed with interchangeable accessories to perform DNA separations in thick agarose gels, immunoelectrophoresis, thin layer or granulated bed electrofocusing, and zone electrophoresis. The 125 x 430 mm cooling platform accommodates multiple separations. The entire platform can be used lengthwise to obtain extremely high resolution, or crosswise for high volume preparative separations. The lid is hinged to the unit for easy opening and closing, and includes a condensation coil.

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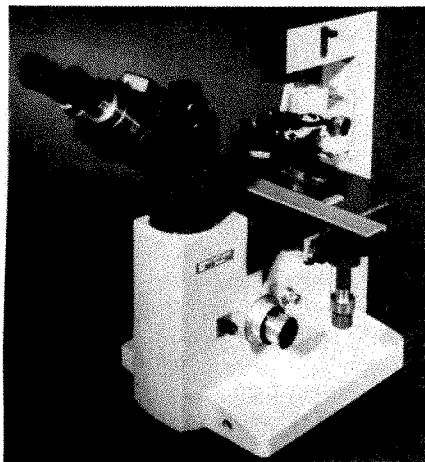


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The Reichert-Jung Biostar microscope

Inverted biological microscope. Reichert-Jung. The Reichert-Jung UK's Biostar has been specifically designed to ensure optimum performance in the inverted mode. The keynotes of the design are ergonomics and adaptability ensuring reduced operator fatigue over long periods of use in a wide range of application. A complete selection of highly corrected objectives for bright-field and phase contrast examinations is offered, including 20 $\times$ and 45 $\times$ lenses with correction collars suitable for use with cover thickness up to 2 mm. High transmission binocular and trinocular bodies feature converging eyetubes for viewing comfort, high eye-point viewing being combined with the large (20 mm) field of view.

Circle No. 72 on Reader Enquiry Card.

Substrate for alkaline phosphatase testing. J. T. Baker announces the availability of a new, high quality *p*-nitrophenylphosphate disodium salt for alkaline phosphatase testing. The main feature of the substrate is the extremely low inorganic phosphate content which is now guaranteed to be less than 0.1%. J. T. Baker also offers a range of standardised DEA- and AMP buffers.

Circle No. 71 on Reader Enquiry Card.

Laboratory dispensers. Misco. A wide selection of Misco dispensers, which can cope with the requirements of most laboratory dispensing tasks, are now available. Precision manufactured and tested, the flat-top glass dispensers are modular in construction with a periscoping scale post for ease of storage and stacking. All parts which come into contact with liquid are made of borosilicate glass and Teflon. The working parts of the dispenser are contained in the glass bottle. The Misco threaded bottom dispenser, together with adaptors, will fit any glass joint or screw-neck container.

Circle No. 70 on Reader Enquiry Card.

Germinators/Incubators. Harrington Enterprises, has introduced a line of germinator/incubators featuring a fibre-reinforced plastic construction. Both interior and exterior are constructed of structural, highly flame-resistant, glass-reinforced, polyester sandwich panels. This method produces equipment with high strength and light weight, with no shock hazard, no corrosion, and no seams to leak. It is easily cleaned and is double insulated. The germinator/incubators, series I-12G and I-20, are also equipped with sophisticated electronic controllers. A wide range of units with variable parameters of temperature, humidity, light spectrum and intensity are available.

Circle No. 69 on Reader Enquiry Card.

Digital temperature alarm unit. British Rototherm. The Temp-A-Lert digital temperature monitor from BR allows the user to pre-set high and low alarm limits. Designed for medical, industrial and laboratory applications, the Temp-A-Lert gives a visual and/or audible alarm if the temperature goes under or over the predetermined limits. The unit, designated TA986, is a desk top model with dimensions of 81 mm $\times$ 156 mm $\times$ 212 mm, and features a Centigrade to Fahrenheit conversion switch with a large 0.5 in. digital display. Accurate readings can be taken within 10 s in liquids, 45 s in humans and slightly longer in ambient air. The unit is supplied complete with a rechargeable nickel cadmium battery.

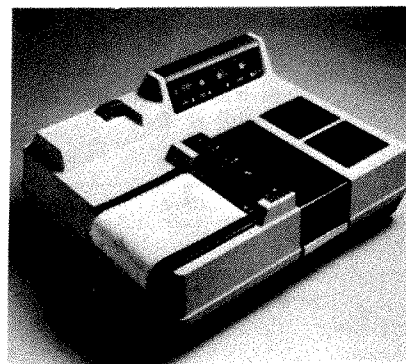
Circle No. 68 on Reader Enquiry Card.

Centrifuge. Burkard. Burkard Scientific announce the latest Koolspin table-top centrifuge, the model 2121100. Now with a 25.5-cm bowl, this version of Koolspin has increased capacity, and a swing-out rotor for Universal bottles (25 ml). Many combinations of carrier, tube and bucket are available in addition to the existing rotors, which all fit the new model. Also, a 55° angle rotor has been introduced to accommodate 105-mm long tubes, in two versions, 16 or 18 mm diameter.

Circle No. 67 on Reader Enquiry Card.

Human chorionic gonadotrophin anti-serum. Northeast Biomedical Laboratories announce the introduction of a new antiserum to human chorionic gonadotrophin (β -subunit). This material has been raised in goats and will be supplied as undiluted serum. Five standard preparations and conjugates are available on a routine basis but other conjugates can be made to order for special applications.

Circle No. 66 on Reader Enquiry Card.



An SP3 infrared spectrophotometer from Pye Unicam

IR Spectrophotometers. Pye Unicam. The new SP3 series of infrared spectrophotometers from Pye Unicam offer the performance benefits of a ratio recording system hitherto associated with high cost research type instrumentation. This has been made possible by basing the design on a rapid response pyroelectric detector and a new high energy ceramic source. All three models in the SP3 range are suitable for both qualitative and quantitative studies. The most simple version, the SP3-100 has a frequency range of 4,000 cm^{-1} to 600 cm^{-1} and is designed for applications where rapid sample throughput and quantitative accuracy are essential.

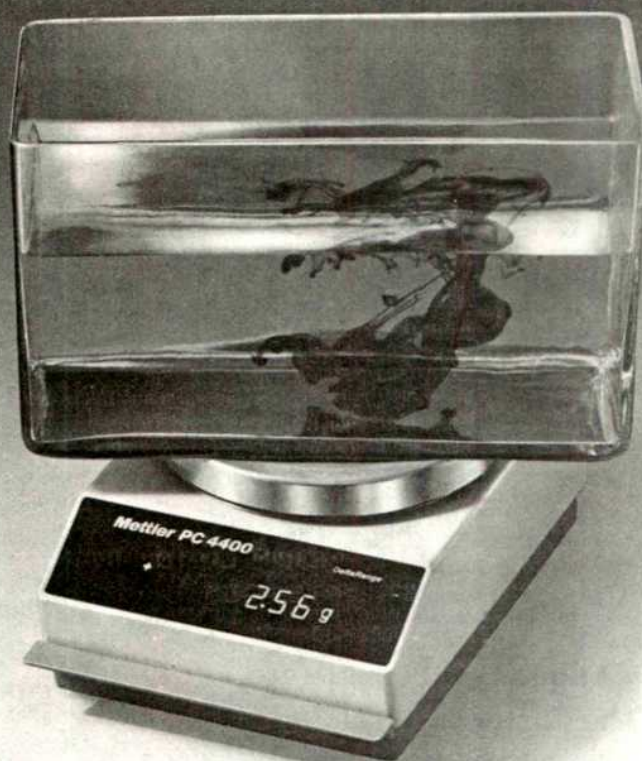
Circle No. 65 on Reader Enquiry Card.

Scanning spectrophotometers. Pye Unicam. Two models have been added to the Pye Unicam SP8 series of UV/visible spectrophotometers. The SP8-250 is a double monochromator version of the SP8-200 microprocessor controlled, research spectrophotometer and the SP8-150 is offered as a higher performing variation of the SP8-100 formula. These four models share with the lower cost SP6 range, the benefits of a major breakthrough in optical technology—master holographic gratings.

Circle No. 64 on Reader Enquiry Card.

High voltage pulse generator. Rofin's 7540 series high voltage pulse generator is intended to operate mainly with infra-red modulator systems such as the Rofin series 7500. The 7540 features various rise times from 1 ns to 70 ns and pulse repetition rates of 10 kHz or 100 kHz depending on the choice of output module. The pulse width is variable from 1 ns to 100 μs at an output of 5 kV p-p. A 3-unit modular design approach provides flexibility and ease of servicing. The 7540 will operate with any electro-optic modulator system or high impedance load. It will drive into load impedances from 200 ohms to many megohms.

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An unconventional balance with an extra measure of accuracy

Mettler DeltaRange breaks through the boundaries that used to restrict the capacity/accuracy ratios of conventional weighing instruments. And Mettler PC electronic top-loading balances are equipped with this new *Mettler DeltaRange* feature.

Just press the control bar of the Mettler PC4400, for example, and you get a 400 g fine range with readings that are 10 times as accurate as those in the coarse range. And you can do it as often as you wish, at any point of the entire weighing range. That is to say, whenever and wherever a 0.01 g readability is needed. On a balance that has a capacity of no less than 4000 g!

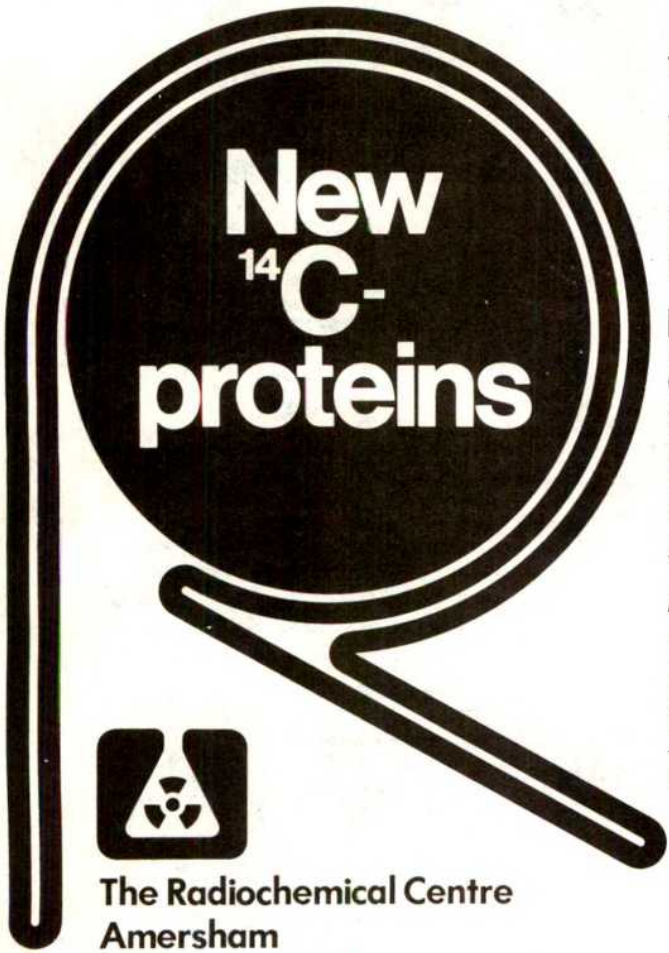
In practice, this means that you can use a heavy container to accurately weigh in as many components as you want. Or that you can weigh one time on a lightweight paper, the next time into a heavy recipient; both times with results that are readable to the second decimal place.

To learn more about the many things your Mettler PC balance can do for you with outstanding speed and precision, send for our detailed brochure. It's an eye-opener.

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How Britain runs its science

This highly successful chart, which appeared in Nature 28 August 1975 (but is still much requested and much referred to), has now been completely revised and updated to include all major changes in the personnel and administration of British science.

Measuring approximately 2' x 3' and containing black-and-white illustrations of prominent figures, copies of this chart are now available at 75p (UK), US \$1.50 (Rest of World).

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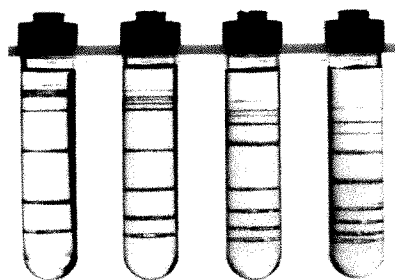
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Density marker beads for Percoll gradients

Density marker beads. Pharmacia. Pharmacia Fine Chemicals announce the availability of density marker beads, for calibration of density gradients of Percoll. Density marker beads are coloured derivatives of Sephadex, each type specially derivatised to have a specific density in Percoll. The beads are used as an external marker, and the shape of the gradient (either preformed or generated in situ) is monitored simply by measuring the distance between the bands.

Circle No. 62 on Reader Enquiry Card.

Programmable calculator. Sinclair. A new, 79 step programmable calculator, designed for advanced professional use, has been introduced by Sinclair Radionics. The Enterprise Programmable comes complete with a three volume program library, carrying case, high energy alkaline battery and comprehensive instruction manual. The three volume programme library includes 316 ready to use programs covering general finance, mathematics, physics, electronics and engineering calculations. In addition to the program library there is provision to record personal programmes on blank cards. The Enterprise Programmable is based on the Enterprise model, which is a compact, portable or desk top unit, complete with a.c. mains adaptor, and the large, bright 8-digit red display features fully floating decimal point or fixed scientific notation. The Enterprise Programmable has seven independent, three function memories, common and natural logs and antilogs, y^x , sin, cos, tan, arcsin, arccos, arctan and degree/radian conversions. Its full 79-step program facility has merged upper case functions, forward/backward stepping, conditional and unconditional branching.

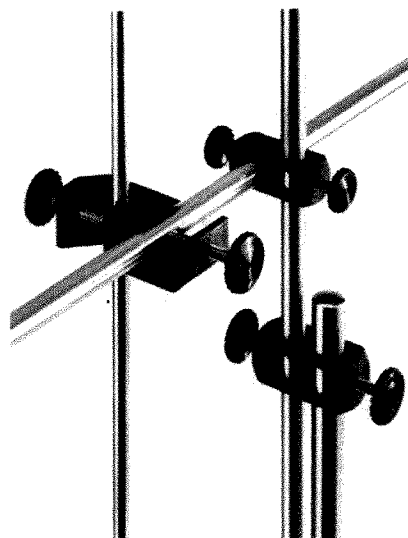
Circle No. 61 on Reader Enquiry Card.

Scientific calculator. Texas Instruments. A new powerful slimline scientific calculator incorporating statistical functions and a unique Constant Memory feature has been introduced by Texas Instruments. The new TI-50 can retain data in its two full arithmetic memories even when the calculator is switched off, and offers 60

algebraic calculator functions, including logarithms and trigonometry, plus statistical operations. Apart from the two memories with their Constant Memory feature, the calculator also uses the Texas Instruments AOS algebraic operating system and the APD automatic switch-off feature.

The calculator has a large 8-digit liquid-crystal display, and incorporates full scientific notation (5-digit mantissa and 2-digit exponent display format) and mantissa expansion capability. The algebraic operating system includes 15 levels of parentheses and up to four pending operations, allowing the user to enter problems from left to right as they are usually written. The APD (automatic power down) feature automatically shuts the calculator off after about 15 min of non-use. The low-power-consumption liquid-crystal display and the internal circuitry help to provide up to two years' of normal operation on a single set of batteries. A 'battery-low' indicator is also provided.

Circle No. 60 on Reader Enquiry Card.



BioScience nylon bossheads

Nylon bossheads. BioScience. New glass-filled nylon bossheads in three sizes have been introduced by BioScience. Closed or open-sided versions are available, the former allowing bars to be held at right-angles or parallel to each other. Made of Maranyl, ICI's nylon compound, an extremely strong but light-weight material, they are virtually indestructible in normal use. The clamping screws are made of stainless steel. They have a good resistance to atmospheric corrosion and to most laboratory chemicals; are non-magnetic and of low thermal conductivity. They are, of course, washable. The larger of the closed type will accept bars up to 12.7 mm diameter. The smaller will take sizes up to 9.5 mm diameter. The open-sided version will hold bars from 3.2 to 12.7 mm diameter.

Circle No. 59 on Reader Enquiry Card.

Disposable ultrafilter. Millipore. The Immersible-CX molecular separator, a new disposable ultra-filter, provides a simple, rapid, and inexpensive means for concentrating and desalting proteins and other macromolecules in small to intermediate volumes of liquid. Each disposable unit contains 13 cm² of Pellicon ultrafiltration membrane and can process from 1 to 100 ml. The membrane, on a porous core through which vacuum is applied to drive filtration, is specially formulated for high flux and minimum nonspecific binding.

Circle No. 58 on Reader Enquiry Card.

In-line membrane filter holder. Sartorius. Recently announced by Sartorius is a 47 mm in-line filter holder (or with fittings—tap sampler) which is constructed in 316 stainless steel, with filter support, underdrain and back pressure screen coated in Teflon. A valve on the inlet side allows air venting or water removal (for example with PTFE filters) and the back pressure is removed or replaced by a twist.

Circle No. 57 on Reader Enquiry Card.

Multisheet chromatography tank. Gallenkamp. A new multisheet chromatography tank has been added to the Gallenkamp range. The tank can be used for one or two way ascending or descending chromatography and is designed to cater for a large number of samples at one time. Provision has been made for up to 10 250 × 250 mm papers on the aluminium frame for simultaneous ascending developments. Alternatively, two troughs are provided to hold paper for descending chromatography. A further pair of troughs can be used if required, and are available as accessories.

Circle No. 56 on Reader Enquiry Card.

Measuring syringes. Hamilton. Six new syringes with removable needles have been introduced into Hamilton's 700 series in sizes ranging from 5 to 500 μ l. All the 700s are now available with the added convenience and versatility of replaceable needles. Absolute liquid delivery is accurate to within $\pm 1\%$. Repeatable accuracy is within $\pm 1\%$. Bent or plugged needles can be easily replaced in the field. And the cost of added syringes is saved by simply replacing the needle. The needle threads securely in place so it cannot be pulled off by mistake. Carry over when changing samples is eliminated because there is virtually no dead volume. The syringe can be sterilised by all normal methods, including autoclaving. Each stainless steel plunger is individually tested and mated to its barrel to assure leak-proof sealing. They are shipped with one standard needle installed.

Circle No. 55 on Reader Enquiry Card.

Substrate for peroxidase staining. Northeast Biomedical Laboratories announce the introduction of a kit designed for use with both peroxidase-labelled antibodies and the PAP (peroxidase anti-peroxidase) complex. It is based on a non-carcinogenic substrate, aminoethyl carbazole. The kit includes the necessary buffer solutions, reagents for blocking endogenous peroxidase and nonspecific staining as well as pre-measured quantities of chromogen and carrier.

Circle No. 54 on Reader Enquiry Card.

Reverse-osmosis system. Millipore has extended its range of wall-mounted reverse osmosis systems. While retaining the advantages of high performance (90–99% rejection) and low operating cost, the new Milli-RO systems incorporate further significant advantages. Precisely designed and engineered to produce water at a rate of 125 and 250 l h⁻¹, the systems are based on a modular construction in 316 stainless steel and are easily dismantled for cleaning or cartridge replacement. The systems automatically flush to drain hourly for self cleaning and will shut off the pump when the feed water pressure is too low. Solid state controls also indicate low pressure cut-off, full tanks, system under flushing cycle and water saving.

Circle No. 53 on Reader Enquiry Card.

HPLC column. Applied Science. Applied Science Laboratories is now offering a new type of highly efficient, stable HPLC columns. These columns typically demonstrate 30,000 to 40,000 theoretical plates per m at a linear velocity of 0.14 cm per s, yet sustain pressures up to 6,000 p.s.i. or more. The columns are constructed of LiChroma stainless steel and are capped with zero or low dead volume unions containing removable frits. Packings are available with a complete range of surface functional groups. Columns can be supplied in 25 cm lengths and various internal diameters.

Circle No. 52 on Reader Enquiry Card.

Vacuum tight glass joints. Laboratory Impex. Unilok G retaining seals are a new laboratory aid bringing improved safety and efficiency to all apparatus assembled with standardised glass joints. The seals are extremely compact yet secure, ensuring gas-tight seals for both positive and negative pressures, without the need for extensive modifications to glassware.

Circle No. 51 on Reader Enquiry Card.

GC/MS Systems. Finnigan. The OWA-series GC/MS systems from Finnigan Instruments feature pre-programmed parameters built into the data system specifically to handle all organics-in-water problems. Despite low cost, the

OWA-series instruments exceed requirements for specificity, sensitivity, data integrity and an EPA-compatible data format/medium. Other features of the new system include low-cost data storage and transmission, and low-cost operation and maintenance.

Circle No. 50 on Reader Enquiry Card.

Gas chromatograph. Packard. The Packard model 430 dual channel integrating gas chromatograph uses a micro processor to control all analytical functions. This multi-user system can accept up to 15 users' analytical programs in store at any one time. An exclusive feature of the new system is its dual channel simultaneous integrator giving retention time, area counts and area per cent, and analytical program listing.

Circle No. 49 on Reader Enquiry Card.

HPLC injection valves. Schoeffel. The Schoeffel Instrument Division of Kratos, offers high pressure injection valves for liquid chromatography. These feature a rotary valve for injection, column switching and recycling, and include a high pressure, septumless syringe loading sample injector, with sample loops available from 10–100 μ l. A full line of accessories such as pneumatic actuators, mounting plates and fittings is also available.

Circle No. 48 on Reader Enquiry Card.

STOP FREEZING STOP SQUEEZING STOP LOSING TIME AND DNA

BRL'S LOW MELTING POINT AGAROSE

For rapid *in situ* redigestion of restriction enzyme cleavage fragments. Freeze – Squeeze¹ or Gel Dissolution – Chromatography² techniques are time-consuming, inefficient methods for recovery of DNA fragments from agarose.

When mapping restriction enzyme cleavage sites of DNA, BRL's Low Melting Point Agarose makes redigestion of large numbers of DNA fragments easy, rapid and economical.

BRL's Low Melting Point Agarose melts at 62°C, gels at 28°C and remains fluid at 30–37°C for several hours. Parker and Seed³ have recently developed a simple, efficient method for *in situ* redigestion of DNA fragments after electrophoresis through low melting point agarose. A detailed protocol for this enzyme-saving, time-saving technique is included with the Product Profile Sheet shipped with each order of BRL's Low Melting Point Agarose.

References

1. Thuring, R.W.J., Sanders, J.P.M., and Borst, P. (1975) *Analyt. Biochem.* **66**, 213–220
2. Sugden, B., Delroy, B., Roberts, R.J., and Sambrook, J. (1975) *Analyt. Biochem.* **68**, 36–46
3. Parker, R.C. and Seed, B. *Methods in Enzymology*, *in press*

Ordering Information

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Circle No. 36 on Reader Enquiry Card.

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Ainsworth

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M MACMILLAN
PRESS



Balzers 07 120 sputtering device

Sputtering device. Balzers. An improved version of the Sputtering Device 07 120 for the preparation of electron microscopy specimens is available from Balzers High Vacuum Limited. The device has a rotary symmetrical magnetic field and, because the target and specimen table are water-cooled, a high sputter rate can be maintained without the specimen temperature rising beyond approximately 25 °C. The specimen surface is easily cleaned, an etching device being brought into operation simply by changing plugs on the unit. A built-in aperture enables samples to be etched and then sputtered without interrupting the vacuum as well as the pre-sputtering of difficult films such as oxides of nickel and chromium.

Circle No. 47 on Reader Enquiry Card.

Aggregation meter. Sienco. An improved version of the Sienco dual sample platelet aggregation meter, featuring reduced sample requirements and improved optics, has been announced. With a minimum sample requirement of only 0.2 ml, as well as standard 0.4 ml, the Sienco Model DP-247-E is especially useful instrument for pediatric and other applications where only small samples are available. The meter depends on optical transmission through a cuvette, generates two simultaneous curves on a single pen or two pen recorder in identical sample conditions. The dual run capability allows the choice of several sample combinations, saves time, and minimises plasma deterioration. A key application for the Sienco aggregation meter is the detection and characterisation of haemostatic disorders associated with haemorrhagic problems (decreased aggregation) and thrombotic tendencies (increased aggregation). The instrument provides excellent aggregation resolution with lipemic plasmas as well as very low platelet count plasmas. In addition to platelet function determinations, the Sienco aggregation meter may also be used to measure plasma coagulation: it is well-suited for either routine clinical use or research into platelet function and blood coagulation.

Circle No. 46 on Reader Enquiry Card.

STEM Philips EM 400 microscope.

Philips. Advanced features for fast, efficient operation—plus unique signal mixing and display facilities—are included in a new STEM unit for the Philips EM 400 transmission electron microscope. Addition of the fully integrated STEM system to the EM 400 gives the microscopist a choice of three basic imaging modes and various analysis modes. Rapid switching between TEM, STEM and SEM is made possible by the provision of preset alignment controls, while up to six signals are push-button selectable. Any combination of the available signals can be superimposed on the viewing screen, permitting ready comparison of various types of information from a given specimen area. Alternatively, images can be displayed side by side in a split-screen presentation. A dual magnification system gives the further option that a signal can be viewed at a selected magnification on one half of the screen, while simultaneously being shown alongside at a zoomed value 1× to 4× larger. Using the split-screen mode, two successive exposures can be made on the same piece of film at different goniometer tilt angles, providing a correctly aligned stereo pair. Independent adjustment of brightness and contrast allows easy choice of different imaging modes. Line time and number of lines are also independently controlled, giving a wide choice of frame speeds and enabling the best scanning conditions to be selected for each application. A special 1000× line time facility produces excellent counting statistics for X-ray analysis. The STEM unit is complemented by a wide range of modular plug-in accessories for the optimisation, manipulation and recording of scanned images.

Circle No. 45 on Reader Enquiry Card.

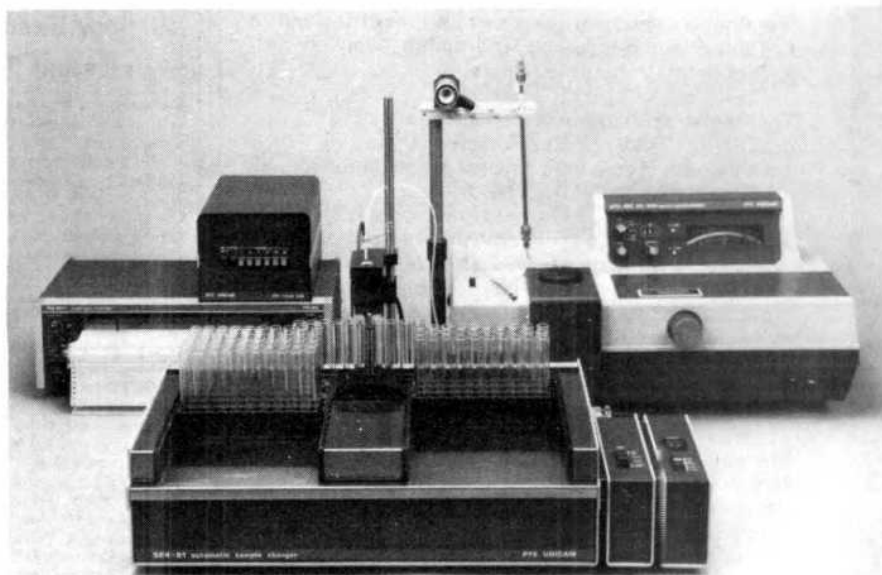
Water-jacketed CO₂ incubator.

Forma. The CH/P System adds a new dimension to solid-state electronic CO₂ control with direct digital CO₂ setpoint to within 0.1% over a full 0–20% range and direct digital display of both setpoint and chamber readout on one easy-to-read LED indicator. The direct digital CO₂ setpoint and digital CO₂ display electronically locks-in for precise CO₂ control. A CO₂ deviation alarm provides an audible and visual warning if CO₂ tension drifts more than 1% from setpoint. Sensor fans gently circulate chamber air to eliminate CO₂ stratification, to improve temperature uniformity and to hasten temperature recovery after door openings. The Model 3325 has a temperature range of 2 °C above ambient to +60 °C, a solid-state direct reference dial, and 37 °C fixed-point selector switch. This unit with two independent chambers, two CH/P System Controllers occupies only 4 square feet of floor space. CH/P System digital CO₂ controllers are now available on all Forma automatic water-jacketed incubators.

Circle No. 44 on Reader Enquiry Card.

Low-cost spectrophotometers. Pye Unicam. The SP6 Series of low cost, single beam UV/visible spectrophotometers now feature major improvements in performance, versatility and construction. Four instruments are available offering the choice of visible or UV/visible range and digital or meter display. New accessories include an automatic cell changer, a thermoelectric heated and cooled holder and a low cost programmable calculator offering autozero and autoconcentration modes or enzyme range and immunoassay computations.

Circle No. 43 on Reader Enquiry Card.



Pye-Unicam SP4-01 sample changer

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APPOINTMENTS VACANT

HUMAN SCIENCES

We have an opportunity for a graduate to contribute to the ergonomics programme of the Institute of Occupational Medicine, which is the coal industry's research centre for medical and allied problems. The programme involves a wide range of ergonomics investigations in coal mining, chemical and other industries. The successful candidate will be appointed to the Human Sciences Group and will initially work in the field of occupational stress.

The Ergonomics Branch is a large multi-disciplinary team with good supporting facilities and is situated at Boreby, nine miles south of Derby.

Salary, dependent on age and experience, will be in the range £4,000 to £5,000. Excellent conditions of employment include generous leave.

Please apply to:

Head of Staff Recruitment/Training,
National Coal Board,
Scottish Area,
Green Park,
Greenend,
Edinburgh EH17 7PZ. 252(A)

THE MEDICAL COLLEGE OF ST BARTHOLOMEW'S HOSPITAL (University of London) West Smithfield, London EC1A 7BE

Applications are invited from good honours graduates or those expecting to graduate in 1979 in Biochemistry or related subjects, for the post of

RESEARCH ASSISTANT

to undertake investigations on the biochemistry of chemical carcinogenesis. The successful candidate will be registered for the degree of PhD.

The appointment, funded by the M.R.C., will be for three years and will be at an initial salary of £3,384 p.a. plus £502 London Allowance.

Enquiries to Professor E. M. Crook, Biochemistry Department, The Medical College of St Bartholomew's Hospital, Charterhouse Square, London EC1M 6BQ.

Applications in writing to The Secretary of the Medical College at the West Smithfield address, quoting reference 829. 220(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a temporary post of

RESEARCH ASSISTANT

to work on the molecular biology of the acquisition of bacterial drug resistance in association with Dr J. H. Parrah in the above Department. The post would be most suitable for a postgraduate with appropriate research experience, although persons graduating this year in Biochemistry, Genetics, Microbiology, or an appropriate combination of these subjects, will be considered. The appointment will be made with effect from October 1, 1979, for a fixed term of two years.

Salary at an appropriate point on the IB Scale for Research and Analogous Staff (£3,384 to £4,882) (under review), according to age, qualifications and experience.

Application forms and further particulars may be obtained from The Registrar, The University of Leeds, Leeds LS2 9JT, quoting reference number 83/19/D. Closing date for applications April 30, 1979. 259(A)

UNIVERSITY OF DURHAM DEPARTMENT OF BOTANY

Applications are invited from candidates with interests in molecular and microbial genetics for the post of

LECTURER IN GENETICS

tenable from October 1, 1979. Experience in recombinant DNA technology preferred.

Salary of the scale £3,883 to £7,716 (under review) plus superannuation.

Applications (3 copies) naming the referees should be sent by May 1, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 1LE (from whom further particulars may be obtained). 218(A)

PLANT BREEDING INSTITUTE

Maris Lane, Trumpington,
Cambridge CB2 2LQ

A vacancy exists for a Scientific Officer/Higher Scientific Officer for project funded by the Home Gro Cereals Authority for the period December 31, 1980. The officer appointed is required to take responsibility for the development of application of methods for the rapid identification of malting quality barley. The work will be of chemical/biochemical nature aimed at determining differences in the structure and metabolism of barley genotypes.

Minimum qualifications required an Honours Degree or equivalent Chemistry or Biochemistry or ordinary degree, H.N.C., with experience.

The appointment will be as Scientific Officer (£2,839 to £4,414 per annum or Higher Scientific Officer (£4,101 to £5,448 per annum). At least five years relevant postgraduate experience required for appointment as Higher Scientific Officer.

Applications with curriculum vitae and names and addresses of three referees should be sent to the Assistant Secretary (Establishment), quoting CER 49, by April 20, 1979. 222(A)

UNIVERSITY OF CAMBRIDGE
DEMONSTRATOR IN PLANT PHYSIOLOGY. Applications are invited for a University Demonstratorship in the Department of Botany to commence on October 1, 1979. The person appointed will be expected to assist in teaching some of the following: integrated cell biology course, cell or whole plant physiology, plant development. The applicant should have research interests in some aspect of plant physiology. The appointment will be in the first instance for 3 years with a maximum tenure of 5 years. The salary is on an incremental scale rising from £4,133 to £5,129 per annum. Applicants, with the names of three referees, should be sent by May 1979 to the Secretary of the Faculty of Biology 'A' Appointments Committee, Department of Applied Biology, Pembroke Street, Cambridge CB2 3PQ from whom further particulars may be obtained.

Registration Officer

We are a major International Company manufacturing ethical pharmaceuticals. The Company has been successful in recent years in identifying a series of valuable new medicines and has a continuing research programme involving further potential new medicines.

The Registration group is a small team of graduates whose principal function is the organisation of scientific data for presentation to the Committee on Safety of Medicines.

A post of Registration Officer in this department is now vacant. Candidates should be graduates in a biological science, should have facility in the organisation of scientific data and the ability to write clearly and concisely on scientific topics. Previous experience of product registration or other experience within the Pharmaceutical Industry would be an advantage.

Please apply to:-

Mrs. J. C. M. Andrews,
Personnel Officer,
Wyeth Laboratories
Huntercombe Lane South, Taplow,
Maldenhead, Berks. Tel. Slough 28311.



Wyeth Laboratories

THE OPEN UNIVERSITY FACULTY OF SCIENCE



Biology Electron Microscope Technician

We require an experienced technician to maintain and operate a recently purchased Joel JEM 100S Electron Microscope and its ancillary equipment housed in a new purpose built EM suite. This sectioning equipment includes Reichert OMU3 and Cambridge Huxley ultrathin sections. Access to scanning electron microscope facilities are also available. The post offers an opportunity to a technician seeking employment in an expanding area of a unit which serves both the teaching and research requirements of the Discipline. Applicants should have a HNC or equivalent and 10-12 years' practical experience preferably in Biological Electron Microscopy. Commencing salary will be within the Technician Grade 6 scale £3,884 to £4,365 per annum (under review). Application forms and further particulars are available from The Recruitment Office (JD438/1), The Open University, P.O. Box 75, Walton Hall, Milton Keynes MK7 6AL, or telephone Milton Keynes 63888. 24-hour answering service. Closing date for applications 23rd April, 1979. 272(A)

PROFESSORSHIPS

in PHYSICS, ELECTRICAL ENGINEERING MATHEMATICS & BALLISTICS

The Royal Military College of Science runs first degree, post-graduate, army staff and special courses on a wide variety of subjects. While the College's primary function is the scientific education of the Army officer at all stages of his career, courses are also attended by civilians, and military personnel from overseas.

The College is a residential establishment occupying a large estate in the Vale of the White Horse, near Swindon, and has an academic staff of over a hundred. Research is an important part of the work, and there is every opportunity for staff to pursue their own research interests and publish findings.

The excellent facilities and equipment include a nuclear laboratory, workshops and ICL 1903A, PDP 11/45 and PDP 11/20 computers with a wide range of peripherals.

Due to retirements, the following posts will shortly fall vacant. The new appointments may be permanent and pensionable, or (with present employee's agreement) on secondment terms. Candidates must have a record of high professional attainment, and have had considerable relevant experience, including some teaching at university level.

Chair in Physics

The Department is responsible for the teaching of physics as required for all courses at the College. In addition, special instruction is given on nuclear radiation and weapons effects. Research interests centre on solid state and nuclear physics, mainly polymer studies (including pulsed NMR), radiation effects on polymers, radiation grafting, nuclear techniques for materials study, solar cells, new tools for atmospheric studies, and applications of photon autocorrelation.

Salary will start between £11,710 and £12,730, according to qualifications and experience. Ref: S/9828/3.

Chair in Electrical and Electronic Engineering

The professor will be responsible for the full range of work of this large Department. In addition to teaching to degree and other academic courses, instruction is given on guided weapons, digital systems, computer hardware and signal processing. Current fields of research interest include antenna design, microwave integrated circuits, a load simulator for large control systems, and digital feedback communication systems.

Applicants for this position must hold, or be eligible for, corporate membership of the IEE.

Salary will start between £11,710 and £12,730, according to qualifications and experience. Ref: S/5109/3.

Associate Professor— Mathematics and Ballistics

The Associate Professor will be responsible for the teaching and research activities of the Mathematics and Ballistics Branch. Its present research programme covers control system theory; boundary value problems in mathematical physics; approximation theory and numerical analysis; quality assurance and reliability; mathematics of wave power machines; internal ballistics; blast waves; transient vibration analysis of gun barrels; and the stability of projectiles, including liquid filled projectiles.

Salary will start between £10,040 and £11,300, according to qualifications and experience. Ref: S/5107/3.

For further details and an application form (to be returned by April 24, 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (an answering service operates outside office hours).

Please quote the appropriate reference.

211(A)



THE UNIVERSITY OF LEEDS

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a temporary post of

TEACHING FELLOW

in the above Department. Candidates should either have a Ph.D. in Biochemistry or a closely-related discipline or be about to submit for a Ph.D. Degree. The person appointed will participate in teaching biochemistry to science and medical students and will also be expected to engage in research, preferably in an area already under investigation in the Department. The appointment will be made with effect from October 1, 1979, for a fixed term of three years.

Salary at an appropriate point on the 1A Scale for Other Related Staff (£3,883 to £6,555) (under review), according to age, qualifications and experience.

Application forms and further particulars may be obtained from The Registrar, The University of Leeds, Leeds LS2 9JT, quoting reference number 83/20/D. Closing date for applications: April 30, 1979. 257(A)

EDINBURGH UNIVERSITY

PHYSICS DEPARTMENT

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for this post to work on the Raman scattering of molecular systems under high pressures. Financial support is available from S.R.C. for the initial phase of this work until April 1980, with good prospects for continued support. Use will be made of the department's excellent Raman scattering laboratory which enjoys interactive computer control. A high pressure cell, capable of over 500 kbar, is nearing completion and exploratory experiments will soon be started. Experience in either Raman or high pressure techniques is desirable, but not essential. The work will be done under the supervision of Dr G. S. Pawley.

Applications, with a curriculum vitae and the names of two referees, should be sent to Professor R. A. Cowley F.R.S., at the Physics Department, Kings Buildings, Edinburgh EH9 3JZ.

221(A)

AGRICULTURAL RESEARCH COUNCIL

Institute for Research on Animal Diseases

A vacancy exists in the Department of Immunology and Parasitology for a

RESEARCH SCIENTIST

to work on cellular immunity related to helminth infections (including liver fluke) of domestic livestock. The person appointed will join a team investigating the development of immunity to parasitic diseases. Studies will include the immunological function of different cell types and the isolation of antigens which will stimulate immunity for the protection of farm animals.

Qualifications: A first or upper second class honours degree, or equivalent with 4 years relevant postgraduate experience in immunology. Applicants must have an interest in parasitology.

Salary Scale: Appointment will be to the Senior Scientific Officer scale £5,154 by 8 to £6,898 per annum. The A.R.C. pension scheme is non-contributory.

Application forms:

Secretary,

Institute for Research on Animal Diseases,
Compton, Newbury, Berkshire.

Quoting Ref: 271.

269(A)

UNIVERSITY OF JOS, JOS, NIGERIA VACANCIES

In the Faculty of Medical Sciences

Applications are invited from suitably qualified candidates for appointments in the Department of Anatomy, Faculty of Medical Sciences of the University of Jos.

A. READER

Qualifications: Candidates should possess a good Medical Degree with postgraduate qualifications in either M.S., M.E., Ph.D. or D.Sc. (Anatomy). Teaching experience in Human Anatomy, Histology and Embryology in any recognised Medical Colleges/Universities at undergraduate and postgraduate levels for at least seven years is essential. Experience in administration and supervision of postgraduate research is desirable.

B. SENIOR LECTURER

Qualifications: Candidates should possess either M.S., M.Sc., or Ph.D. (Anatomy/Histology). Postgraduate teaching experience in Anatomy, Histology and Embryology in Medical Colleges/Universities for M.B., B.Sc. Classes for at least three years is essential.

C. LECTURER I

Qualifications: Candidates should possess postgraduate qualifications in either M.S. or M.Sc. (Anatomy). Postgraduate teaching experience in Anatomy, Histology or Embryology is desirable. Candidates with postgraduate qualifications in general surgery, that is M.Sc. Surgery or F.R.C.S. could be considered if they hold established teaching post in Anatomy in recognised Medical Colleges/Universities.

D. SALARY

Reader GL. 14—N8,868 by 320, N9,828 per annum.
Senior Lecturer GL. 13—N7,764 by 320, N8,724 per annum.
Lecturer I GL. 11—N6,444 by 180, N6,984 per annum.

E. CONDITIONS OF SERVICE

Successful candidates will be on probation for the first two years and afterwards confirmed to retiring age if their services are considered satisfactory. Other fringe benefits include pension scheme, car allowance, part furnished accommodation or housing allowance in lieu of accommodation. Twenty-five per cent contract addition is payable to all expatriates.

F. METHOD OF APPLICATION

Candidates should send their Curriculum Vitae in six (6) copies giving the following details; Name, Age, Nationality, Marital Status, Educational Institutions attended with dates, Work experience (i.e. post held, duration and salary earned), names of three referees at least two of whom should be competent to attest to the candidates professional/academic standing and characters. Candidates should request their referees to send their confidential reports under confidential cover to the address below. All applications should be sent to:

REGISTRAR,
UNIVERSITY OF JOS,
P. M. B. 2084,
JOS, NIGERIA

Applicants resident in the UK should also send a copy of their C.V. to the Principal Assistant Secretary (Recruitment) at the Nigerian Universities Office, 180 Tottenham Court Road, London W1P 9LE. 251(A)

UNIVERSITY OF WALES university college of swansea

SENIOR RESEARCH ASSISTANT

High Performance Semiconductor Oxides

A Senior Research Assistant is required for an S.R.C. grant investigating chemical changes in semiconducting oxides exposed to reducing environments, with the aim of developing gas detectors which will exhibit specificity to certain gases. Instruments which incorporate these detectors will be designed within the Electrical Engineering Department.

Applicants should have a good honours degree and preferably research experience in solid state chemistry or an immediately relevant subject.

The appointment is for three years, commencing as soon as possible and the remuneration will be up to £4,382 per annum plus USS/USDPs benefits.

Prospective candidates may contact Dr K. J. Gallagher of the Chemistry Department but further particulars and application forms (two copies) should be obtained from the Personnel Officer, to whom they should be returned as soon as possible.

RESEARCH DEMONSTRATORS

Applications are invited for two vacancies of Research Demonstrator in the Department of Genetics involving laboratory and tutorial teaching in Genetics. Candidates should have, or expect in the near future to have, a Ph.D. and be active in research. For one post (a) experience in evolutionary or human genetics, and for the other (b) experience in microbial genetics is desirable.

Appointment (a) is available from April 1, 1979, or as soon as possible thereafter, (b) from October 1, 1979. Both appointments will be on a scale up to £4,382 (under review) together with USS/USDPs benefits. Closing date for applications, Friday, April 13, 1979.

Further particulars for the above posts and application forms (2 copies) may be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea SA2 8PP, to whom they should be returned by the above dates. 256(A)

UNIVERSITY OF BRISTOL DEPARTMENT OF PHYSICS TECHNICIAN GRADE 5 £3,186 to £3,720 per annum (scale under review)

Applications are invited for the above post which falls vacant April 1979. The requirement is for a technician to assist in the teaching laboratory of a graduate course in Physics of Materials in which a range of mechanical testing equipment, optical microscopes, metallographic and vacuum apparatus, furnace and X-ray facilities are housed. The successful applicant, under supervision of a member of the academic staff, is responsible for maintaining this equipment and assisting in its demonstration to graduate students.

Preferred qualifications would be at HNC level or equivalent. Applications, stating age, experience and qualifications should be made to: Dr D. Dingley, H. H. Wills Physics Laboratory, Royal Fort, Tyndall Avenue, Bristol BS8 1TL, from whom further particulars can be

THE UNIVERSITY OF LEEDS DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a temporary post of

TEACHING ASSISTANT

In the above Department from candidates with suitable postgraduate or postdoctoral experience. The person appointed will participate in teaching biochemistry to science and medical students and will also be expected to engage in research in an area already under investigation in the Department. The appointment will be made with effect from October 1, 1979, for a fixed term of two years.

Salary at an appropriate point on the grade 1B Scale for Other Related Staff (£3,384 to £5,604) (under review), according to age, qualifications and experience. Application forms and further particulars may be obtained from The Registrar, The University of Leeds, Leeds LS2 9JT, quoting reference number 83/21/D. Closing date for applications, April 30, 1979. 258(A)

UNIVERSITY OF NAIROBI KENYA

Applications are invited for posts of SENIOR LECTURER IN DEPARTMENT OF METEOROLOGY. Applicants should have a Ph.D. in Meteorology with a minimum of three years research and teaching experience at a University level in at least one of the following branches: Dynamic Meteorology, Synoptic Meteorology, Climatology, Pollution Meteorology, Agrometeorology, Hydrometeorology, Physical Meteorology and Cloud Physics. The appointees teach under graduate and postgraduate courses and also organise and supervise research at postgraduate level. Salary scale K22,988 to K23,984 per annum (K2 equals £131 Sterling). The British Government may supplement salaries by £5,430 per annum (Sterling) for married appointees or £3,648 per annum (Sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowance and holiday visit passages SSSF or FSSU, family passages, medical and scheme, housing allowance, various allowances. Details applications (two copies) including curriculum vitae and naming three referees should be sent by air mail not later than May 3, 1979 to the Registrar, (Recruitment and Training) University of Nairobi, P.O. Box 3019, Nairobi, Kenya. Applicants resident in U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. 270(A)

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG CHAIR OF MICROBIOLOGY

Applications are invited for the Chair of Microbiology, within the School of Biology, in the Faculty of Science. The interests at present of this division of Microbiology are: (a) Industrial Microbiology, (b) Microbial Ecology, (c) Microbial Genetics, (d) Immunology and Virology. Applicant with experience in the above, or other microbiological specialist areas will be considered.

The salary allocated to the post is in the range R12,949 to R16,546 per annum, the initial salary to be determined according to qualifications and experience. A salary review is expected in April 1979.

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion or colour. Further particulars relating to this practice and to the above post are included in an information sheet obtainable from the London Representative, University of the Witwatersrand, Chichester House, 77A High Holborn, London WC1V 7HE or from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, to whom applications should be lodged.

BIRZEIT UNIVERSITY

An independent Arab University in the Israeli-occupied West Bank of Jordan has the following faculty openings for the academic year 1979/1980: Biology — Biochemistry, Chemistry (Physical, inorganic, organic), Mathematics (Algebra, Analysis, Mathematical Statistics), Physics (Experimental and Theoretical). English is the basic language of instruction and candidates should have a Ph.D. preferably or a master's degree with experience. Starting annual salary for Ph.D. is 2,700 Jordanian Dinars (current exchange rate, 1 J.D. = about £17). Optional summer teaching extra. Fringe benefits include: Relocation allowance (15% of salary), Transportation also provided. All applications should be addressed to Dean of the Faculty of Science, Birzeit University, Birzeit, West Bank Via-Israel.

26(A)

POSITION AVAILABLE

DIRECTOR, Institute of Animal Resources Ecology, University of British Columbia, position vacant as of July 1, 1979. The Institute is a postgraduate centre for teaching and research with emphasis on innovative approaches and multi-disciplinary constructs in ecological science and resource management. Because the Institute has strong roots in biology, the applicant must have understanding of biological sciences but need not necessarily be a zoologist or ecologist. He should be prepared to serve the Institute's main goal of innovative research that bridges gaps between traditional disciplines, methods and institutions. Applicants and curriculum vitae, including at least three references, should be sent to Dr A. R. Thompson, Director, Westwater Research Centre, University of British Columbia, 1933 West Mall Annex, Vancouver, B.C., Canada, V6T 1W5, to arrive not later than May 15, 1979.

W22(A)

**IMMUNOLOGY
POSTDOCTORAL
RESEARCH ASSOCIATE
DARTMOUTH
MEDICAL SCHOOL**

Hanover, New Hampshire, USA

Immunologists are invited to apply for a postdoctoral position to work with a group on a project concerned with the proliferation, maturation, and differentiation of T-lymphocytes. The emphasis is on the interaction of soluble regulatory factors with their target cells. Experience in cellular immunology, protein biochemistry, and/or radioimmunoassay is desirable. The position is for two to three years and is available after July 1, 1979. The salary is according to qualification and experience (\$12,000 to \$18,000/yr.). Applications including curriculum vitae, a description of research experience, relevant reprints and the names and addresses of two referees should be sent to Dr Kendall A. Smith, Dartmouth Medical School, Hanover, NH 03755, USA.

W21(A)

**BEDFORD COLLEGE
(University of London)
LECTURESHIP IN
GEOLOGY**

Applications are invited for a Lectureship in Geology from October 1, 1979 from persons with experience and research interests in the field of petrology involving mineralogy and geochemistry. It is expected that the successful applicant will be conversant with geochemical analytical techniques, particularly in the X-ray field, and will undertake the supervision of the Department's geochemical laboratories. Gross salary scale £4,477 to £8,752 per annum (under review). Particulars and application forms, returnable by April 29, are available from Senior Assistant Secretary (Personnel), Bedford College, Regent's Park, London



DEPARTMENT OF PHYSICS

Applications are invited for the post of

LECTURER

in the Department of Physics. Candidates should have an active research interest in Quantum Physics and experience with the use of fields and groups. The person appointed will be expected to interact effectively with the Theoretical High Energy Physics group and to play a full part in the Department's programme of teaching in theoretical physics.

Salary scale £3,883 to £7,754 per annum plus U.S.S. The initial salary will depend on qualifications and experience. Further particulars may be obtained from Mrs E. C. P. Sears, Staffing Section, The University Southampton SO9 5NH to whom applications (7 copies from United Kingdom applicants) should be sent not later than May 18, 1979. Please quote reference number 1403/A.

253(A)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF ORGANIC CHEMISTRY

Applications are invited for a temporary post of postdoctoral

RESEARCH FELLOW

in the above department to undertake work with Dr R. B. Herbert, on a project sponsored by the M.R.C. on the structure and biosynthesis of vitamin D derivatives in plants. Candidates should already possess or expect to obtain a Ph.D. degree in the near future. The appointment will be made for a fixed term of two and a half years from October 1, 1979, or from a date to be arranged.

Starting salary in the range £3,883 to £4,382 of the I.A. scale for Research and Analogous Staff (£3,883 to £6,555) (under review), according to age, qualifications and experience.

Applications (quoting reference number 45/9/D) should be made as soon as possible to the Registrar, The University, Leeds LS2 9JT (from whom further particulars may be obtained).

267(A)

**UNIVERSITY OF
WESTERN AUSTRALIA
Perth**

**SENIOR RESEARCH
OFFICER IN CLINICAL
IMMUNOLOGY
DEPARTMENT OF
PATHOLOGY**

This N.H. and M.R.C. funded position is intended for a science graduate with experience in immunopathology, biochemistry, immunology or cell biology to join a research team working on autoimmune disease with particular reference to contractile protein and receptor antigens, immunoregulation and histocompatibility. The Department also provides a comprehensive clinical immunology service. Salary range \$A15,786 to \$A20,737 per annum.

Applications in duplicate stating full personal particulars, qualifications and experience to reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia, 6009 by May 1, 1979. Candidates should request

CSIRO AUSTRALIA

Institute of Animal & Food Sciences

Chief of Division

**Division of Food Research
Sydney NSW**

Applications are invited for the position of Chief, Division of Food Research, from scientists who have an established record of personal research achievement and leadership in food science or related disciplines. The position has become vacant on the appointment of the former Chief, Mr. M. V. Tracey, to the position of Director of the Institute of Biological Resources, CSIRO.

CSIRO is Australia's largest and most comprehensive research organization, having approximately 7,000 employees of whom 2,400 are research and professional scientists. Its broad charter covers research into problems of primary and secondary industries and also such fields of community interest as human nutrition, the environment and the development and use of natural resources. The Organization has recently grouped its research activities in five Institutes and one Bureau, and the Division of Food Research is one of the Divisions within the Institute of Animal and Food Sciences.

The components of the Institute are the Divisions of Animal Health, Animal Production, Food Research, Human Nutrition, the Molecular and Cellular Biology Unit, the Wheat Research Unit and the Centre for Animal Research and Development in Indonesia.

The Division of Food Research has three main laboratories: the Food Research Laboratory in Sydney, NSW, the Meat Research Laboratory in Brisbane, Queensland, and the Dairy Research Laboratory in Melbourne, Victoria. There is also a Food Research Unit in Hobart, Tasmania. The headquarters of the Division are located in the Food Research Laboratory. The total staff of 338 includes 162 professional scientists.

The Division of Food Research conducts research programs in food science and technology directed generally towards improvements of the quality of fresh and processed foods both for Australian consumers and for export markets. Specific programs are concerned with the micro-biological status and safety of foods; the nutritional status, quality and structure of foods; the minor components of foods of sensory and physiological significance; and the development of improved procedures for the handling, storage, transport, and processing of foods in the commodity groups: meat, dairy products, seafoods, poultry, eggs, fruits and vegetables.

A Chief of Division is responsible for the leadership, development, scientific direction and integration of the research programs of the Division, and is encouraged to promote active collaboration with other Divisions, industry and research bodies working in associated areas. A recent review of the work of the Division of Food Research recommended the strengthening of relations with the Australian food industry.

The salary for the position is negotiable but will not be less than \$A34,374 per annum.

The terms of the appointment will be discussed with the short-listed applicants at interview. The appointee would be eligible to contribute to the Commonwealth Superannuation Scheme, subject to certain conditions.

Further information on the Division is in a booklet available on request from the Secretary, CSIRO, at the address below.

Dr. K. A. Ferguson, Director of the Institute of Animal and Food Sciences, and Chairman of the Appointment Committee, will be visiting North America in April/May 1979 and the United Kingdom in early June 1979 and will be available for discussions concerning the appointment with applicants and other interested parties. Those interested should contact either the Minister (Scientific), Australian Scientific Liaison Office, Canberra House, 10-16 Maitland Street, London WC2R 3EH, England, or the Counsellor (Scientific), Australian Embassy, 1601 Massachusetts Avenue NW, Washington DC 20036, USA. It may be possible to arrange such discussions at a number of points in the United Kingdom and the USA. Interested scientists should ensure that the appropriate overseas office is contacted in ample time to enable suitable arrangements to be made.

Applications stating full personal and professional details and names of at least two professional referees and quoting reference number 300/729 should reach:

The Secretary
CSIRO, PO Box 225
DICKSON ACT 2602
AUSTRALIA

by 27 April, 1979 (248A)

**Science Research Council
Daresbury Laboratory**

The Daresbury Laboratory has a vacancy in the theory group for a

SOLID STATE THEORETICIAN

The position is a permanent one at Higher Scientific Officer level.

The successful applicant will be expected to take a strong interest in numerical computations of relevance to experiments planned for the new facilities. Applicants should possess a Ph.D in solid state theory or expect to obtain one during 1979.

At the laboratory a purpose-built X-ray and ultraviolet source and a major nuclear structure facility based on a 30 MV tandem Van de Graaff are under construction. An IBM 370/165 computer is installed at the laboratory, and a computational science group has recently been established working mainly in the quantum chemistry area.

Appointments will be made at salaries between £4101 and £5448, the level at which the HSO is appointed depending on age, ability and experience.

Closing date: 1 May 1979

For further information please write to or telephone the head of the theory group, Dr. J.B. Pendry (Tel. No. (0925) 65000, Extension 223). Applications, from either sex, should be sent together with curriculum vitae and the addresses of two referees, quoting reference number DL/665/1 to:

The Personnel Officer

DARESBUY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

195(A)

**UNIVERSITY OF
SHEFFIELD**

TECHNICIAN (Grade 5)

required for the University Surgical Unit at the Northern General Hospital. The work of the department includes an interest in clinical nutrition and surgical metabolism. Applicants should possess an H.N.C. or equivalent qualification.

Salary on scale £3,186 to £3,720 per annum (under review).

Please write to the Administrative Officer (Personnel), (Ref. S1215/N), The University, Sheffield S10 2TN. 261(A)

**UNIVERSITY OF READING
DEPARTMENT OF ENGINEERING**

Applications are invited for a

LECTURESHP

in **ENGINEERING SCIENCE** for a fixed term of three years. Applicants should have a higher degree and/or appropriate postgraduate experience and should be interested in both the electrical and mechanical branches of engineering. Experience of the instrumentation and control of mechanical systems would be an advantage. The successful candidate would be required to lecture in the undergraduate courses and to take an active part in research.

The person appointed should take up duties on October 1, 1979.

Further information may be obtained from the Registrar (Room 214, Whiteknights House), The University, Whiteknights, Reading RG6 2AH, by whom applications should be received not later than May 11, 1979. 274(A)

**FREIE UNIVERSITÄT
BERLIN**

Klinikum Steglitz

**IMMUNOLOGY RESEARCH
UNIT**

Position open for M.D. or Ph.D. with experience in *Immunology*. Available for two years but can be extended. The appointee will have to work on mechanisms of lymphocyte activation and regulation of immune response by activated cells. Salary approximately £10,000 per annum (minus tax), depending on experience, age and family status.

Applications to Professor Diamantstein, Immunology Research Unit, Klinikum Steglitz, Freie Universität Berlin, 1 Berlin 45, Hindenburgdamm 27, Germany. 27(A)

**FACULTY POSITION
AVAILABLE IN RETINAL
HEMODYNAMICS AND
BLOOD FLOW STUDIES**

University of Illinois

Eye and Ear Infirmary
and

Abraham Lincoln School
of Medicine

The University of Illinois
at the

Medical Center, Chicago

Equal Opportunity/Affirmative
Action Employer

Applications are invited for a faculty position at the assistant or associate professor level. The major responsibility will be supervision of research of vascular flow in the retina, utilising a unique non-invasive computerised ophthalmoscopy system. Active interchange with engineers and clinical ophthalmologists will be fostered. An advanced degree (Ph.D. or M.D.) is a prerequisite.

Those interested should direct inquiries together with a curriculum vitae and bibliography to:

Morton F. Goldberg, M.D.
Professor and Head
Department of Ophthalmology
University of Illinois Eye and
Ear Infirmary
1855 West Taylor Street
Chicago, Illinois 60612

**THE EAST OF SCOTLAND
COLLEGE OF AGRICULTURE**

ANIMAL BREEDING SPECIALIST—GRADE III/IV

ANIMAL PRODUCTION ADVISORY AND DEVELOPMENT DEPARTMENT

(Ref: N 237)

Applications are invited for the above post in the Animal Production Advisory and Development Department. Applicants should have postgraduate experience in Animal Breeding and an interest in the problems and methods of on-farm livestock improvement. The post will particularly concern breeding and development work with dairy cattle and sheep, although advisory expertise will be required for all farm livestock.

Salary, on the scale for Grade IV or Grade III according to experience and qualifications, as follows: Grade IV, £2,998 to £3,942; Grade III, £4,178 to £6,898. Non-contributory pension scheme.

For further particulars and application forms write to: The Secretary, The East of Scotland College of Agriculture, West Mains Road, Edinburgh EH9 3JG. Closing date for return of completed applications April 20, 1979. 233(A)

**THE AUSTRALIAN
NATIONAL UNIVERSITY**

Applications are invited from suitably qualified persons for appointment to the following position:

**THE JOHN CURTIN SCHOOL
OF MEDICAL RESEARCH**

**DEPARTMENT OF
MICROBIOLOGY**

RESEARCH FELLOW

The appointee will work with Dr G. Air and Dr W. G. Laver on the molecular biology of influenza virus variants. The project involves selection of influenza virus variants with monoclonal antibodies, then immunological analysis of their proteins and nucleic acids.

The appointment, which is funded by a grant from the National Institutes of Health, U.S.A., is for one year in the first instance with probable extensions for a further two years. It is hoped that the successful applicant would be able to take up the appointment in the near future.

Salary: Commencing salary will be in accordance with qualifications within the range \$A15,786 to \$A17,716. Current exchange rates are \$A1 : 56p : \$US1.13.

Other Conditions: A limited grant towards travel and removal expenses may be made. Assistance with housing is provided for an appointee from outside Canberra.

The closing date for receipt of applications is April 12, 1979. They should be sent to the Registrar, P.O. Box 4, Canberra, ACT 2600, Australia. 229(A)

Theoretical Physicists

The South African Atomic Energy Board has its headquarters at the National Nuclear Research Centre, Pelindaba, some 20 miles west of Pretoria.

The recently established Plasma Research Group hopes to put the first local tokamak machine into operation during 1979. Similar apparatus elsewhere has yielded promising results, and the Group is hopeful that, with a machine of this nature, controlled fusion will soon be demonstrated as a usable source of energy for the future. Research is planned on the instabilities of the tokamak plasma by employing a number of diagnostic techniques. Furthermore the Board has for several years conducted research in the field of nuclear physics and has a team of theorists actively engaged in the fields of nuclear theory and elementary particle theory.

The Board has vacancies for physicists who are qualified to Ph.D. level in the above fields of research.

General

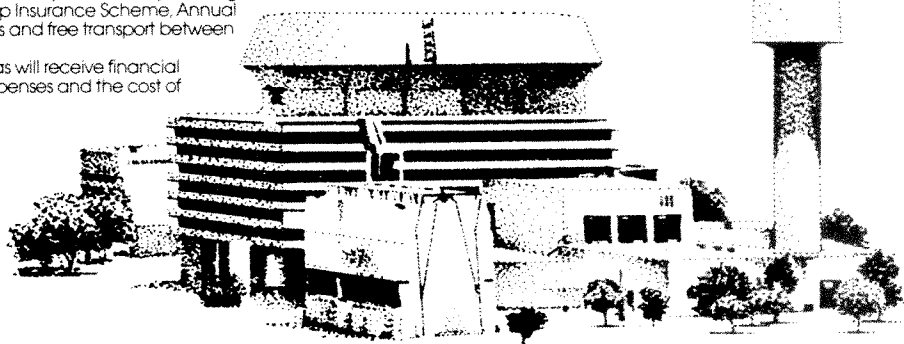
Salary offered will be in accordance with qualifications and experience. Fringe benefits include: Medical Benefit Scheme, Assisted Housing Loan Scheme (after one year's service), Housing Subsidy Scheme, Pension and Group Insurance Scheme, Annual Leave Bonus, ample leave privileges and free transport between Pretoria and the Board's site.

A person appointed from overseas will receive financial assistance in regard to travelling expenses and the cost of

transporting his furniture and effects including a motor car. He is required to make use of all assistance given by the South African Department of Immigration and the amount received from the said Department will be deducted from the financial assistance offered by the Board. He is also required to serve the Board for two years in consideration of the financial assistance received. A non-South African appointee must undertake to apply for South African citizenship as soon as he qualifies. In certain cases the Board is prepared to consider appointments on contract for periods of 3 years.

Written applications, giving full details of qualifications, experience, and present salary, etc. should be addressed to: The Head, Personnel Administration, Atomic Energy Board, Private Bag X256, Pretoria 0001, Republic of South Africa.

ATOMIC ENERGY BOARD PELINDABA



187(A)

UNIVERSITY OF SHEFFIELD RESEARCH TECHNICIAN (Grade 5)

required for duties in the Space Physics Group within the Department of Physics for an initial period of three years from April 1, 1979. The successful applicant will be primarily concerned with the design, development, operation and maintenance of complex and sophisticated apparatus, equipment and instruments used in research into phenomena associated with the propagation of very low frequency radio waves.

Qualifications or experience should be of H.N.C. or equivalent standard. A current driving licence is essential and duties may include some travel both within the U.K. and abroad for periods of up to several weeks.

Salary on scale £3,186 to £3,720 per annum (under review).

Please write to the Administrative Officer (Personnel), (Ref. S1214/N), The University, Sheffield S10 2TN. 193(A)

UNIVERSITY OF BIRMINGHAM DENTAL SCHOOL Department of Oral Pathology RESEARCH FELLOW

Postdoctoral Research Fellow required to continue a project into the development of *in vivo* methods for testing the toxicity of dental materials. Previous experience of cell culture and quantitative cytochemical techniques would be an advantage, but is not essential. The project is for three years and would be carried out in parallel with other *in vivo* projects currently in progress.

Salary on the scale £3,883 to £6,555, plus superannuation; maximum starting salary £5,129.

Further particulars available from the Assistant Registrar, The Dental School, St Chad's Queensway, Birmingham B4 6NN, to whom applications, including a full curriculum vitae and the names of three referees should be sent by April 30, 1979. 247(A)

UNIVERSITY OF BIRMINGHAM ASSISTANT SAFETY ADVISERS

Applications are sought for two new posts of Assistant Safety Advisers from science graduates or professionally qualified persons, preferably with experience of health and safety work. The duties will be concerned with the use, handling, storage and disposal, in the one case of biologically hazardous materials and in the other of hazardous chemicals including carcinogens, poisons and dangerous drugs. If necessary additional training will be provided.

Salary in the scale £6,317 to £7,754 per annum (under review) with superannuation benefits.

Further particulars and application forms available from The Senior Assistant Secretary, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom application should be made by May 7 1979.



SCIENCE BOOKS EDITOR

This is a new post in the book review Department of The Times Higher Education Supplement. The job will entail commissioning and editing reviews of academic science books, page layout and production work. Applicants should have a good degree and copy-editing or sub-editing experience.

Please send full career details to:

The Employment Manager (Ref: J/7)
Times Newspapers Limited
200 Gray's Inn Road
London WC1X 8EZ

276(A)

BEDFORD COLLEGE (University of London) LECTURER IN BOTANY

Applications are invited for a Lectureship in Botany, from October 1, 1979. Applicants should have a research interest in the field of higher plant physiology and have a Ph.D. degree of a British University or its equivalent; some postdoctoral experience would be an advantage. Gross salary range £4,477 per annum to £8,752 per annum (under review). Particulars and application forms (returnable by April 19, 1979) available from Senior Assistant Secretary (Personnel), Bedford College, Regent's Park London NW1 4NS (Tel: 01-486

BEDFORD COLLEGE (University of London) LECTURER IN ORGANIC CHEMISTRY

Applications are invited for a Lectureship in Organic Chemistry from October 1, 1979. Applicants should have a Ph.D. degree of a British University or its equivalent; some postdoctoral experience would be an advantage. Gross salary range £4,425 per annum to £8,700 per annum. Particulars and application forms (returnable by April 23) available from Senior Assistant Secretary (Personnel), Bedford College, Regent's Park, London NW1 4NS (Tel: 01-486 4400).

BIRKBECK COLLEGE (University of London)

Applications are invited for the post of LECTURER IN EXPERIMENTAL PHYSICS with special reference to either Solid State Physics/Electronic or Nuclear and Particle Physics. Initial salary up to £4,655 per annum on scale £3,910 to £7,754 per annum (under review) plus London allowance of £450 per annum. Further details and application forms should be obtained from the Secretary, Birkbeck College, Malet Street, London WC1E 7HX. Closing date: April 19. 234(A)



PLANT BREEDERS AND AGRICULTURAL SEED SPECIALISTS

of
KING'S LYNN AND CHESTER
invite applications from
AGRICULTURAL TECHNICIANS

As part of the continued expansion of our Research and Development activities and Plant Breeding programmes we intend to appoint the following qualified male or female Agricultural Scientists with post-graduate experience in Plant Breeding or closely allied subjects as well as some agronomists.

Applications are invited from persons who will be able to work within a team structure, be prepared to travel widely and who can be available to start work during 1979.

Salaries and terms will be relevant to age, previous experience and will be negotiable.

BASED AT DOCKING, NEAR KING'S LYNN, NORFOLK
Plant Breeding Laboratory Manager

The successful applicant will be required to take over existing projects related to quality screening in wheat and barley and to service the other requirements of the breeding programmes. In our view a broad biological background is essential, and experience in one or more research and development functions supporting plant breeding would be advantageous.

This is a senior appointment involving liaison with research and development in Sweden, control of other scientific staff and establishing the functions and staffing of this section of the business in new premises currently being adapted. Therefore serious consideration will be given to scientific officers who can adapt themselves to commercial life and accept the responsibilities which this post presents.

Plant Breeder

The successful applicant will be required to take over the present kale and rape breeding programmes and arrange trials of maize and other forage crops as well as assisting in all aspects of winter cereal breeding programmes.

Pure Stock Multiplier

The successful applicant should have considerable experience in the production of pure stocks of cereal seed, either in the commercial field or at a research institute or other station.

A degree is not a pre-requisite of this applicant but Official Crop Inspector's and Seed Sampler's qualifications are desirable, and a good knowledge of the Seeds Regulations a distinct advantage. This appointment will be in the Variety Stock Control Department and presents good prospects.

BASED AT FARNDON, NEAR CHESTER, CHESHIRE
Plant Breeder

An additional plant breeder is required to assist in the Spring barley breeding work. The person appointed will be involved in every aspect of the programme, including international co-operation.

Forage Crop Agronomist

The execution of Agricultural and Amenity herbage variety trials at Farnndon and elsewhere will form a major part of this job. Other duties will involve participation in the management of the Breeding Station Farm and liaison with herbage breeders in other countries.

All the above appointments will qualify for the Company's contributory Pension and Life Assurance Scheme as well as free B.U.P.A. membership, and other fringe benefits.

Applications, which will be treated in confidence, should in the first instance be made with curriculum vitae, references and details of present position and salary, to Mr. D. G. Stiles, Company Secretary, The Miln Marsters Group Limited, Waterloo Street, King's Lynn, Norfolk, PE30 1PA. 243(A)

UNIVERSITY OF OXFORD

DEPARTMENT OF ZOOLOGY
ANIMAL ECOLOGY RESEARCH GROUP

**APPOINTMENT OF A DEPARTMENTAL
DEMONSTRATOR IN ANIMAL ECOLOGY**

Applications are invited for the post of Departmental Demonstrator in Animal Ecology from October 1, 1979. The successful candidate, who should recently have received a Ph.D. or be in his final year before submission, will be required to take part in ecology teaching and to undertake research in animal ecology.

The appointment will be for three years in the first instance with the possibility of renewal for a maximum of a further three years. The salary will be on University Scale £3,384 per annum (pre-doctoral) rising to a maximum of £4,466 per annum with membership of U.S.S.

Applications, including the names of two referees, should be submitted to Dr J. Philipson, Animal Ecology Research Group, Department of Zoology, South Parks Road, Oxford OX1 3PS, by April 30, 1979. 224(A)

**UNIVERSITY OF
STIRLING**

BIOLOGY DEPARTMENT

**LECTURESHIP/SENIOR
LECTURESHIP**

A vacancy exists in the Biology Department at Lecturer/Senior Lecturer grade to be filled by September 1, 1979. The successful applicant is expected to be involved in the teaching of plant ecology including field courses. An interest in numerical techniques and/or genetics would be an advantage.

Salary scales: Lecturer £3,883 to £7,754; Senior Lecturer £7,506 to £9,263 (both under review).

Applications, together with the names of two referees should be sent to the University Secretary (N), University of Stirling, Stirling FK9 4LA, from whom further particulars may be obtained. Closing date: April 20, 1979. 273(A)

(University of London)

Keppel Street, WC1E 7HT

**ROSS INSTITUTE OF
TROPICAL HYGIENE
LECTURER IN**

ENVIRONMENTAL HEALTH

The successful candidate will join an interdisciplinary group working on the evaluation and planning of health care in developing countries. He or she will be required to work on environmental health in primary health care, and should have appropriate overseas experience and relevant post-graduate training. The post will carry teaching and research responsibilities at this School and overseas.

The appointment, which will be initially for a period of up to three years, will be made to the university lecturer salary scale (£3,883 to £7,754 plus £450 London Allowance) currently under review. Application forms, which should be returned by May 18, 1979, and further information are available from F. C. Ball, Assistant Secretary. 246(A)

**UNIVERSITY COLLEGE
LONDON**

ICRF TUMOUR IMMUNOLOGY
UNIT

**TECHNICIAN/
RESEARCH OFFICER**

with an interest in electron-microscopy and ultrastructural research to work initially on a project concerned with histamine release from mast cells. Experience in thin section cutting preferred, but not essential. Degree/H.N.C. plus laboratory experience required.

Salary range £3,261 to £4,902 plus London Allowance.

For application form please telephone Mrs Nelson, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2 on 01-242 0200, extension 305. 225(A)

**UNIVERSITY COLLEGE
LONDON**

DEPARTMENT OF
CHEMISTRY

**POSTDOCTORAL
RESEARCH ASSISTANT**

required from approximately October 1, 1979 to work in collaboration with Dr T. M. Cresp on the total synthesis of biologically active sesquiterpenes. Salary in range £3,909 to £4,158 plus £450 London Allowance: U.S.S. The post is tenable for one year with possible renewal for a second year. Applications, consisting of curriculum vitae and names of two referees, should be sent to Dr T. M. Cresp, Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ, U.K. 183(A)

**UNIVERSITY OF
EAST ANGLIA**

Norwich

CLIMATIC RESEARCH UNIT

**A CLIMATOLOGIST/
METEOROLOGIST/
ENVIRONMENTAL
SCIENTIST**

with statistical experience is required as soon as possible to work until March 1981 on a project concerning the identification of changes in variability and the frequency of climatic extremes in the European Sector. Qualifications: Ph.D. with preferably at least two years postdoctoral experience. Salary will be within the range £4,382 to £6,080 on the Grade 1A scale for Research and Analogous staff plus F.S.S.U./U.S.S. benefits.

Applications (one copy) giving full particulars of age, qualifications and experience, together with the names of three referees, should be sent not later than April 7, 1979 to Administrative Assistant, Climatic Research Unit, University of East Anglia, Norwich NR4 7TJ, from whom further particulars may be obtained. 205(A)

**UNIVERSITY OF SURREY
DEPARTMENT OF METALLURGY
AND MATERIALS TECHNOLOGY**

Applications are invited
for the post of Redland

RESEARCH FELLOW

tenable in the Department of Metallurgy and Materials Technology at the University of Surrey.

The research programme carried out by the Fellow will relate closely to the research and development interests of Redland Limited, and the Fellow normally carries out a number of subsidiary investigations requested by the Company's Research and Development Centre. The post affords an excellent opportunity for the incumbent to familiarise himself with industrial research and to make the transition from academic to the industrial environment.

Applicants should have a Ph.D. or equivalent experience in some area of material science, or solid state chemistry. Preference may be given to candidates with interests or experience in silicate chemistry, Portland cement and ceramics. In view of the nature of the appointment, candidates having the ability to formulate new experimental approaches to unresolved problems would be preferred. The Fellow appointed will undertake research in materials of interest in construction and building and the main concern will be with property structure relationships of interest in developing new or improved materials.

The appointment would be made for two years in the first instance, renewable thereafter on a yearly basis.

Remuneration will depend on age and experience and will be in the range £3,883 to £5,129 (under review), on scales related to the University lecturers' scales. Applications, from men and women, in the form of a curriculum vitae, with the names and addresses of three referees, should be sent to: Professor J. E. Bailey, Department of Metallurgy and Materials Technology, University of Surrey, Guildford, Surrey GU2 5XH. 206(A)

**UNIVERSITY OF EXETER
DEPARTMENT OF PHYSICS**

Applications are invited for a post of
LECTURER

in Physics, tenable from October 1, 1979. The research field of interest is Medical Physics. Preference will be given to applicants with experience in hospital departments.

Salary on the Lecturers' scale £3,883 to £7,754 p.a. (under review).

Further particulars from Miss Doelen Watson, University of Exeter, Northcote House, Exeter, to whom applications (8 copies) should be sent by April 24, 1979. Please quote reference No. 3199. 208(A)

UNIVERSITY OF MALAWI CHANCELLOR COLLEGE

Applications are invited for the post of

LECTURER IN PHYSICS

Applicants should have a good honours degree in Physics or in Electronics and preference will be given to those with a higher degree and with some teaching and research or industrial experience. The appointee will be expected to teach at all levels of the five-year honours degree programme. Salary scale: Lecturer K3,500 to K6,600 per annum (£1 sterling equals K1.65). The British Government may supplement salary in range £3,450 to £3,654 per annum (sterling) for married appointee or £2,340 to £2,544 per annum (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. If no British Government supplement is available, the University may pay an addition of between K1,320 to K1,656 per annum (taxable in Malawi). Family passages; superannuation scheme; Gratuity 15 per cent to 25 per cent; biennial overseas leave; housing and various allowances. Detailed applications (two copies) with curriculum vitae and naming three referees to be sent to Registrar, University of Malawi, P.O. Box 278, Zomba, Malawi by May 5, 1979. Applicants resident in U.K. should send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 217(A)

UNIVERSITY OF MALAWI CHANCELLOR COLLEGE

Applications are invited for the post of

LECTURER IN ANALYTICAL CHEMISTRY

in the Department of Chemistry. Applicants should have an honours degree in Chemistry, and preferably a higher degree in analytical chemistry. They should also have had at least five years in the practice of analytical chemistry, preferably in industry. Salary scale: Lecturer K3,500 to K6,600 per annum (£1 sterling equals K1.65). The British Government may supplement salary in range £3,450 to £3,654 per annum (sterling) for married appointee or £2,340 to £2,544 per annum (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. If no British Government supplement is available, the University may pay an addition of between K1,320 to K1,656 per annum (taxable in Malawi). Family passages; superannuation scheme; Gratuity 15 per cent to 25 per cent; biennial overseas leave; housing and various allowances. Detailed applications (two copies) with curriculum vitae and naming three referees to be sent to Registrar, University of Malawi, P.O. Box 278, Zomba, Malawi by May 5, 1979. Applicants resident in U.K. should send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 213(A)

QUEEN MARY COLLEGE

University of London

PLANT BIOLOGY AND MICROBIOLOGY DEPARTMENT

Applications are invited for a

TEMPORARY LECTURESHIP

in Mycology or Fungal Plant Pathology for one year from September 1. Preference will be given to candidates with strong interests in Microbial Ecology. Salary on scale £3,883 to £7,754 per annum (under review plus £502 London Allowance. Please apply by letter, enclosing curriculum vitae, list of publications and names of three referees, to The Registrar (N), Queen Mary College, Mile End Road, London E1 4NS, not later than April 9. 214(A)

Science Research Council Daresbury Laboratory

COMPUTATIONAL SOLID STATE SCIENTISTS

The Theory and Computational Science Division of the SRC Daresbury Laboratory has vacancies for work in the computer simulation of solid state structures, both perfect and defective. It is intended that the programme of work be carried through in collaboration with ICI Corporate Laboratory, Runcorn and University groups principally at Oxford and University College London. The posts offered are as follows:

RESEARCH ASSOCIATE

This post is offered for a period of two years, on a salary scale of £3695 to £6216, depending on age, qualifications and experience. The successful man or woman, who will probably have a Ph.D. in Physics or Chemistry, will be expected to devote attention to the research aspects of the programme. The work will involve in part the construction and/or modification of large scale computer programs.

SCIENTIFIC OFFICER

This is a permanent position offered on a salary scale of £2839 to £4415, depending on age, qualifications and experience. The successful man or woman, who will have a good degree in Physics or Chemistry and possibly some post-graduate experience, will be responsible for providing support for University groups wishing to make use of the computer programs made available by the project, and will work in close liaison with the Research Associate mentioned above.

The laboratory has powerful computing facilities available, including an IBM 370/165 on site, and a link to two IBM 360/195 computers at the SRC Rutherford Laboratory.

The Theory and Computational Science Division is presently engaged on programmes in solid state and surface physics, the theory of molecular structure, X-ray crystallography and continuum processes in atoms and molecules.

Closing date: Tuesday 1st May 1979.

Further information can be obtained from Dr. V. R. Saunders,

Tel. Warrington 65000 (Ext. 204).

Please write or telephone Warrington 65000 (Ext. 467) for an application form, quoting Ref. No. DL/664/1 to:

The Personnel Officer

DARESBURY LABORATORY

Science Research Council

Daresbury, Warrington WA4 4AD

P96(A)

PURDUE UNIVERSITY RESEARCH ASSOCIATES THEORETICAL CHEMISTRY

Two openings exist for theoretical chemists to study molecular liquids and solutions with applications in organic chemistry. A Ph.D. in chemistry or physics and background in computing and molecular quantum or statistical mechanics are required.

Start anytime in 1979. Salary is \$12,000/year. Appointments are renewable.

Send vitae and names of references to: Prof. Wm. L. Jorgensen, Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, U.S.A. An equal opportunity/affirmative action employer. W19(A)

M.R.C. CLINICAL RESEARCH CENTRE

(Northwick Park Hospital)

Watford Road, Harrow, Middx. HA1 3UJ

TECHNICIAN required in the Division of Communicable Diseases to participate in a research programme to investigate changes in tissue antigens in association with human cancers. The work will be carried out in an immuno-chemistry laboratory and is suitable for those interested in training in precise methods of immunochemical quantitations and in methodologies including the purification of antigens and antibodies, radioimmunoassays and immunofluorescence.

Qualifications: H.N.C. or science degree

Salary: within the range £3,615 to £5,034 inc. London weighting. Further details and application form from Mrs J. Tucker-Bull. Please quote reference 108/2/4070. Closing date April 27, 1979. 190(A)

**THE AUSTRALIAN NATIONAL UNIVERSITY
JOHN CURTIN SCHOOL OF MEDICAL RESEARCH
DEPARTMENT OF IMMUNOLOGY
PROFESSORIAL FELLOW**

Applications are invited for a position of Professorial Fellow in the Department of Immunology, John Curtin School of Medical Research. An appointment will be made to undertake research either in the field of Cancer Biology or in an area of Developmental and Reproductive Immunology.

Applicants interested in research into Cancer should have a comprehensive background in Cellular Immunology, particularly in relation to the response of the immune system to cellular antigens. Applicants who have worked in Reproductive Physiology or Developmental Biology should have an interest in foetal/maternal interactions and in the developmental aspects of the immune system.

Closing date: **31 May, 1979.**

**RESEARCH SCHOOL OF BIOLOGICAL SCIENCES
DEPARTMENT OF POPULATION BIOLOGY
POSTDOCTORAL FELLOW**

Applications for the above position which will become vacant in January 1980, are invited from biologists with interests in chromosome banding techniques, gel-electrophoresis or electron microscopy. The successful applicant will join a small group investigating the genetic basis and consequences of divergence via chromosomal rearrangements in natural populations of grasshoppers. Particular emphasis will be placed upon the study of patterns of recombination in experimental hybrids, both at the cytological and genic level. The karyotype of the species complex under investigation contains an array of markers, including pericentric chromosomal rearrangements, variation in heterochromatin distribution and biochemical polymorphisms and involves all members of the genome. It is ideally suited to an integrated analysis of the recombination process.

Closing date: **30 June, 1979.**

SALARY

Salary will be in accordance with qualifications and experience within the range: Professorial Fellow \$A30,713 per annum; Postdoctoral Fellow—a fixed point between \$A15,786 and \$A20,606 per annum. Current exchange rates are: \$A1 : 56p. : \$US1.13.

TERM OF APPOINTMENT

Professorial Fellow, until age 65 with the option of retiring at any time after age 60; Postdoctoral Fellow, one to two years. A shorter term may be negotiated for applicants on leave from other institutions.

OTHER CONDITIONS

Reasonable appointment expenses are paid. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with housing is provided for an appointee from outside Canberra.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain the further particulars from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF. 271(A)

**UNIVERSITY OF MALAWI
CHANCELLOR COLLEGE**

Applications are invited for the following posts in the DEPARTMENT OF BIOLOGY:—

1. **LECTURER IN ANIMAL PHYSIOLOGY/BIOCHEMISTRY.** The appointee will be required to teach comparative animal physiology and molecular and cellular biochemistry courses at upper levels within the degree programme. Research in a number of important areas is possible and encouraged but must be suited to facilities available in a developing country.

2. **LECTURER IN GENETICS.** The appointee will be required to teach introductory, population and molecular genetics within the degree programme. Research is encouraged and considerable opportunities exist in applied aspects of this field. Consequently, preference will be given to applicants with interests and experience in applied genetics.

3. **LECTURER IN POPULATION ECOLOGY.** The appointee will be required to teach both introductory and advanced topics in ecology within the degree programme. It is hoped that he/she will be able to contribute to the Country's active research in limnology and fisheries. Thus, preference will be given to applicants who have strong interests and experience in aquatic ecology—particularly fisheries.

4. **LECTURER IN VERTEBRATE ECOLOGY.** The appointee will be expected to develop a teaching and research programme in vertebrate zoology. Special interests within this area such as systematics, behaviour, and wildlife management will be an advantage.

Applicants must have a postgraduate degree in the relevant fields and previous teaching and research experience in a developing country will be an advantage. Highly qualified applicants may be considered at Senior Lecturer level.

Salary scales: Senior Lecturer K5,500 to K8,000 per annum. Lecturer K3,500 to K6,600 per annum (£1 sterling equals £1.65). The British Government may supplement salaries in range £3,450 to £4,128 per annum (sterling) for married appointees or £2,340 to £2,976 per annum (sterling) for single appointees (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. If no British Government supplement is available, the University may pay an addition of between K1,640 to K1,690 per annum for Senior Lecturers, K1,320 to K1,656 per annum for Lecturers (taxable in Malawi). Family passages; superannuation scheme; Gratuity 15 per cent to 25 per cent; biennial overseas leave; housing and various allowances. Detailed applications (two copies) with curriculum vitae and naming three referees to be sent to Registrar, University of Malawi, P.O. Box 278, Zomba, Malawi, by May 5, 1979. Applicants resident in U.K. should send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 216(A)

**UNIVERSITY OF
MELBOURNE
SENIOR LECTURESHIP
(LIMITED TENURE) IN
OVERSEAS AGRICULTURE
(ANIMAL PRODUCTION)
in the
SCHOOL OF AGRICULTURE
AND FORESTRY**

The University's General Development Grant is funding a new three-year project called "Training and Research in Overseas Agriculture". This project provides for the appointment until April 30, 1982 of a Senior Lecturer in Overseas Agriculture who would contribute directly to the project by giving lectures and conducting research in some aspects of Animal Production of importance in developing countries, and participate generally in research and teaching in Animal Production, thus allowing present members of staff to contribute to the new project. The appointee will teach in courses offered to undergraduate and postgraduate students in agriculture and veterinary science.

Applicants should hold postgraduate qualifications in Agricultural or Veterinary Science and should preferably have had experience in a developing country. Applicants with specialist qualifications and experience in Animal Nutrition or Animal Genetics will be particularly welcome.

SALARY: \$A21,180 to \$A24,657 per annum.

Enquiries to Professor N. M. Tulloh, in the University.

Further information regarding details of application procedure and conditions of appointment is available from the Staff Officer, University of Melbourne, Parkville, Victoria, 3052, Australia, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications close on April 22, 1979.

237(A)

**ROYAL POSTGRADUATE
MEDICAL SCHOOL
University of London**

CLINICAL PHARMACOLOGY

SCIENTIFIC OFFICER required to work on a number of research projects, centred on hypertension. The projects will include radioligand binding studies of receptors, and catecholamine turnover. The candidate should possess a degree or equivalent in a related subject. Experience in general biochemical techniques is required and a working knowledge of at least one of the projects is desired.

Salary on scale £3,887 to £6,169 a year, starting salary dependent on experience.

Application form and further particulars may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS quoting reference number 20/103/N. 236(A)

**UNIVERSITY OF
DUNDEE
DEPARTMENT OF
PHYSICS**

Applications are invited for a two-year appointment as

RESEARCH ASSISTANT

to work in the above Department as a member of a research group directed by Professor W. E. Spear and concerned with the development of amorphous silicon devices.

The post would offer a challenging opportunity to someone with a good degree or professional qualification in physics or electrical engineering and with some research or development experience.

The starting salary will depend on qualifications and experience but could be in the region of £5,000 per annum (scale under review). The post is superannuable under U.S.S.

Applications in writing, quoting Reference EST/42/79J and containing a full curriculum vitae and the names of two referees, should be lodged by April 19 with The Secretary, The University, Dundee DD1 4HN. 197(A)

**UNIVERSITY OF
EDINBURGH
DEPARTMENT OF BOTANY
Applications are invited for a
POSTDOCTORAL
RESEARCH FELLOW**

to work with Dr. A. Trewavas on a three-year project supported by the A.R.C. The work will involve preparation of cDNA to cellulase mRNA to investigate the mechanisms of transcriptional control induced by auxin. Experience of techniques in this area of work would be an advantage but is not essential.

Salary is on the scale £3,883 to £6,555 per annum.

Applications (C.V. and two referees) may be made to Dr A. Trewavas, Department of Botany, The King's Buildings, Edinburgh, EH9 3JH, from whom further details may be obtained. Please quote Reference 5011. 115(A)

**LUDWIG INSTITUTE FOR
CANCER RESEARCH
(London Branch)**

(in association with the Royal Marsden Hospital and the Institute of Cancer Research, Sutton, Surrey)

UNIT OF MOLECULAR BIOLOGY
Applications are invited from suitably qualified and experienced

BIOCHEMISTS

to fill one or other of two appointments in the new Unit which will be established shortly under Dr P. S. Rudland. The principal focus of research will be on experimental aspects of mammary cancer.

For post (a), previous experience of protein and nucleic acid biochemistry is essential.

For post (b), previous experience of protein purification techniques and cell biology is essential.

Further particulars may be obtained from the address given below. Candidates for these appointments, which will commence in or before September 1979, should have gained appropriate postdoctoral experience which will be reflected by the salaries likely to be offered £5,129 to £6,080, plus London Weighting (equivalent to M.R.C. non-clinical Scientific Grade II). The appointments will be for an initial period of three years. University Superannuation Scheme available.

Apply with curriculum vitae and names of two referees to The Secretary, Ludwig Institute for Cancer Research (London Branch), The Haddow Laboratories, Royal Marsden Hospital, Belmont, Surrey SM2 5PX, before April 27, 1979. 166(A)

**UNIVERSITY OF BRISTOL
DEMONSTRATORSHIPS
IN GEOLOGY**

Applications are invited for two postgraduate University Demonstratorships in the Department of Geology. Successful applicants will be registered for a higher degree in one of the following research fields:

- (1) Metamorphic and geochemical studies in S.W. England or N. Norway.
- (2) Hydrogeology or engineering geology in S.W. England.
- (3) Submarine geology of the Western Approaches.
- (4) Lower Old Red Sandstone sedimentology, S. Wales.

The posts are tenable for three years from August 1, 1979. Duties will include twelve hours demonstrating per week. Salary £3,384 to £3,883 per annum (under review), with U.S.S. Superannuation. Preference will be given to applicants possessing a good honours degree with relevant experience and/or hold, or are expecting, a M.Sc. degree.

Applications, indicating field of research interest, to The Secretary, Department of Geology, Queen's Building, University of Bristol, University Walk, Bristol, BS8 1TR (from whom further particulars may be obtained), with names of two referees, by May 1, 1979. 242(A)

**UNIVERSITY OF
STIRLING
CHAIR OF EARTH
AND ENVIRONMENTAL
SCIENCE**

The successful applicant will lead teaching and research in a small but rapidly expanding team concerned with the earth's surface and man-environment relationships. He or she must be of high academic standing, with wide experience in the subject and broad interests so as to co-ordinate the activities of specialist staff and members of other departments contributing to the interdisciplinary teaching of Earth and Environmental Science. It is hoped the appointee will have a special interest in natural resources, land use or other applications of environmental science.

Further particulars are available from The University Secretary (N), University of Stirling, Stirling FK9 4LA, to whom applications should be made, together with the names of three referees, by April 27, 1979. 210(A)

**UNIVERSITY OF
NOTTINGHAM
SCHOOL OF AGRICULTURE
DEPARTMENT OF
APPLIED BIOCHEMISTRY
AND NUTRITION
POULTRY RESEARCH**

Opportunities exist in two areas: (i) food intake and the control of energy metabolism, and (ii) mechanism of the decline in shell quality with age, for research leading to higher degrees. Applicants must have, or expect to have, upper second class honours degrees, or better, in animal science (nutrition), physiology or biochemistry. Reply with a brief academic history and names of two referees to Dr K. N. Boorman, School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD. 202(A)

**MEDICAL RESEARCH
COUNCIL
LABORATORY ANIMALS
CENTRE
TECHNICIAN**

There is a vacancy in the Genetics Department for a technician to assist in the institution of systems of genetic monitoring of small laboratory animals. Experience in immunological and electrophoretic techniques would be an advantage. An H.N.C. or equivalent qualification is essential.

Incremental salary in the range £3,261 to £4,680 plus £354 London Weighting. Contributory superannuation scheme.

Applications to The Chief Technician's Office, Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM5 4EF. 244(A)

**THE
QUEEN'S UNIVERSITY
OF BELFAST
POSTDOCTORAL
FELLOW
IN CHEMISTRY**

Required to work in collaboration with Dr D. R. Boyd on the synthesis of optically active metabolites of polycyclic aromatic hydrocarbons. The post is available for one year from October 1, 1979 or such other date as may be agreed.

Salary range £3,885 to £4,385 per annum.

Applications with curriculum vitae and the names and addresses of two referees should be addressed to the Personnel Officer, The Queen's University of Belfast BT7 1NN, Northern Ireland. 198(A)

NUCLEAR STRUCTURE PHYSICISTS

The Daresbury Laboratory, an establishment of the Science Research Council situated in the north Cheshire countryside, has 2 vacancies for Experimental Nuclear Structure Physicists in the Nuclear Structure Division.

The successful men or women will be involved in the research programme on a 30 MV tandem electrostatic accelerator at present under construction.

The duties of the posts will be to carry out research in conjunction with university users on the Nuclear Structure Facility. In addition they will provide the overall co-ordination and scheduling of the scientific programme, organisation of effort within the Laboratory for supporting the programme, and will assess the future needs for instrumentation and other facilities.

These posts require scientists with a good knowledge of either nuclear structure physics or of a related field, so that a high level of expertise in nuclear structure physics could be gained rapidly. Successful candidates will have experience in designing and using scientific equipment, and preferably some experience in the organisation of the work of teams of physicists engaged on experiments.

Applicants should have several years of experience and possess good academic qualifications, preferably a good honours degree in a relevant discipline. Appointments will be made, according to age, experience and qualifications, on the following scales:

Higher Scientific Officer £4,101 - £5,448 (under review)

There is a non-contributory superannuation scheme, a generous leave allowance and a flexible working hours scheme.

Closing date: 1st June 1979

Please write, or telephone Warrington 65000 Ext. 467 for an application form quoting reference No. DL/666/T to:

The Personnel Officer

DARESBURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

194(A)

**UNIVERSITY OF WARWICK
LECTURESHIP IN
ENVIRONMENTAL
SCIENCES**

Applications are invited for a Lectureship in the Department of Environmental Sciences, tenable from October 1, 1979. The appointment will be the fifth academic post in a Department whose research largely concerns microbial ecology and biochemistry and biodegradation processes. Applicants should have interests in these or related fields. Initial salary within the first four points of the Lecturer scale: £3,883 to (£4,631) to £7,754 per annum (under review), with superannuation benefits. Further particulars and application forms from the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No. 34/A/79. Closing date for receipt of applications is April 27, 1979. 231(A)

**THE UNIVERSITY
OF MANITOBA**

The University of Manitoba invites applications for the position of Professor and Head of the Department of Statistics. Currently there are twelve faculty members in the Department which offers undergraduate and graduate programmes, and the laboratories and facilities provide a stimulating environment for research and teaching.

Salary will be appropriate to the qualifications of the appointee. The 1978-79 floor for full professors is \$27,540.

Canadian citizens, landed immigrants and others eligible for employment in Canada at the time of application are especially encouraged to apply.

Applications should be made as soon as possible, with a deadline of June 15, 1979, giving curriculum vitae, publication list and names of three referees to: Dr R. D. Connor, Dean, Faculty of Science, University of Manitoba, Winnipeg, Manitoba. W20(A)

**UNIVERSITY OF
ST. ANDREWS
DEPARTMENT OF
ZOOLOGY**

Applications are invited for a Lectureship in the Department of Zoology tenable from September/October 1979. The successful candidate will be a member of the Department of Zoology, with research facilities either at the Gatty Marine Laboratory or the Zoology Department, dependent on the candidate's own research. Preference will be given to an experimental biologist.

Salary at appropriate scale £3,883 to £7,754 (under review), starting salary not above £5,604, plus U.S.S.

Applications should preferably be in typewritten names of three referees to: The Personnel Officer, University of St. Andrews, St. Andrews, Fife, KY16 9SS. 198(A)

**THE UNIVERSITY
OF LEEDS**
DEPARTMENT OF PLANT
SCIENCES

Applications are invited for a
temporary post of
LECTURER

with a special interest in
AGRICULTURAL CHEMISTRY
in the above Department, available
from October 1, 1979. The
appointment will be made for a
fixed term of three years.

Candidates should have a good
background in chemistry and phys-
ics, preferably with an under-
standing of the function and fate
of pesticides. However, candidates
with other interests should not be
deterred from applying. The duties
of the successful candidate will in-
clude teaching agricultural chem-
istry to students in the agricultural
sciences and botany. He/she will
also be expected to develop an
active research programme. The
department has good research
facilities.

Salary on the scale for lecturers
£3,883 to £7,754 (under review),
according to age, qualifications and
experience.

Application forms and further
particulars may be obtained from
the Registrar, The University,
Leeds LS2 9JT, quoting reference
number 53/10D. Closing date for
applications April 27, 1979.

266(A)

**UNIVERSITY COLLEGE
CARDIFF**

Applications are invited for the
post of

RESEARCH ASSISTANT

in the Department of BIO-
CHEMISTRY. The successful candi-
date should have a good Honours
degree in biochemistry or a related
subject and will work with Dr J. Kay
on chicken papain as a rennet substi-
tute with studies on its production,
stability and activity. The project will
involve techniques such as enzyme
purification, affinity chromatography,
milk clotting assays and protein
structural investigations.

The appointment which will be for
one year, in the first instance, renew-
able for a further two years, is
financed by a grant from the Agricul-
tural Research Council. Salary range
£3,384 to £3,883 (under review).

Applications (two copies), including
a curriculum vitae together with the
names and addresses of two referees,
should be forwarded to the Vice-
Principal (Administration) and
Registrar, University College, P.O.
Box 78, Cardiff CF1 1XL. Closing
date April 30, 1979. Reference 1744

199(A)

**ASSISTANT PROFESSOR
DIRECTOR
INSTITUTE OF
MARINE SCIENCE
UNIVERSITY OF ALASKA**

The University of Alaska, Fair-
banks, invites applications for the
position of Director, Institute of
Marine Science and Division of Marine
Science. Applications should be sent
to the Registrar, University of Alaska,
Fairbanks, Alaska 99775. Further par-
ticulars and application forms may be
obtained from the Registrar, University
of Alaska, Fairbanks, Alaska 99775.

UNIVERSITY OF LEEDS
DEPARTMENT OF
CARDIOVASCULAR STUDIES
TECHNICIAN IN CHARGE

required to supervise cardiological
physiological techniques. The post
involves administration, quality
control, training of staff, and co-
ordination of routine diagnostic
technical investigations in two hos-
pitals, and requires an ability to
control a large technical staff (30
plus technicians) and be responsible
to hospital consultants. Experience
in the technique of cardiological
investigation is essential. Prior
enquiry to the department is en-
couraged. The level of qualification
looked for is H.N.C. or equivalent,
together with substantial relevant
experience.

Salary is on the scale £5,085 to
£5,517 (Grade 8B). Salaries are
presently under review.

Applications to: Professor R. J.
Linden, Department of Cardio-
vascular Studies, New Medical
School, University of Leeds, Leeds
LS2 9JT. 260(A)

**ASSISTANT:
STRUCTURAL GEOLOGY,
ETH ZURICH**

Applications are invited for a pos-
ition as Assistant to Professor Ramsey
to set up a laboratory for the study of
model simulations of tectonic struc-
tures. Applicants should hold a Ph.D.
or equivalent degree, and have a sound
background in computer techniques.
Some previous experience of physical
and numerical modelling would be
useful. The position starts September
1, 1979, initially for three years, with
the possibility of extension beyond this
period.

Further information in the nature
of the work, and the research activities
of the structural geology group will be
sent on request. Applicants should
send a curriculum vitae, and the
names of two referees to Professor
J. G. Ramsay, Geol. Inst. ETH, ETH-
Zentrum, 8092 Zurich. 28(A)

**UNIVERSITY OF
NEWCASTLE UPON TYNE**
CHAIR OF
INORGANIC CHEMISTRY

Applications are invited for the
Chair of Inorganic Chemistry from
candidates whose teaching and research
interests lie broadly within that field.
The appointment carries with it the
Headship of the Department for an
initial period of five years. Salary will
be on the professional range. Member-
ship of a University superannuation
scheme will be required.

Further particulars may be obtained
from the Deputy Registrar, University
of Newcastle upon Tyne, 6 Kensington
Terrace, Newcastle upon Tyne NE1
7RU, with whom applications (15
copies) giving the names of not more
than three referees, should be lodged
not later than April 20, 1979. (Appli-
cants from outside the British Isles
may submit one copy only.) 254(A)

**THE UNIVERSITY
OF MANCHESTER**
DEPARTMENT OF
GEOGRAPHY

Applications are invited for a two-
year RESEARCH ASSISTANT post
concerned with glacioclimatological
studies in Norway and Switzerland.
Applicants should hold, or expect this
summer to obtain, a first or upper
second class degree in earth sciences,
physics or chemistry. Opportunities to
register for a higher degree. Post
tenable from October 1. Salary range
per annum £3,384 to £3,634. Particu-
lars and application forms (returnable
by April 9) from the Registrar, The
University of Manchester, M13 9PL.

Computer Manager

The Public Health Laboratory Service Board invites
applications from suitably qualified candidates with
enthusiasm and drive for the position of Manager to
take charge of a newly-formed Data Processing Unit.

The Board provides a microbiology service at 52
laboratories throughout England and Wales, at the
Central Laboratory, Colindale, N.W.9, and from
April 1, 1979, at the new Centre for Applied Micro-
biology & Research, Porton Down, Wiltshire. A
number of computer projects already operate and these
are to be brought under centralised control together
with future developments. A CTL computer is now
being installed at Porton Down.

The Manager will be responsible for planning the
development of a coherent, organised service. He/she
will be based at Colindale though at first, during the
commissioning and programming of the new computer,
much time will be devoted to the unit at Porton Down.

The successful candidate is likely to be a graduate
with sound experience of modern computing methods.
He/she, while responsible to the Board's Headquarters
officers, will work with senior scientific and medical
staff of the different units.

The appointment will be under N.H.S. terms and
conditions of service with a salary on scale 23, £6,955
to £8,423 plus £354 London Weighting.

A copy of the job description may be obtained from
Dr R. A. Bassett, P.H.L.S. Board, 61 Colindale
Avenue, London NW9 5EQ (Tel: 01-200 1295) to
whom applications with full career
details and names and addresses of
three referees should be sent by April
23, 1979. 189(A)

**PH
LS**

**Public Health Laboratory
Service Board**

AGRICULTURAL RESEARCH COUNCIL

Institute for Research on Animal Diseases

SCIENCE COMPUTER PROGRAMMER

required to join Statistics Section involved in the development of database
management and statistical computer systems. The successful applicant
will work with statisticians in the section and collaborate with research
staff. The Institute has remote links to an ICL 4/70-72 computer at
Rothamsted Experimental Station.

Qualifications: degree, HNC or equivalent, with experience in computer
science, mathematics or statistics, at least 5 years' relevant experience
for appointment to HSO.

Salary scales: Scientific Officer £2,839 per annum to £4,415 per annum
or Higher Scientific Officer £4,101 per annum to £5,448 per annum
depending on qualifications and experience. Non-contributory pension
scheme.

Application forms:

Secretary,

Institute for Research on Animal Diseases,
Compton, Nr. Newbury, Berks. RG16 0NN.
Quoting Ref: 346.

271(A)

FUNGUS BOOK AUTHOR needs
enthusiastic assistant to do field work
and text research. Applicant needs
degree, and driving licence. London
based. £3,000 p.a. Please reply to Box
No 249(A), c/o Macmillan Journals,
3 Dymally Buildings, Holborn EC1N
2NR. 249(A)

RESEARCH GROUP requires labo-
ratory scientific officer to assist
genetic and cell biology work
involving tissue culture. Candidates
with relevant experience preferred. Ap-
plications should be sent to:
Administrative Assistant, Paediatric
Research Unit, The Prince of
Wales Research Laboratories, Guy's Hosp
Medical School, London Bridge, S

MEDICAL RESEARCH COUNCIL

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

Applications are invited for a three-year POSTDOCTORAL appointment in the DIVISION OF PARASITOLOGY, available from October 1, 1979. The successful candidate will be required to develop monoclonal antibody techniques for the analysis of antigens of malaria parasites. There is particular interest in parasite antigens present in the erythrocytic membrane, and in isoantigenic changes in this membrane resulting from the presence of the parasite. Experience in monoclonal antibody techniques and/or immunochemistry or membrane biochemistry desirable.

The appointment will be in the salary range £4,408 to £6,080 per annum according to age and qualifications, plus £450 per annum London Allowance. Superannuation provision.

Applications, giving details of qualifications, experience and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA before April 30, 1979, quoting reference P/1. 275(A)

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL

in association with Chelsea College and the Royal Dental Hospital of London School of Dental Surgery (University of London)

LECTURER/SENIOR LECTURER IN BIOCHEMISTRY

Particulars available from the Establishment Office, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, to whom applications, with the names of three referees, must be sent to arrive by April 23, 1979. 245(A)

UNIVERSITY OF ABERDEEN CHAIR OF ZOOLOGY

Applications are invited for the above Chair which falls vacant on January 1, 1980. Preference may be given to Zoologists with experience in the experimental laboratory approach to animal biology. The successful candidate may be called upon in due course to exercise the responsibilities of Head of the Department of Zoology.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by April 30, 1979. 223(A)

WELSH PLANT BREEDING STATION UNIVERSITY COLLEGE OF WALES, ABERYSTWYTH N.E.R.C.

RESEARCH STUDENTSHIP

Applications are invited for a N.E.R.C. Research Studentship tenable for three years from October 1979 on ONE of the following research topics:

1. Variation within and between grass species in reaction to environmental stress (Supervisor: Dr A. H. Charles).

2. Ecological genetics of natural populations of perennial ryegrass (Supervisor: Dr M. D. Hayward).

The candidate, who should possess or expect to gain a good Honours degree in a relevant discipline, will be registered with the University of Wales for a higher degree.

Applications, including the names of two referees, should be sent before April 18 to The Secretary, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB from whom further particulars can be obtained. 238(F)

UNIVERSITY OF NOTTINGHAM

RESEARCH STUDENTSHIP

in Laser Spectroscopy of short-lived molecules

Applications are invited for a three-year Research Studentship tenable from October 1, 1979. The successful candidate will be expected to read for a Ph.D. The research project involves laser spectroscopy of gaseous molecular ions, and laser-induced reactions of ions. Emphasis will be placed on problems of astrophysical and atmospheric interest. Applicants should possess or expect to obtain a 1st or upper second class Honours degree in Chemistry or Physics, and should write to Dr P. J. Sarre, Department of Chemistry, University of Nottingham, University Park, Nottingham, by April 20, 1979. 207(F)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL STUDIES SCHOOL OF AGRICULTURE,

SUTTON BONINGTON, LOUGHBOROUGH, LEICS.

N.E.R.C.

RESEARCH STUDENTSHIP

Applications are invited from suitably qualified students for a N.E.R.C. studentship tenable from October 1st 1979. Candidates should have or expect to receive at least an Upper Second Class Honours degree in the Plant Sciences. The project is concerned with plant adaptation and colonisation in relation to nitrogen supply. The work will be supervised jointly by Professor W. J. Whittington and Dr G. C. Blackwood from whom further details can be obtained at the address given above. 201(F)

UNIVERSITY OF LONDON KING'S COLLEGE DEPARTMENT OF ZOOLOGY

N.E.R.C. STUDENTSHIP

Applications are invited from biologists holding or expecting to obtain a first class or upper second class honours degree for a N.E.R.C. Postgraduate studentship for research on:

(a) The epidemiology of rodent pipriplasmiasis (Professor F. E. G. Cox).

(b) The parasitic relationship between gall forming arthropods and their tree hosts (Dr B. D. Turner).

Further particulars and application forms may be obtained from the Secretary, Department of Zoology, King's College, Strand, LONDON WC2R 2LS, quoting reference 191667/N. 182(F)

UNIVERSITY OF YORK

DEPARTMENT OF BIOLOGY

POSTGRADUATE STUDENTSHIP

Applications are invited for a N.E.R.C. supported postgraduate studentship in the Department of Biology. One research area for which the studentship may be awarded is the investigation of a B-chromosome polymorphism in populations of *Allium schoenoprasum*.

Applicants should have, or expect to obtain, a first or upper second class honours degree.

Further details are available from Dr S. M. Bougourd, Department of Biology, University of York, Haslington, York YO1 5DD. 255(F)

WELSH PLANT BREEDING STATION UNIVERSITY COLLEGE OF WALES, ABERYSTWYTH A.R.C.

RESEARCH STUDENTSHIP

Applications are invited for an A.R.C. Research Studentship tenable for three years from September/October 1979 on ONE of the following research topics:

1. Competitive interactions in herbage crops (Supervisor: Dr J. Hill).

2. Cold hardiness in forage grasses (Supervisor: Dr C. F. Eagles).

The candidate, who should possess or expect to gain a good Honours degree in a relevant discipline, will be registered with the University of Wales for a higher degree.

Applications, including the names of two referees, should be sent before April 16, 1979 to The Secretary, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB, from whom further particulars can be obtained. 239(F)

ASSISTANTSHIPS

THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF MICROBIOLOGY

POSTDOCTORAL

RESEARCH ASSISTANTSHIP

Applications are invited from men and women for a Postdoctoral Research Assistantship financed by the S.R.C. to work on the mechanism of methanol assimilation in yeasts, involving isotopic work with whole cells and purification and study of enzymes. Applicants should possess or expect soon to possess a Ph.D. in microbiology or biochemistry. Tenable immediately until March 31, 1982 but consideration will be given to applicants expecting to complete their Doctorates by October 1979. Salary up to £4,382 a year on Range 1A. Particulars from Professor J. R. Quayle, Department of Microbiology, University of Sheffield, Sheffield S10 2TN, to whom applications, quoting two referees, should be sent by April 9, 1979. Quote ref: R 268/G. 268(P)

STUDENTSHIPS



THE UNIVERSITY OF ASTON IN BIRMINGHAM

DEPARTMENT OF GEOLOGICAL SCIENCES

N.E.R.C. RESEARCH STUDENTSHIPS

Applications for the above studentships leading to a Ph.D. degree are invited in the following fields:

- Structure, stratigraphy and litho-geochemical haloes around mineral deposits at Parye Mountain, Anglesey (CASE Award).
- Metal sulphide ores—studies of the rates and mechanisms of exsolution processes.
- Microstructure and microchemistry of heterogeneous meteorites (CASE Award).
- Engineering geological mapping in the Birmingham area.

Applications and requests for further details to be sent to Professor D. D. Hawkes, Department of Geological Sciences. 262(F)

The University of Aston in Birmingham

Gosta Green

Birmingham B4 7ET

MEDICAL RESEARCH COUNCIL

Clinical and Population Cytogenetics Unit

Applications are invited for graduates with good honours degrees and from prospective graduates for

MRC RESEARCH STUDENTSHIPS

that have been requested for October 1979. Awards will be in the fields of meiosis immunoglobulin production *in vitro* and computer pattern recognition. Studentship will be for a maximum period of 3 years and successful applicants will be expected to register for the degree of Ph.D. at the University of Edinburgh.

Application details and summaries of the projects are available from the Administrative Officer, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU. 279(F)

ASSISTANTSHIPS—cont.

UNIVERSITY OF DURHAM
DEPARTMENT OF CHEMISTRY
Applications are invited for chemists
or chemical physicists for an
**S.R.C. POSTDOCTORAL
SENIOR RESEARCH
ASSISTANTSHIP**

for sixteen months from as soon as possible, to work in collaboration with Dr J. Yarwood on the interaction and dynamics of hydrogen bonded systems in the liquid phase. These studies, which include infrared, far-infrared, Raman and neutron scattering studies are also supported by a N.A.T.O. research grant and would be carried out at Durham, the Technical University, Braunschweig and at A.E.R.E. Harwell. Some experience in one branch of molecular spectroscopy is essential and an interest in the chemical physics of molecular motions and interactions in the liquid phase would be an advantage.

Initial salary £3,883 or £4,133 per annum on National Research Range 1A plus superannuation.

Applications (three copies) naming three academic referees should be sent by April 18, 1979, to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars can be obtained

278(P)

UNIVERSITY OF BRISTOL
DEPARTMENT OF
ZOOLOGY
**RESEARCH ASSISTANTSHIP
IN REPRODUCTIVE
NEUROENDOCRINOLOGY**

Applications are invited for a post-graduate research assistantship to work with an established research group on a project associated with the neural and neuroendocrine basis of seasonal breeding cycles in mammals. The post, which is financed by a grant from the Agricultural Research Council, is available from October 1979 and will last for three years. Candidates should have, or expect to obtain, a first or upper second class Honours degree. The commencing salary (scale 1B) will be £3,384 per annum plus U.S.S. (scale under review).

Further information may be obtained from Professor B. K. Follett, Department of Zoology, University of Bristol, Woodland Road, Bristol RS8 1UG. Applications, including the names and addresses of two referees, should be sent to him by April 13, 1979.

240(P)

UNIVERSITY OF
LEICESTER
DEPARTMENT OF GEOLOGY
**POSTGRADUATE
RESEARCH ASSISTANTSHIP
IN GEOPHYSICS**

Applications are invited for a post-graduate appointment to work on the development of model seismology for undergraduate teaching. The project will entail the development of a model for the examination of seismic wave propagation through simulated geological structures using seismic reflection and refraction techniques. It will also involve the development of processing programmes demonstrating procedures used in industrial and academic seismic data processing schemes.

The post is available for six months commencing July 1, 1979 and would suit a geophysics or physics graduate with a good honours degree, who is practically minded and has a strong interest in computing.

SALARY RANGE £3,884 to £4,133 per annum.

Applications (one copy) with curriculum vitae and the names of two referees should be sent to Dr P. K. H. McIntyre, Department of Geology, University of Leicester, Leicester LE1 7RH, before May 31, 1979.

Further particulars are available

UNIVERSITY OF
NOTTINGHAM
SCHOOL OF AGRICULTURE
SUTTON BONINGTON
LOUGHBOROUGH

**TWO
RESEARCH ASSISTANTSHIPS**

tenable from October exist in the Department of Agriculture and Horticulture. The work is supported by the Agricultural Research Council for a three-year period on the following topics:

1. Puberty and reproduction in the gilt (supervised by Dr D. J. A. Cole).
2. Voluntary feed intake in the growing pig (supervised by Dr D. J. A. Cole and Professor D. Lewis).

Candidates will have the opportunity to register for a higher degree. Salary £3,384 to £3,883 per annum.

Applications including a full curriculum vitae and the names and addresses of two referees should be sent to Dr D. J. A. Cole at the School as soon as possible 200(P)

COURSES

**SOUTH EAST THAMES
REGIONAL HEALTH
AUTHORITY**

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UNIVERSITY OF SURREY**

The Authority offers special training posts as Basic Grade Biochemists with the opportunity to study for an M.Sc. in Clinical Biochemistry.

Posts are available from October 1979 for a two year sandwich M.Sc. Course at the University. In the case of fresh graduates, a good honours degree, e.g. Biochemistry or Chemistry (or its equivalent) is essential.

During the parts of the course spent away from the University, students must work in a specified hospital laboratory in the area of the Authority. The certificate of proficiency will normally be issued to candidates successfully completing the two year course.

University application form together with the postgraduate prospectus, and separate Regional Health Authority application form and sponsorship details are obtainable from the Academic Registrar, University of Surrey, Guildford. Applicants must give past examination grades.

Closing date for receipt of applications. April 23 (Ref 953). 265(D)

SYMPOSIA

**FEBS SYMPOSIUM
ON DNA**

Liblice, near Prague,

Czechoslovakia

24-29 September 1979

Topics: Recombinant molecules of DNA; DNA-protein interactions, DNA recombinations *in vivo*, DNA repair

Information from Drs S. Zadravil and J. Sponar, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Flemingova nám.2, 166 10 Prague 6, Czechoslovakia

The closing date for applications is May 15, 1979 250(M)

FELLOWSHIPS

**UNIVERSITY OF
EDINBURGH**

Applications are invited for the William Gordon Sogge Brown Research Fellowship in Mathematics, tenable in the University of Edinburgh for one or two academic years from October 1, 1979. Stipend is in the Research Salary range 1A, which is equivalent to the Lecturer's scale. Applications (before April 30, 1979) should be addressed to Professor A. G. Mackie, Department of Mathematics, University of Edinburgh, James Clerk Maxwell Building, King's Buildings, Mayfield Road, Edinburgh EH9 3JZ, U.K., from whom further particulars can be obtained. Please quote Ref: 237(F)

UNIVERSITY OF
SOUTHAMPTON
SCHOOL OF BIOCHEMICAL
AND PHYSIOLOGICAL
SCIENCES
**POSTDOCTORAL
RESEARCH FELLOWSHIP
PHYSIOLOGY/
PHARMACOLOGY**

Applications are invited for a POSTDOCTORAL RESEARCH FELLOWSHIP IN PHYSIOLOGY. Candidates should have a scientific and/or medical qualification and research experience, preferably with publications, in an aspect of Physiology and/or Pharmacology. It would be helpful if the applicant had particular interest in cardiovascular and/or renal physiology and in the mechanism of action of angiotensin. The successful applicant will join research teams actively involved in transport studies renal physiology and hypertension and/or studies into the physiological effect of burns. The Research Fellow may be called upon to participate in research supervision and from time to time in a small amount of teaching of courses in both the Faculty of Science and in the Faculty of Medicine. Excellent facilities for research in all aspects of physiology, pharmacology, histology and histochemistry are available. The appointment is for one year in the first instance, is renewable and will commence as soon as possible. Further particulars are obtainable from Professor K. A. Munday.

Salary Range: £3,883 to £4,882 per annum (under review). USS benefits. Initial placing according to qualifications and experience. Applications giving date of birth, curriculum vitae and the names and addresses of three referees, should be sent by April 20, 1979 to: Mrs P. Vaughan-Smith, The University, Southampton SO9 5NH, quoting reference 1061/R.

162(E)

UNIVERSITY OF
GLASGOW
**RESEARCH FELLOWSHIP
IN
SOLID STATE PHYSICS**

Applications are invited for a University Postdoctoral Research Fellowship in experimental Solid State Physics. The current research interests of the Solid State group are the Physics of Amorphous Materials and Electron Physics. In the case of the latter subject the group has just received a large S.R.C. grant to purchase a U.H.V. Scanning Transmission Electron Microscope.

The Fellowship is tenable for up to three years. The initial salary will be from £3,883 to £5,367 per annum depending on age and experience, on Range 1A of the salary scales for Research and Analogous Staff (under review). Superannuation benefits will apply.

Applications stating age, academic qualifications, research experience and the names of two referees should be submitted not later than April 30, 1979, to Professor J. C. Gunn, Department of Natural Philosophy, University of Glasgow, Glasgow G12 8QQ, from whom further details of the research work of the Solid State Physics group may be obtained.

In reply please quote Ref. No. 4408/M. 263(E)

**THE UNIVERSITY
OF MANCHESTER**

Applications are invited from graduates or those expecting to graduate in 1979 for:

**RESEARCH STUDENTSHIP
IN SCIENCE AND MEDICINE**

Value as for S.R.C. studentships, renewable after first year.

Particulars and application forms (returnable by June 1) from the Registrar, The University, Manchester M13 9PL. Quote ref: 55/79/N. 106(F)

UNIVERSITY OF
GLASGOW
**RESEARCH FELLOWSHIP
IN PHYSICS**

Applications are invited for a number of University Postdoctoral Research Fellowships in Physics from persons whose research interests lie in the following fields:

Experimental Gravitation (Grav Wave Detection), High Energy Physics, Nuclear Structure, Theoretical Plasma Physics, Nuclear Structure.

The Fellowships are tenable for up to three years from October 1, 1979. The initial salary will be from £3,367 to £5,367 per annum depending on age and experience on Range 1A of salary scales for Research and Analogous Staff (under review). Superannuation benefits will apply.

Applications stating age, academic qualifications, research experience and the names of two referees should be submitted not later than April 1979, to Professor J. C. Gunn, Department of Natural Philosophy, University of Glasgow, Glasgow G12 8QQ, from whom further details of the research work of the Department may be obtained.

In reply please quote Ref. 4409/M. 264(E)

INSTITUTE OF CANCER RESEARCH

Postdoctoral Scientist required for three-year Fellowship in the Department of Cytogenetics and Immunogenetics, at Fulham Road, London SW3, to work with human premalignant and malignant cells *in vitro*, study will involve the genetics of cytogenetically marked cell populations maintained and manipulated in tissue culture and xenograft systems. Experience in tissue culture and cytogenetics essential. Salary in scale £3,883 to £6,080 p.a. (M.R.C. Scientific N Clinical) plus London Allowance £450 p.a. Applications in duplicate with the names of two referees to: Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 2B, quoting ref 300/G/3 204(A)

UNIVERSITY OF
BRADFORD
**RESEARCH FELLOW
in Visual Science**

Applications are invited for two-year Postdoctoral Fellowship for research on the Characteristics of Normal and Defective Colour Vision. The successful candidate will be attached to the new Chair in Visual Science and will work under the direction of Professor J. D. Moreland. It is hoped to make the appointment as soon as possible. Salary within range £3,883 to £6,555 per annum (under review). Superannuation. Further particulars and application forms (to be returned ASAP) from the Registrar, Post Ref VS/RF/R/X University of Bradford BD7 1DP West Yorkshire 192(E)

**THE WELLCOME
RESEARCH LABORATORY
Beckenham, Kent
POSTDOCTORAL
IMMUNOLOGIST**

Applications are invited for a 1 year research fellowship suitable for postdoctoral scientist. The research project is oriented towards the study of the mechanisms of MHC-restricted cell mediated cytotoxicity and of immune cell interactions in chicken with special reference to use of embryonally generated chimera. Applications, with brief details of qualifications and experience should be sent to Dr J. Ivanyi, Department of Experimental Immunobiology, Wellcome Research Laboratory, Langley Court, Beckenham, Kent B3BS. 226(E)

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Immunoferritin: Electron microscopic localization of the nicotinic acetylcholine receptor in membrane vesicles from *Torpedo California* (electric ray) organ tissue with Fab₂ fragments of antiacetylcholine receptor and subsequently with ferritin-conjugated second antibody. The section was stained for 20 sec with lead citrate. Ferritin molecules (arrowheads) cover the outer surface of closed membrane profiles. (Tarrab-Hazdai R, et al: *Proc Natl Acad Sci USA* 75 2497-2501, 1978.)

Immunoperoxidase: Localization of neuraminidase in the crypt bases of polymorphs in the stroma of human small intestine. The tissue section was incubated with antineuraminidase, followed sequentially with second antibody and peroxidase antiperoxidase. Peroxidase was demonstrated with 3, 3', 5, 5' diaminobenzidine. (Courtesy of MJ O'Brien, G.I. Research Laboratory, Mallory Institute of Pathology, Boston.)

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Cover picture

The Viking Orbiter 1 spacecraft has provided the first photographs of a cyclone system on Mars. Data collected by IR mapping sensors suggest that the clouds consist mainly of water vapour rather than the expected CO₂ ice particles. Details of the martian meteorology observations are reported on page 531. Wind effects on Mars as determined from Viking Orbiter and Lander results are detailed on page 533.

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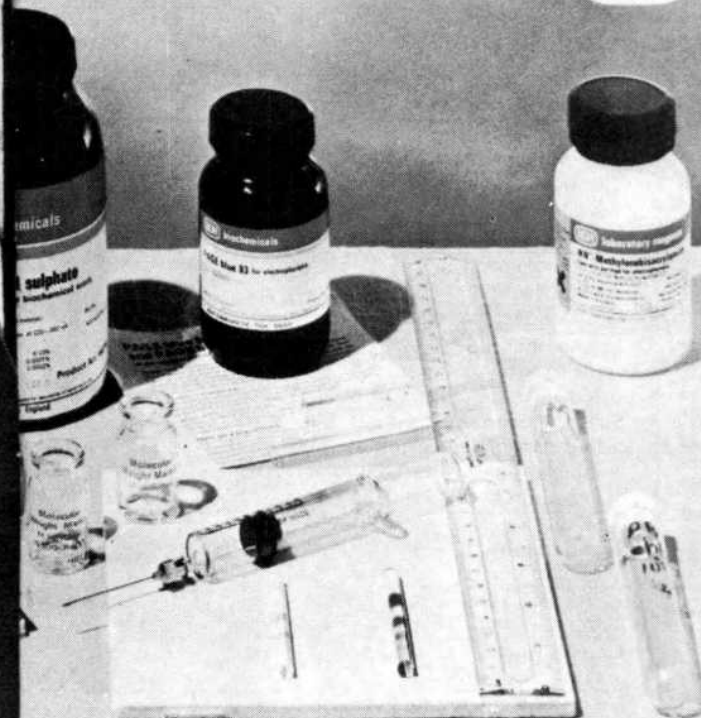
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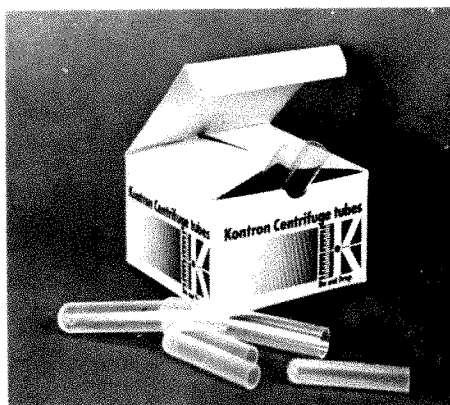
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5 April 1979

A quiet case for European science

WHATEVER issues the forthcoming election in Britain brings up, two things are reasonably certain: that the nation's place in the European Economic Community will be widely discussed and that science and technology policy will not. But, argues André Danzin in a newly published book *Science and the Second Renaissance of Europe* (Pergamon), the future of Europe is so much bound up in science and technology that if the public were kept adequately informed "scientific and technical policy would be expressed in terms of electoral concern".

What are we to make of Western Europe? Danzin, who is chairman of the European Committee for Research and Development (CERD) an advisory committee of twenty-one individuals, paints a very realistic picture of the deep problems, but also the immense potential of the region. The real resource is people and their cultural identity, but in recent years, limitations on space, materials, energy and money have dimmed the spirit. By almost any criterion, the nine of the EEC have failed to keep pace with the United States or Japan. (Danzin is excessively scrupulous about regarding the EEC as an entity, and almost never talks about individual nations; hence the economic and growing scientific strength of West Germany is neutralised by poor performances elsewhere in the community.)

He does not believe there is evidence that Europe is falling behind in its possession of knowledge; it is the "lack of concern" that is hobbling progress. And for Danzin, progress is undoubtedly closely tied for the foreseeable future to science and technology, and more specifically to the world of information. He shows a striking graph (admittedly from an American source) which demonstrates the colossal recent growth in numbers of those who could be said to be working in 'information' rather than agriculture, industry and personal services. It is estimated that more than 50% of the working population is now engaged in acquiring, storing, processing and transmitting information, or in working in allied support industries. And there is no sign that the trend which has lifted this figure from 15% to 50% in thirty years is yet over.

If there is every sign that information is becoming such an important commodity, and if there is no indication that Europeans are individually less well-endowed with intellectual capacity than, say, Americans or Japanese, why, Danzin asks, should Europe take the risk of being outstripped by its rivals "in the only specialised field where it is not seriously handicapped—brainwork and inventive capacity"? And yet R&D expenditure per capita in Europe is less than half that in the US, with signs of the gap widening; whilst in Japan (where financial comparisons are inappropriate owing to much lower unit costs of Japanese research scientists) the number of specialists working in

R&D as a fraction of the total population is double the European figure.

The troubles are not all simply in money and numbers of researchers, however. Danzin is highly critical of the sort of structures that individual European nations have evolved in the academic, bureaucratic and industrial world and which they now carry over into the machinery of the EEC itself. Rigidity, resistance to innovation, personal immobility; these are the constraints which prevent Europe responding flexibly to a changing world, and which contrast so unfavourably with American experience. For the businessman and industrialist the attraction of Europe ought to be, but as yet is not, the possibility of selling in a market larger than any other in the world. For the scientist and technologist it is the diversity of Europe which ought to be attractive—the many different traditions each bringing different skills and approaches to bear on problems. But this diversity can only be harnessed if there is an easy flow of people and ideas around Europe. With the exception of certain large projects, this cannot be said to be happening at present—if anything Europeans pair off with Americans rather than with fellow Europeans. Language is, of course, a barrier, and there is no sign (in Britain at least) of any improvement on this score. But it is more than language; there is much insularity of attitudes.

Danzin does not propose a crusade for the Europeanisation of science—his final chapter is entitled "Proposals for Mild Treatment". He notes that the research budget of the Community is only around 1% of the total for individual nations, but believes community-wide research has made encouraging progress, despite "pointlessly cumbersome" decision-making circuits. He would like to see the European research budget grow to a "substantial" level with a new executive body of more flexibility, able to take quick decisions and fund on a multi-annual basis. The new body would have not just to incorporate the existing European laboratories but also to stimulate them to take part in a "vast system of extra-mural exchanges".

Some will be disappointed that after such an astute analysis of the European problem, and after such a ringing declaration of the importance of science and technology, Danzin does not come up with some highly radical solutions. But this is the work of a realist; developments have to be evolutionary, and they have still to meet the approval of nine selfish governments, so proposals for a bonanza will get nowhere. On the other hand, a revolutionary change of attitudes costs nothing and can be effected almost overnight. A careful reading and re-reading of Danzin's quiet book by all of those concerned about the future of the EEC and its institutions would undoubtedly change a lot of minds. □

Recombinant DNA research: private actions raise public eyebrows

Rival scientific groups, often working towards common goals, share a close network of financial supporters.

David Dickson reports

LAST Thursday, the US Court of Customs and Patent Appeals confirmed its earlier decision that patent applications could be granted on two separate microorganisms, the one developed by scientists with General Electric, the other by Upjohn Co.

For the second time in 18 months, the court reversed a decision by the Patents and Trademarks Office refusing the patent applications on the grounds that the microorganisms were products of nature. The re-evaluation was made necessary by a Supreme Court ruling last June that the Appeals Court should reconsider its verdict in the light of a decision that a particular computer program was not patentable.

Three of the five Appeals Court judges, in repeating their previous position, said that they failed to see any connection between the computer program and the microorganism cases. And it could therefore be some time before a final verdict—the outcome of which is being awaited before patent applications are considered for a growing list of recombinant DNA inventions—is reached, since the Patent Office may well choose to take the case back to the Supreme Court for final clarification.

Whatever the outcome, however, growing confidence in the US business community that the development of recombinant DNA technologies promises large profits has led to a steady flow of venture capital to support research. No one has yet made very much money, but high commercial expectations have helped raise the value (on paper) of the five small private companies most deeply involved to a figure estimated at more than \$150 million.

In the rush for commercial pay-offs, however, some of the tactics used are beginning to raise various concerns on Capitol Hill, such as whether private industry's voluntary compliance with the National Institutes of Health's guidelines provides an adequate basis for ensuring the same degree of safety in private industry as the guidelines currently require in university laboratories.

Some of the issues are:

- Private corporations have already financed research by US scientists in various European laboratories to carry out experiments for which the facilities required by the guidelines at the time were not available in the US.

- A major pharmaceutical company is providing backing for two of the leading three teams investigating the possible production of human insulin by bacteria (and has tried—unsuccessfully—to gain a license to develop the results of the third team).

- A transnational recombinant DNA company has been set up by a Canadian multinational company with a group of leading scientists from Europe and the US. Capital for the company has been raised on both sides of the Atlantic, and the arrangement permits experiments to be located in the country offering the most acceptable environment for the research.

- As reported in *Nature* last week, at least one US company is already planning to conduct experiments involving more than ten litres of culture without receiving prior formal approval from the NIH, as the current guidelines require of federally-sponsored research.

The rapid growth of the small research companies, representing a direct marriage between university research workers and finance raised on the venture capital market, bears many similarities to the growth of the electronics industry ten years ago. Although most of the large pharmaceutical companies are building up their in-house capability to do such research, in most cases they have been less aggressive and slower to accept the commercial potential of the new technology than outside investors who are more prepared to take speculative risks.

In addition, rather than selling the licence to develop patents arising from their research direct to the major

companies, a number of scientists have preferred to set up their own commercial ventures, often using the money they have raised to licence the patents from work they have carried out on federally funded grants. "A fact of life is that the vast majority of talent in the field is in the universities at the present time," says Professor David Jackson of the University of Michigan.

Five relatively small companies currently occupy the centre of the stage. These are:

- Cetus Corporation, set up in Berkeley, California in 1971 on an initial capital of \$5 million, and currently carrying out research on a contract basis for industry in a number of areas, including biomedical, chemical, food and agricultural technologies. Cetus has opened a P3 facility, and its current value on paper is estimated by president Dr Peter Farley as around \$65 million. Almost half of this stock is owned by Standard Oil and National Distillers Company.

- Genentech, Cetus' rival across the bay in San Francisco, which claims to be "the leading company in the application of recombinant DNA techniques to problems in human health care". Set up in 1976 by Robert Swanson and Dr Herbert Boyer, Genentech is also interested in a wide range of applications, but has gained most publicity for its work on somatostatin and insulin.

- Biogen, a company incorporated in Luxembourg, which was set up last year by Mr Dan Adams, then head of the venture capital division of International Nickel. Biogen has nine scientist co-founders from both sides of the Atlantic, and has so far concentrated mainly on working towards pharmaceutical products such as insulin, interferon and a possible hepatitis vaccine. Adams estimates the current value to be about \$50 million and Biogen is currently constructing laboratories in Switzerland to P3 containment levels.

- Genex, established in 1977 by Dr Jackson of Michigan and two others. It is concentrating on industrial processes involved in, for example, the food and chemical industries. Genex plans to open a laboratory in Rockville, Maryland in the near future, and to announce a major deal with "one of the fortune 500 companies", within the next four weeks according to president Dr Leslie Glick.

- Bethesda Research Laboratories, set up three years ago and already a major



supplier of restriction enzymes. It started moving into recombinant DNA research last summer, and has opened a P2 facility also in Rockville (rapidly becoming the "route 128" of molecular biology, largely because of its proximity to NIH).

In addition, collaborative research, in Waltham, Massachusetts, which already supplies materials such as synthetic genes required for research to a number of the companies listed above, is now contemplating entering the field directly, according to its president, Dr Orrie Friedman.

At the scientific level, research groups supported by the various companies are frequently exploring different paths towards the same goals, characterised by the fierce—though usually friendly—rivalry common to all fields of science. Some of these goals include the production by bacterial means of insulin, interferon, growth hormone, and various industrial enzymes.

At the financial level, however, the situation is slightly different. Apart from Bethesda Research Labs, which is financed by a private family trust, the other four companies share some common financial backers in what one participant acknowledges to be a "somewhat incestuous" relationship.

For example, Kleiner and Perkins, an established West coast venture capital firm which supplied the backing for electronics companies such as Fairchild Industries, has provided substantial support for both Cetus and Genentech, although Dr Farley of Cetus says that the standard oil support now puts the company apart from the other three. Similarly, New Jersey, which receives its own funds from companies such as Aetna Life Insurance, Emerson Electric, and the Monsanto Chemical Company, has a stake in Genentech, and last year provided a substantial amount of capital to Genex. Mr Gerald Large, president of Innovent, is now a director of Genex.

Perhaps the most extensive involvement has been that of International Nickel, (INCO), a Canadian based mineral company, which set up a venture capital division operating from New York in 1975 to support "worthwhile entrepreneurial investment opportunities in Canada, the US and Europe." Soon after it began operations, the then head of the division, Mr Dan Adams, selected recombinant DNA technology as a fruitful field for investment "I felt that this was the most exciting field of technology for investment for the immediate future," he said last week.

In line with this conviction, INCO bought a small amount of stock in Cetus in 1976 valued at about \$500,000

and subsequently in 1977 bought a larger, 10% share in Genentech. Then in 1978, with advice from Genentech—(which is now demanding a share in the equity in return), and a scientific board brought together by Dr Walter Philip Sharp of MIT, INCO set up a Gilbert of Harvard University and Dr new company, Biogen, with Adams as president (Adams has since left both INCO and Biogen, but is looking for new areas to start a similar venture).

INCO was incorporated in Luxembourg, "which has some attractive tax advantages", according to Adams, and sponsors research in the laboratories of a number of members of its scientific boards, such as that of Dr Charles Weissman, of the Institute for Molecular Biology at the University of Zurich.

Financing provided largely by INCO through Biogen has already allowed Gilbert to carry out experiments into the production of human insulin at the Microbiological Research Establishment at Porton Down in the UK, since at the time the P4 containment facilities required by the NIH guidelines were not available in the US.

Of the major pharmaceutical companies, Eli Lilly has been the most aggressive in pursuing potentially valuable research results (as well as the most vocal critic of attempts to legislate basic research in the private sector). The company currently controls 80% of the \$140 million domestic insulin market, and is keen to maintain this position.

Last Autumn, Eli Lilly entered an agreement with Genentech clinched soon after scientists in the latter company announced they had produced human insulin from bacteria (although the insulin produced in this way has yet to be shown to be biologically active) under which the two companies will carry out a joint long-term manufacturing and marketing programme.

In addition, Eli Lilly has entered a separate agreement with Dr Howard Goodman and Dr Bill Rutter of the University of San Francisco in the Department of Biochemistry and Biophysics. The pharmaceutical company has provided over \$250,000 to support the laboratory's research into the production of human insulin, money which last year allowed research workers from the laboratory to carry out experiments in France which could not be done in the US.

In return for providing financial support, the company expects first refusal on the license to develop any patentable results which arise from the research.

In addition to the above two agreements, Eli Lilly recently bid unsuccessfully to purchase from Harvard University the rights to develop the patent filed by Gilbert for research

developed in connection with other research teams at Harvard (the British drug company Boots, also expressed interest in licensing the patent but Harvard decided that the patent should go to Biogen).

Within this complex of financial and corporate interests, those involved are taking pains to emphasise that all research—at least that which is carried out in the US, and at least with respect to the technical aspects of containment levels—is conducted in accordance with the criteria in the NIH guidelines.

For research outside the US, however, it is generally accepted—even by NIH itself—that these guidelines need not apply, strictly but that the research should be done in compliance with local requirements (making countries with relatively low requirements attractive).

However, evidence that companies are already more than willing to move abroad to carry out research that cannot be done in the US is beginning to worry US lawmakers, which have so far held back from legislating over the private sector. "This is certainly something we are keeping a close eye on" a staff aide to Senator Adlai Stevenson's science subcommittee said last week.

In return, companies are worried about a proposal by the Food and Drug Administration to introduce regulations that would require all research leading to products for which licenses are applied to have been conducted under the NIH guidelines. They are suggesting that, if the work is carried out abroad, the compliance should be with local guidelines. "It would be silly for FDA or NIH to try to police the world" said Adams.

Meanwhile other critics of existing policy and of the NIH sponsored decision processes are concerned that without legislation explicitly covering the private sector, there will be no way of guaranteeing the safety of the procedures that this sector adopts. In the wake of last week's nuclear power incident at Harrisburg public credibility of claims of minimal danger is running low (already scientists at Tufts University have demonstrated that a "disabled" E. Coli strain classified as EK2 is capable of surviving up to 90 hours in the human gut, far longer than was initially anticipated).

"The question remains who is going to control both the process and the products of this research," says Dr Susan Wright of the University of Michigan. "Given that great uncertainties still exist, and the fact that risk assessment experiments have not yielded uniformly negative results and have indicated some areas for renewed concern, it is important that Congress does all that it can to guarantee the safety of those involved." □

COGENE plays up benefits, plays down risks

IN A surprise statement last Sunday, the foreign secretary of the Royal Society, Mr Michael Stoker, announced the lifting of the ban on reporting of what promises to be the most important meeting on the risks and benefits of recombinant DNA techniques since Asilomar, the conference held in California in 1975 during which scientists called for strict control of the use of recombinant DNA techniques.

COGENE, the Committee on Genetic Experimentation of the International Council of Scientific Unions (ICSU), had previously issued a directive that the whole proceedings at Wye College, Ashford, Kent, UK would be off the record. The directive was the result of what was felt to be unbalanced press coverage concerning recombinant DNA risks, particularly after Asilomar.

Dr Stoker, however, when opening the meeting, asked participants to

"tear up that note". There had, he said, been a change of attitude on the organising committee. But the note had already had its effect—only three journalists were present and they were from specialist scientific magazines, including *Nature*.

We are therefore now able to report that COGENE is shifting its attention from risks to benefits. Supported by UNESCO and the World Health Organisation, it is planning to hold a series of two to three week teaching courses in recombinant DNA techniques in developing countries beginning in January 1980. COGENE chairman Dr Bill Whelan said: "We have the money and we just want to find a country that would appreciate our help." Recombinant DNA techniques could be of great benefit in Third World countries, Whelan believes, particularly in agriculture, where they could help in a rapid assessment of the

molecular genetics of local crops.

Meanwhile, however, COGENE is slowing up its attempts to assess the risks of the technology. "We have run out of good ideas for risk assessment experiments", said Dr Anna-Marie Skalka, who chairs the COGENE subcommittee on risk assessment. However, Professor Mark Richmond, a member of the UK's Genetic Manipulation Advisory Group (GMAG) who will report on his own assessment experiments at the meeting, believes that the real problem is finding willing researchers to do the work. "I took it up to show willing", he said, but the work takes up time, lab space and people especially when there is a wide feeling in the scientific community that the risks are minimal. "If the UK Medical Research Council wants people to assess risks it may have to force them to do it", he said.

Robert Walgate

GMAG states the position on 'self-closing'

At the end of last week, GMAG issued a statement designed to satisfy the "urgent need to clarify certain issues" and to remove "any uncertainty of ambiguity about present requirements". As expected some self-cloning experiments—those using *Escherichia coli* K12 and other well-characterised non-pathogenic laboratory strains of *E. coli*, *Bacillus subtilis*, *Bacillus stearothermophilus* and *Saccharomyces cerevisiae*—are exempted from statutory notification.

In general, however, self-cloning experiments are still considered to be within GMAG's remit. "The group is satisfied" says the statement "that many self-cloning experiments are without conceivable hazard", but it "interprets the definition of genetic manipulation to cover self-cloning experiments". It also acknowledges "that there has been uncertainty on this point and that some users have interpreted the definition to exclude such experiments". The definition of genetic manipulation used by GMAG is "the formation of new combinations of heritable material by the insertion of nucleic acid molecules produced, by whatever means outside the cell, into any virus, bacterial plasmid or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation".

In future, therefore, all self-cloning experiments with systems other than those specifically designated as exempt, should be notified to GMAG which "will advise on containment". Those working with exempt organisms are

asked to provide voluntarily "a limited amount of information about their plans to help (GMAG) keep in touch with all developments in the field". This should include "species and strains, the place of work and the scientists responsible".

GMAG is also seeking to amend regulations to cover the use of "products of genetic manipulation which are capable of self-propagation". Voluntary notification of the use of these products is requested so that GMAG can advise on containment. However, exemptions from this will include the products of those self-cloning experiments which are exempt and the insertion of *Xenopus* genes coding for 28S, 18S and 5S ribosomal RNAs into prokaryotic DNA. Users are invited to propose other products of recombinants which could be added to this list. Special consideration is also to be given to scaled up work involving the growth of self-propagating products in volumes of 10 litres or more. Arrangements for this type of work are still under discussion.

GMAG still feels unable, according to the statement, to accept some of the principles that the United States and other countries have adopted, following the NIH lead. Nevertheless it will study the NIH guidelines to see if there are some procedures which could be adopted in the UK in the hope that collaboration with other national authorities will bring about some uniformity of procedure.

GMAG's terms of reference are restated as advising all those concerned in any way with genetic manipulation,

continually assessing new genetic manipulation techniques (and new containment methods) and advising on appropriate action. It is also required to "maintain appropriate contacts with relevant government departments, the Health and Safety Executive and the Dangerous Pathogens Advisory Group", and to keep records of containment facilities and the qualifications of biological safety officers. □

UK minister criticises COGENE meeting

Mr David Ennals, UK Secretary of State for Health and Social Security, expressed his concern last week at the original intention to ban reporting of this week's COGENE meeting. "Basically, I'm opposed to secret conferences", he told the genetic engineering subcommittee of the House of Commons Select Committee on Science and Technology. "Far too much in the field of science is done behind closed doors".

The subcommittee was holding its first public session after a recent visit to the US. Mr Ted Leadbitter cited a recent US survey which had found that of 18 countries using recombinant DNA techniques, the US and the UK had the most stringent regulations. Might this encourage valuable work to go abroad, he asked? Mr Ennals said he thought the most important thing was to "watch the hazards carefully" if we did not want to upset the balance between risk and benefit. GMAG he thought was flexible enough to do this. □

European physicists discuss the "great projects"

A SELECT group of European physicists met in Rome last week to learn of Europe's plans for future "very large capital projects" in astrophysics, sub-nuclear physics, synchrotron radiation and nuclear fusion—projects which, the organisers say, could dominate research and development until the end of the century. They were there by invitation of the European Physical Society, whose president, Antonino Zichichi, had decided that the meeting was needed to inform key physicists with an interest in any one of the projects of the plans for the others. All the projects are likely to compete, at least to some extent, for future funds.

Professor L. Woltjer of the European Southern Observatory (ESO) headquarters in Geneva, told the meeting that after the present generation of ground-based single dish telescopes of diameter 3-6m, the future of Europe's optical astronomy will rest with the joint NASA/ESA Space Telescope, due for launch from the Space Shuttle in 1984, and a ground-based Very Large Telescope (VLT).

So far the latter is only an idea but it is being considered seriously by the ESO. The current plan is that it would have a diameter of about 16m, would probably be sited at the ESO's site in Chile and would become operational in 1990: its cost is estimated at about \$100 million. As it is unlikely that a single dish of 16m diameter could be built, the VLT would probably be a multi-mirror tele-

scope.

The Space Telescope and the VLT will be complementary, according to Professor Woltjer. The former will be used for gathering data on very faint sources which could never be observed by a ground-based telescope because of the masking effect of the Earth's atmosphere. However, because it is relatively small (2.4m diameter), the Space Telescope will be limited in the number of photons it can collect. The VLT with its large collecting area, will be capable of collecting many more photons and will therefore be suitable for detailed spectroscopic analysis of relatively bright sources.

In radio astronomy, Europe is already active in very long base interferometry (VLBI), the aim of which is to produce a two-dimensional map of radio sources. There are plans to extend the VLBI network by building more receivers and also by using one of the European Space Agency's future communications satellites as a reference point for phase and amplitude information. The French are looking at the possibility of using VLBI at optical wavelengths. A major problem however, is that the engineering tolerances of the receivers have to be very high because of the small wavelength.

Fusion scientists are also planning their next move. According to Professor Palumbo from Brussels, an international team met last January to start discussing the machine to follow the Joint European Torus (JET) which is

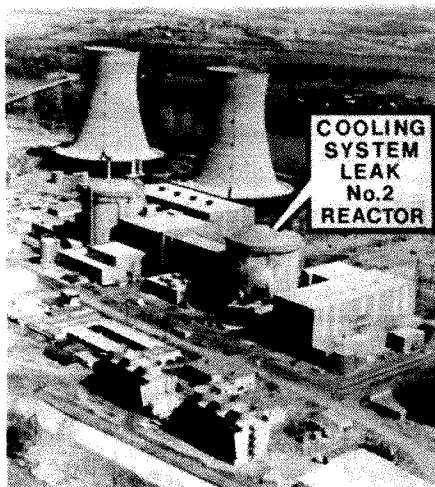
just getting underway at the Culham Laboratory in the UK. Just the week before the meeting, he said, the EEC had increased the budget for European fusion research by 20 million accounting units.

Two plans for major future particle accelerators were discussed. One is Europe's plan to build a large electron-positron storage ring (LEP) (see *Nature* 12 October 1978, page 469 and 14 December 1978, page 658), the other is the Soviet Union's plan to build a 3 TeV proton synchrotron. Work on the latter has been going on for "a long time" according to Professor Siderov of Novosibirsk who presented the project instead of Professor K. P. Myznikov of Moscow who did not attend the meeting. He could not say when the machine would be built or how much it would cost, but seemed confident that it would be built at some time. Professor M. Vivargent of CERN said that the design for LEP currently favoured would cost about SwFr 1000 million and should be built by 1988. More should be known at the end of this year, however, when the report of the latest study group would be ready.

Like LEP, the European Synchrotron Radiation Facility (ESRF), is also awaiting the publication of a report at the end of this year which should also lay down the type of machine the physicists would like. It will then be up to the politicians to decide whether or not that is economically feasible. **Judy Redfearn**

Harrisburg leak 'the worst accident in nuclear history'

THE type of human and mechanical failure which occurred in the nuclear power plant near Harrisburg, Pennsylvania last week and led to the discharge of a cloud of radioactive gas into the surrounding environment, has a 50:50 chance of happening in 400 reactor years, according to Dr Chauncey Starr, Vice-President of the US Electrical Power Research Institute. Speaking at a press conference earlier this week, Dr Starr said that "on the technical side this accident, while no-one wanted it, has a statistical probability that falls within the predicted probability of this type of incident. We have had 400 reactor years of operation of nuclear power and the earliest statistical analyses indicated that low level releases of radiation due to equipment failure would have this kind of statistical probability".



The accident resulted from an overheating of the core of the reactor causing some damage to the fuel rods. The emergency cooling system had flooded the core with water, but the contaminated water, some of which escaped through the roof of the plant as steam, was largely responsible for

the escape of fission products such as xenon, krypton and iodine into the local environment.

A critical problem still to be resolved is a 1,000 cubic ft hydrogen bubble trapped in the reactor core. Efforts to redissolve the bubble have failed and the radiation levels inside the plant are too high to release the pressure manually.

After a second release last Saturday a reading of 1,200 mrem per hour was taken at the gas vent, 25 mrem per hour south of the plant site and 15 mrem per hour within a five mile radius. Street and doorstep measurements in Harrisburg ranged from 2 to 5 mrem per hour. A normal chest X-ray produces 30 mrem of ionising radiation. Thus the inhabitants of Harrisburg, 10 miles from the plant are receiving the equivalent of from one to five chest X-rays per day with plant officials predicting several more days of radiation leaks. The possibility still remains that 600,000 people in a 20-mile radius around the plant may have to be evacuated. □

Finland turns to wood and peat for energy

ONE of the last acts of Finland's outgoing government was to accept, on 15 March, the report of a special commission on energy resources, which advocates 40% self-sufficiency by 1990. This programme, which will entail increased use of peat and wood, is effectively a reversal of a previous policy which envisaged a decrease of self-sufficiency from 29% in 1977 to 23% in 1990.

Since Finland's wood processing industry consumes approximately one quarter of all its energy and one third of its electricity, it would seem ecologically appropriate that it provide some of its energy needs. This it already does by providing some electricity generating plants (which in 1977 produced 7.5 TWh of electricity) with fuel in the form of wood wastes. Finland's Centrist Party, however, would like to go further than the commission's proposals and is suggesting an ultimate energy self-sufficiency of 50%. It says that proper use of wood wastes could replace up to 30% of Finland's oil imports. It is also advocating the growing of 'energy woods'—willow and other non-commercial timber. Finland's Forestry Research Institute is at present researching into this possibility, which would have the advantage of using otherwise unprofitable marginal and scrub lands. The main problem however is finance since planting and management of such 'energy woods' is highly labour intensive.

According to the 1977 forecasts, Finland's annual consumption of primary energy sources was expected to rise from 23.0 to 33.2 Mtoe by 1990—an increase of some 44%. Of these totals, oil consumption was expected to rise from 12.2 Mtoe in 1977 to 15.9 Mtoe by 1990. In 1977, approximately 70% of Finland's oil imports came from the Soviet Union, and it was hoped that the increase would also come from there.

Recently, however, the Soviet Union has been unable to meet Finland's increased demands, apparently due to similar increases from domestic and Comecon consumers, and difficulties in bringing the new Siberian fields on-stream. At the same time, OPEC prices have continued to rise, and the Soviet Union raised its prices proportionally—14% last December, and a further 8–9% last week.

Furthermore, Finland's two supertankers are not allowed to pass through the narrows between Denmark and Sweden if loaded to their full 100,000 tonne capacity. A major rethinking of energy policy seemed timely.



Making a grab for home-grown timber

Being a relatively flat country, Finland has only a limited number of sites suitable for hydroelectric plants, and these are already exploited virtually to full capacity (12.1 TWh in 1977). Nuclear power at present provides some 7% of all generating capacity from the first set at the Loviisa power station. A second set is under construction at Loviisa and two sets at Okiluto. The possibility of a third nuclear power station with a total capacity of 1 GW is still under discussion, but no decision is expected until the early 1980s.

Finland, incidentally, does not seem to have a vocal anti-nuclear lobby. However, on accepting the Soviet tender for the Loviisa plant, the Finns demanded far more stringent safety precautions than were then normal for Soviet reactors, including containment buildings (seen by the Soviet designers as a capitalist trick for inflating construction costs), redundant cooling systems and improved computerised controls. When the Soviet Union was unable to supply these additions, the Finns went shopping in Holland, West Germany and the USA.

Nuclear power, however, depends on foreign suppliers, not only for the construction of the station (the Soviet Union for Loviisa and Sweden for Okiluto) but also for nuclear fuel. If Finland is to become relatively more self-sufficient, wood and peat will become considerably more important.

Until very recently, peat which at present accounts for only 1% of Finland's energy production, had not been considered a commercially viable alternative. Earlier this month, however, the then Minister of Trade and Industry, E. Rantala announced a new agreement between Neste Oy, the Imatra Power company and the Navire Company on the study and adoption of wet carbonisation. This process removes most of the water from the peat, giving a fuel that is comparable with coal and contains only some 10% of water. Liquefaction of peat, said Mr Rantala, is also to be studied and the next supplementary budget is expected to allot additional funds for peat research.

Vera Rich

Hungary's careful use of pesticides for plant protection

"CHEMICAL pesticides, even if used extensively, never harm the wild life economy, provided that they are applied professionally." So said Mr Ferens Hargetai, head of the plant protection department of the Hungarian Ministry of Agriculture, at a seminar in Cambridge, England last week.

Hargetai's statement might well alarm some ecologists, particularly when joined to his definition of environmental protection. By this, he said, "we mean, whether confessed or not, the human being rather than the environment itself. Speaking about environment protection, one never thinks of the protection of the Colorado beetle or house fly, though these also belong to the environment in the same way as the singing birds or the national parks do".

However Dr Illes Desi, of the National Institute of Hygiene, suggested at the seminar, that developing an appropriate pesticide strategy was by no means simple. "More pesticides", he told *Nature*, "mean more food—but also more pollution."

The Cambridge seminar was part of the UK-Hungary trade and technology cooperation programme, and it was the Hungarians who asked specifically for a meeting on pesticides. Hungary has an excellent record of awareness of pesticide hazard. Mr Hargetai said it was the first country in the world to ban DDT, aldrin, dieldrin and HCH. "We found that the concentration of DDT in the population was very high—12 to 16 ppm. This was among the highest in the world. So it had to be stopped".

In the same year (1968), a set of requirements for the registration of pesticides was drawn up. In 1972, a new system was introduced classifying pesticides from the point of view of environmental hazard. This meant certain pesticides could not be used within a given distance of a river; some may only be applied by trained experts.

These experts play an important role in Hungarian agricultural planning. By law, every state farm must have on its staff a "plant protection engineer". Cooperatives and the few remaining private farms can (and in the case of dangerous pesticides, must) hire the relevant experts from the government-sponsored Plant Protection Service.

This service is concerned with the whole range of plant management problems including research into crop pests and diseases. In 1975 a forecasting system began which has reduced considerably the amount of pesticide needed. □

Breakthrough by PLUTO: The PLUTO collaboration, working on the e^+e^- storage ring at DESY in Hamburg, announced last week the discovery of new evidence to lend support to Quantum Chromodynamics (QCD) as the proper theory for strongly interacting particles. Recent data at DESY shows evidence for a three-jet structure in the decay of the u -quark just as predicted by some QCD models. QCD model calculations for e^+e^- collisions show that the u -quark decays into 3 gluons which give rise to 3 jets of high momentum particles. The identification of such multi-jet events is very difficult because of severe backgrounds from other processes. The PLUTO analysis is based on a sample of over 2,000 events collected in the u -quark resonance region. Earlier studies of such jet structures have been inconclusive. Only after many refinements of its data analysis procedures has the group been able to confirm the existence of 3-gluon jets.

Boost for biotechnology: A UK working party on biotechnology is being set up to review developments in this rapidly expanding field and to recommend actions which will help British industry. The sponsors of the working party are the Advisory Council for Applied Research, the Advisory Board for the Research Councils and the Royal Society. The chairman will be Dr A. Spinks of ICI. The group is likely to take a very wide look at the subject in disciplines such as photosynthesis, recombinant DNA, fermentation biomass, cell fusion and so on; applications are likely to range through pharmaceuticals, waste disposal, food technology, metal mining, oil production and many others.

Health check on UK radiation workers: The Medical Research Council (MRC) is to conduct epidemiological studies for the UK Atomic Energy Authority (UKAEA) to investigate the effects of long term exposure to low levels of radiation on UKAEA workers. The research team will probably survey the health records of all present and past UKAEA workers and visit all its institutions. Workers employed by British Nuclear Fuels Limited (BNFL) will not be included in the survey as BNFL are carrying out an independent survey. According to Dr Tony Vickers of MRC Headquarters, the head of the study has still to be appointed and results will not be published for at least three years. The whole survey will be monitored by a steering sub-committee of MRC's Protection against Ionising Radiation Committee, chaired by Dr R. H. Mole, a former director of Harwell's Radiobiology unit.

FOE requests reactor safety information: In the wake of the Harrisburg accident (see page 497) Friends of the Earth has renewed its request to the UK Secretary of State for Energy, Tony Benn, to publish the Health and Safety Executive's full report on pressurised water reactor safety. Citing the recent disclosure of the Union of Concerned Scientists "nugget file" of "astonishing" safety deficiencies at US power stations, the closure of five power stations due to a factor of six deficiency in the design of safety piping and the continuing Harrisburg events, FOE criticises the HSE for refusing to release detailed safety studies of PWRs or of the British Gas Cooled Reactor (AGR). In addition, FOE requests Benn to submit the full reports to "a proper review process" by independent analysts.

Meanwhile, British Nuclear Fuels has announced that the latest Windscale leak (29 March) has been located in an out-of-use building that had been used to store highly active wastes before their transfer to buffer storage tanks. BNFL is now investigating the route by which the leak

reached the borehole and is satisfied that "there is no hazard to workers on the site or to people in the nearby community". The radioactivity measured in the borehole was 0.1 curie per litre. It was not possible to obtain the energy of the radiation for conversion to dosage (rems).

US urged to centralise assessment of cancer risks: A single scientific organisation responsible for identifying and characterising potential carcinogens across a range of government agencies has been recommended in a report prepared by the staff of the US Office of Science and Technology Policy. Such an organisation, which it is suggested should be based on an expansion of the National Toxicology Programme established last year by the Department of Health, Education and Welfare, would have two main responsibilities: to identify those substances which pose a potential carcinogenic risk to humans, and to characterise in quantitative terms the nature of the risk and the degree of certainty of current estimates.

Priority for scientific analysis should be given to chemicals for which cost-benefit analysis performed with existing data suggests that regulation be indicated, but leaves too much uncertainty to justify immediate action. "Priority setting and coordination of testing ideally should be done by a single group with input from all relevant agencies" says the report.

Environmentalists criticise secrecy of recombinant DNA meeting: Three US environmental groups have issued a statement criticising this week's meeting on recombinant DNA research organised by the Royal Society and COGENE for placing a cloak of secrecy around its deliberations. The meeting, participants of which had been told that they should not report directly on the proceedings, has been discussing the development of guidelines by different countries to regulate this research. Leslie Dach, of the Environmental Defense Fund, said that "it is a disservice to the discipline of science that the press and the public be excluded from a meeting that is concerned with a new technology of potential global impact." (The ban on reporting was suddenly lifted at the start of the meeting, see page 496).

EDF, Friends of the Earth and the Coalition for Responsible Genetic Research have claimed that the \$200 attendance fee is a barrier to the public against attending the conference. "An international group such as UNESCO cannot afford to condone exclusion of the news media and other observers from an issue as important and debatable as genetic engineering," said Lorna Salzman of Friends of the Earth (COGENE is organised under the auspices of the International Council of Scientific Unions, which is supported by UNESCO).

US study claims benefits of reducing air pollution outweigh costs: The US Environmental Protection Agency has issued a report saying that the costs of cleaning up air pollution from factory chimneys is considerably less than the economic benefits including the reduction in health expenses. According to the EPA, the annual health spending of the US would be reduced by \$40,000 million if pollution from factories was reduced by 60%, although this figure has already been queried by the business community. The report was prepared by research workers at the University of Wyoming, the University of New Mexico and the University of Southern California. It stated that a 30% improvement in the air quality of the Los Angeles region would produce an annual benefit of \$500 per household by raising real estate values.

Swiss go to polls again on nuclear issue

Local democracy continues to block nuclear power in Switzerland. Geoff Milnes explains the latest debate over waste disposal.

THE further development of nuclear power in Switzerland is grinding to a halt because of the refusal of local authorities to allow test borings in the search for suitable repository sites for radioactive waste. This is for many people an unwelcome effect of direct democracy and local autonomy—two characteristics of political life in Switzerland of which the country is justly proud. Local resistance to test borings has led to a polarisation which has spread to the whole question of the role of nuclear energy. This became clear in the campaign leading up to the plebiscite on 18 February, in which 49% of the voters were in favour of a ruling which would probably have brought nuclear development to a complete halt, by demanding popular control of the siting of nuclear facilities by the local population surrounding the proposed sites.

Moreover the situation will remain critical if a revision of the law which recently passed through parliament is turned down in the next referendum, in May.

The present law governing nuclear power plants was put into force in 1959 with the idea of encouraging the peaceful use of atomic energy by the electricity companies, which are mixed

private and cantonal bodies run on a private enterprise basis. Licensing was placed in the hands of the *Bundesrat* (the seven-man cabinet) with no direct democratic or parliamentary control. There was little controversy until mid-1974 when newspapers reported surreptitious drilling by a little-known organisation, NAGRA, looking for repository sites for radioactive waste in a number of localities—without the permission of the local authorities.

This action, together with the obvious and painful dependence of Switzerland on foreign energy supplies emphasised by the oil crisis of 1973/74, a series of frightening projections of future energy requirements, and plans for building 10-15 nuclear power stations which appeared at about the same time, resulted in a tremendous upsurge of public interest and concern.

NAGRA (Nationale Genossenschaft für die Lagerung Radioaktiver Abfälle) is a cooperative organisation founded in 1972 by the "waste producers"—the companies operating or planning to operate nuclear power stations (six companies in all) and the confederal government (responsible for radioactive wastes derived from hospitals, industry and research)—each with one delegate on the administrative board.

This board contracts research in various directions, financed in proportion by the companies and the government—in waste treatment and conditioning, in the development of repository concepts, and in geological studies. The latter has turned out to be the politically critical area.

Switzerland is one of the few countries without a geological survey—and there is no tradition of free flow of geological data, since most studies are carried out by private companies in competition with each other. The NAGRA research was thus contracted out to a private geological bureau. Also, NAGRA became a member of a further institution, KUS (Konsortium für Untertagsspeicher), set up to coordinate all work on underground storage, including the storage of oil and natural gas. In 1974, none of these bodies were in the public eye and the complicated inter-relationships made it difficult for the individual landowners to grasp for what and for whom the geological investigations, including test drilling, were to be carried out, when permission was being sought for land access.

Whether this confusion was a result of a basically nuclear legal situation (NAGRA's view) or whether it was the result of a deliberate policy of secrecy and stealth (the anti-nuclear front's opinion) is hard to judge.

What the geologists say about where to bury the waste

A RECENT book* assesses the alternatives for disposing of the radioactive waste from Switzerland's nuclear power plants, assuming that by the year 2000 a final capacity of $5 \times 1,000$ MWe will be reached. From the geological point of view, radioactive wastes can be put into two categories, according to the length of time that isolation from the biosphere is required.

The first category, low-active waste (NAA), consists of contaminated materials from the normal running of power stations (filter residues, worn-out parts, tools, clothing, etc) and the radioactive debris produced on demolition after the projected 40-year running life. This material contains only short-lived radio-nuclides in small amounts and needs isolation for a few hundred years. The total volume will probably be about $85,000 \text{ m}^3$.

The second category is the high-level α -bearing waste (HAA) which originates from the reprocessing of

the spent fuel. Switzerland depends on foreign plants for fuel reprocessing, but must assume that high-level wastes from these plants will be returned to the country for disposal after an initial storage period. With present reprocessing procedures, which seem unlikely to change drastically in the near future, HAA will contain quantities of plutonium and α -emitters with long half lives which require containment times of at least 100,000 years. However, the total amount of HAA will probably be small (about 400 m^3).

However, several factors make Switzerland far from ideal for the location of radioactive waste repositories, particularly for HAA. These include the complicated geological structure, the humid climate and the complex hydrogeological conditions, the possibility of renewed extensive glaciation and the continuing tectono-seismic activity.

Near-surface mined cavities for low level waste: NAGRA has been contracting research in this direction for a number of years. Research has concentrated on anhydrite as the host rock for NAA repositories. Pure

anhydrite is suitable for cavern construction and has good sorption characteristics and thermal conductivity. It is also generally impermeable to ground-water near the surface, because of a "self-healing" effect caused by its rapid conversion to gypsum (with a volume increase of 60%) when water percolates along cracks and fractures. Anhydrite-bearing formations are widespread in Switzerland, though occurrences of pure anhydrite of sufficient size have not yet been found. The anhydrite occurrences known are all very impure and heterogeneous, often chaotically mixed with other rocks or found along fracture zones with rapidly varying thickness. Investigation on other potential host formations (shale, granite) have not yet been carried out. So, although a reasonable solution can be expected in this direction, the research and development programme is still in its infancy.

Deep borehole repositories for high level waste: The book proposes a fanned array of deep boreholes, penetrating a 350 m thick host formation at a depth of 1-2½ km. Various potential host rocks—rock salt, anhy-

Nevertheless, when the news broke that test borings in connection with the search for suitable underground repositories for nuclear wastes were in progress in several places without official knowledge or consent of the Gemeinde in question, a great cry went up.

Since 1974, no further test borings have been possible and the resolution of this stalemate has become a key issue in the nuclear power controversy. The core of the legal problem seems to be that the *Bundesrat*, under the law of 1959 which is still in force, is only responsible for site licences and permits once a site has been selected. NAGRA has always emphasised, however, that the test borings were exploratory, preceding site selection.

The *Bundesrat* has thus decided that they are governed by the normal laws applied to mining, search for oil, etc. for which the Canton is responsible, after consultation with the Gemeinde concerned. NAGRA lawyers maintain that the preliminary investigations must be considered as an integral part of the nuclear facilities themselves, coming under confederal law, and so the NAGRA put in applications for permits to carry out the borings to the Confederation Energy Office in 1976. These are still pending.

This is not the only legal bone of contention. The other important one concerns the licensing of the power stations themselves. The anti-nuclear

movement, supported by eminent lawyers, maintains that the proof of safety of nuclear power stations, a necessary condition for the granting of construction licences, includes also the safe disposal of the radioactive wastes, and that this cannot be produced prior to repository site selection, i.e. before test borings have been made.

This is just one of a series of irregularities which lead them to the conclusion that the nuclear plants now in operation (three, with a total capacity of 1,000 MWe) and the two 1000 MWe units at present under construction were illegally licensed. In the general atmosphere of public discontent, as illustrated by last month's vote, the *Bundesrat* has considered it politically inopportune to license further works under the old law (the Kaiseraugst works, whose site was occupied by demonstrators for 6 weeks in spring 1975, is one whose licence is still pending). Instead, it set a commission to work to revise it.

The new law, which passed through parliament in a number of turbulent sessions in spring 1978, contained several improvements and clarifications most of which intended to place the nuclear industry under stricter and more democratic control, with regulations which, in contrast to the old law, should rather discourage nuclear power growth unless absolutely necessary.

For the "borehole stalemate", however, the new law brings some relief—

test borings for power stations or waste repositories can be forced through by the confederal authorities by expropriation of land, if necessary. But the situation is still tense.

A section of the anti-nuclear movement decided that the stalemate suited their purposes well and have forced a referendum against the revision of the 1959 law (voting takes place on 20 May). In this, they have laid themselves open to accusations of illogical obstructionism by the nuclear power supporters—it is pointed out that one of the opponents' main arguments against nuclear power is that the problem of radioactive waste disposal is not solved, and yet their activities are directed towards preventing the very investigations necessary to reach a solution.

The anti-nuclear group is intent on preventing the waste disposal problem—which must be solved whether more nuclear plants are put into operation or not—from being approached in the scientific way it deserves. In so doing, it risks alienating that part of the anti-nuclear movement which takes a broader view of the whole energy problem and which is interested only in nuclear power being kept within carefully and democratically controlled limits. Be that as it may, the Swiss voters will be asked to decide another nuclear issue in May and an important public debate continues. □

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drite, shale, marl, gneiss, granite—are possible, but few are thought to occur at convenient depths in sufficient thickness (probably only gneiss and granite). Potential sites are more likely to be found outside the mountain areas, i.e. under the Swiss Plain (Molasse basin). In the Alps, the deep structure is very complicated and difficult to predict and the danger of a new Ice Age and rapid glacial erosion in the next 100,000 years cannot be ignored.

A repository at 1–2½ km depth in a suitable area is unlikely to be affected by "external" effects—human influences, meteorite impact, volcanic eruption, glacial erosion—during that period. The relatively high seismic risk in some areas is a more serious problem because the fracturing associated with earthquake activity could affect the underground water circulation, which is critical for the integrity of any repository. Information about the numerous hot spring and mineral water occurrences indicate high permeabilities and rapid groundwater circulation in some deep formations. It will be necessary to gather detailed information on the flow types, velocities and chemistry of the

groundwater and on the sorption properties of the surrounding rocks in the vicinity of the repositories. These are local parameters, not predictable from surface studies—their investigation requires exploratory deep drilling and long-term, on-site experiments. This is a costly and time-consuming business and is by no means sure of success. Ten to 15 years of directed research will be required before the feasibility of this concept can be settled.

The alternative to the two concepts summarised above is cooperation in an international programme such as the "seabed" programme. Whether the two concepts can be realised also depends also on social and political aspects. The situation is aggravated by the lack of an established geological survey and because the research, being carried out mainly with private financial support in a piecemeal fashion, is not published and not made available to the general public. It seems clear, however, that even with the most favourable political developments, the scientific and technical problems are such that a practical solution and definitive site selection can hardly be expected



The Alps: their complicated structure may make them unsafe for long-term storage.

within the next decade, particularly for the HAA category.

**Geologische Aspekte der Endlagerung radioaktiver Abfälle in der Schweiz* (Bürgisser et al. 1979), Schweiz. Energie-Stiftung, Report Nr. 6.

correspondence

Energy unit for global use

SIR,—Very large powers of 10 occur in discussions of global energy, one example being the exajoule (10^{18} joule). As this number is larger than the number of seconds since the big bang ($\sim 5 \times 10^{17}$) its size is difficult to appreciate, and a more intuitive unit is desirable.

Annual energy consumption is dimensionally power, and a good unit of power is a 60W continuously burning light bulb. (One could make it a 100W bulb, but that is slightly less modest.)

Furthermore it makes sense to report power per capita of the world population. Table 1 then expresses some important energy flows: the figures for 2000 AD and 2020 AD are based on exhibit 19 of Bloodworth, I. J. *et al.*, World Energy Demand to 2020, Executive Summary (World Energy Conference, London, 1977).

The table shows the low average efficiency of photosynthesis: there is evidently scope for the development of high energy yielding crops.

To see the proposed unit in action, we ask to what extent the deserts of the world—a somewhat fuzzy concept—could supply world energy requirements by direct conversion of solar radiation (to electricity, say). This is not a practical idea at present, but it is nonetheless important to have answers to such a question.

Assuming rather poor devices of 1% efficiency and an insolation at one tenth of the solar constant, one finds that the deserts would give an adequate yield for some decades to come (Table 2). Although there is in fact a great deal in hand at present, this advantage is being eroded.

The numbers for the energy consumption are reasonably small when expressed in these units and it is hoped that they carry an intuitive meaning.

Yours faithfully,

P. T. LANDSBERG

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Military structure is off-target for science jobs

SIR,—David Horrobin's military solution to the problems of job opportunities and job security (8 March, page 118) could more aptly be described as the final solution, for if implemented, his ideas would further encourage short-term grant funded work using basic scientists as technicians and assistants. The lack of prospects offered would simply discourage those wishing to enter research as a profession and would commit research to a supportive role in advancing the careers of teachers and, in medical research, clinicians. But what is the problem he is trying to solve? If it is the "unequivocal... decline in research productivity between the years of 35 and 45" then his efforts are misguided: I challenge the myth that research scientists are more susceptible to 'burning out' than any other professional group. The "decline in research productivity" reflects a combination of withdrawal of short-term funding support (experience comes expensive) and the scientist's disillusionment with the abysmal career prospects.

In his proposed career structure we would know by the age of 35-40 whether or not we were suitable. If not, the alternative would be "the comfortable knowledge that they would have three years... to plan a second career." Who on earth is going to employ a middle-aged scientist stigmatised by his so-called "decline in research productivity." Like the universities, industry and government recruit largely at the graduate level and so even if they wished to pick up the academic 'casualties' they would not be able to do so. These proposals would also curb any independent thought, for the research worker would be continuously trying to please and impress the departmental 'Field Marshal' in the hope of being awarded another five years in

the despatches for efforts above and beyond the call of duty. Lastly, he resorts to the expedient of the gratuity payment. These always seem very generous at the time but, as many workers have recently found, the money is very soon spent, leaving the dole queue and senescence as the only feature on a bleak horizon.

Certainly scientists need a stimulus to work. But this should take the form of improved career prospects with adequate peer review by which effort, achievement and responsibility are rewarded; the game of Russian roulette in which the trigger is pulled every five years is no basis for a profession.

Yours faithfully,

MICK KADLUBOWSKI

Association of Researchers in
Medical Sciences,
London, UK.

Sergei Kovalev: remember a colleague

SIR,—Soviet biologist Sergei Kovalev is now in the fourth year of a seven-year term in a strict regime labour camp. He was sentenced in December 1975 for making known various violations of human rights by the Soviet state. Past support by scientists around the world has helped him to receive needed medical assistance and has ameliorated the harsh punishment given to him in the labour camp. Renewed efforts are now required to support him, lest he fade from the public eye and be left to the "mercy" of his jailers.

Scientists at the State University College at Buffalo and State University of New York at Buffalo have contributed funds and have purchased a journal subscription in Kovalev's name. It is sent regularly to him at his prison address. Colleagues at other institutions might well do likewise. Letters to Soviet authorities may also be helpful.

As spring approaches most of us in the northern hemisphere are going about our research and teaching and sharing the love of our families. Let us remind ourselves of our colleague, Sergei Kovalev, who is freezing in a Siberian labour camp because he dared to speak out in favour of human rights. We owe him a great debt and should do all that we can to help him in his time of need. As a symbol of our concern I suggest that each of us send a letter or greeting card to Dr Kovalev at:

Pochtovy Yashchik
No 5110-1BC
Moskva
USSR

This will let him know that he is not forgotten and will remind Soviet authorities that we have not forgotten our colleague. A blizzard of greeting cards would be an appropriate message at the end of this winter season.

Yours faithfully,

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Table 1 Important energy flows (Earth averages)

	60W bulbs per capita	Joules per annum
1975 [World population 4×10^9]		
Food consumption	2	1.5×10^{19}
Energy consumption	33	2.5×10^{20}
Photosynthesis yield	410	3.1×10^{21}
Solar energy incident on Earth	396,000	3.0×10^{24}
2000 AD [World population 6×10^9]		
Energy consumption	51	5.8×10^{20}
2020 AD [World population 7×10^9]		
Energy consumption	76	1.0×10^{21}

Table 2 Possible power yield from deserts

Year	Yield (60W bulbs p.c.)	Requirements from Table 1 (60W bulbs p.c.)
1975	141	33
2000	94	51
2020	81	76

Mean insolation is 135 W m^{-2} ; efficiency of devices 1%.

news and views

Testing solar cells in space

from Andrew Holmes-Siedle

THE silicon photovoltaic cell was, as the Americans say, one of the first 'spin-offs from the space program'. There are now intensive development projects world-wide with the object of developing economical terrestrial photovoltaic power plants. At the same time, US scientists are still attempting to develop cells to give even higher efficiency in space. The problems faced in the latter work are worth mention for three reasons. First, the increased understanding of cell action, engendered by this work, will benefit all solar-cell designers; second, the problems faced represent interesting examples of the sophisticated engineering of semiconductor materials; third, some recent measurements of silicon cells, mounted on unmanned vehicles in space and monitored remotely, have yielded unexpectedly useful information in a different field, namely data on the characteristics of the near-earth radiation environment.

A paper by Richard Statler and Delores Walker of the US Naval Research Laboratories (in the *Proceedings of the International Energy Conversion Engineering Conference*, San Diego, August 1978) describes the design, exposure in orbit and interim

results of a solar-cell experiment in orbit at an altitude of 22,000 km. The satellite was the second Navigational Technology Satellite, NTS-2. This unmanned satellite is intended as a test-bed for new technology. It was launched on 23 June, 1977 and will be monitored for at least 3 years. The small array of cells, flown as an experiment, was independent of a large array of conventional cells which powered the vehicle. Various new types of cell were mounted in groups on the surface of the satellite and various thin, transparent cover glasses were applied in order to absorb the worst of the particle radiation. However, many energetic particles cannot be fully stopped. These penetrating particles degrade the performance of the cells. Significant information concerning the numbers of particles can be obtained from this degradation, as will be described later.

New techniques for increasing cell efficiency were being tested on this flight. For example, the texturing of the front surface of the silicon wafer allows more sunlight to be captured, the product is called a 'non-reflective' cell; very shallow n-on-p junctions give improved response to short-wavelength

sunlight and the product is called the 'violet' cell; the rear surface of the cell can be treated to repel current carriers and the product is called the 'back field' cell, the back surface of the cell can be polished to reflect penetrating infrared light back into the conversion region, the product is called the 'back reflector' cell; finally, solid-state processing of the silicon itself can improve the diffusion of current by removing any existing defects or by preventing the formation of point defects ('radiation damage') by particle radiation. The 'lithium-doped p/n' cell is an example of a radical modification of processing which has the sole object of reducing the sensitivity of the cell to radiation damage.

Four different manufacturers supplied a variety of developmental designs using the above techniques. The table lists the types of silicon cell flown, their photovoltaic properties and their degradation after 223 days in space.

At the top of this table come advanced designs from each of the major manufacturers of cells intended for use in space, showing that several alternative high-efficiency designs can survive well in space. The Solarex cells had excellent initial efficiency but suffered incidental electrode problems, so that no verdict can be passed on these. The only major difference between cells 6 and 9 was the technology for sticking on the cover glass for space solar arrays. This difference is far from trivial, since ultraviolet light from the Sun can seriously affect the adhesive. The use of a fluorocarbon polymer adhesive (on cell 6 only) gave rise to the lowest percentage degradation of any cell and this finding may represent a major step forward in space technology. If we wish to use the usual silicone adhesive, we are forced into the extra expense of depositing an ultraviolet-rejecting film on the cover-slide (compare cells 1 and 7). Cell 10, despite its low position on the absolute scale, represents a modest success for the older p-on-n junction technology. The structure is intrinsically more sensitive to radiation but the results

Table 1 Maximum power output of silicon solar cells after 223 days in orbit on NTS-2 (n-on-p junctions of area 4 cm² unless otherwise stated)

	$P_{\text{max}}(t)$ (mW)	% Power lost in 223 days	Manufacturer	Type
1.	63.2	12.2	Comsat	Textured, non-reflecting (with ultraviolet filter)
2.	60.5	13.6	Spectrolab	Reflecting back surface
3.	57.6	13.5	OCLI	'Violet' cell
4.	53.9	15.5	Spectrolab	'Sculptured' surface
5.	52.9	12.7	Spectrolab	Main array cell type
6.	50.5	8.8	Spectrolab	'Sculptured' (with PTFE special adhesive)
7.	49.6	33.6	Comsat	Textured, non-reflecting (no ultraviolet filter)
8.	48.5	13.9	OCLI	Conventional cell type (silica cover with silicone adhesive)
9.	47.6	11.0	Spectrolab	'Sculptured' (with silicone adhesive)
10.	47.0	15.8	Spectrolab	p/n lithium-doped
11.	40.3	13.9	OCLI	Conventional cell type (glass cover with special bonding method (ES)).
12.	33.3	46.5	Solarex	Vertical junction

demonstrate in space the laboratory finding that lithium atoms incorporated in the lattice can migrate and inactivate the defects created by particle radiation (Faith *IEEE Trans. Nucl. Sci.* NS-12(6) 371; 1972). Without the presence of lithium, another 12% of degradation would have been experienced. As regards silicon cell technology in general, one can say that the table shows that, given the economic incentive, silicon technology can be made to give still further advances in performance. A cell made from AlGaAs also did well in this experiment.

The NTS-2 experiment gave some useful information concerning radiation fluxes in space. The environment of the Van Allen belt has been crudely characterised but controversy continues about the absolute values of particle fluxes in orbits such as those of NTS-2 (a circular orbit of altitude 20,190 km, with the plane inclined 63.5 degrees to the plane of the equator) and of geostationary communication satellites (circular, 35,863 km, inclination 0). The main problem lies in measuring the spectra and spatial spread of the high-energy electrons which are known to circulate in geomagnetic trapping regions between altitudes 500 and 40,000 km. It is also difficult to follow the fluctuations of these values with time over several years. At the same time, engineers designing spacecraft are urgently in need of more precise predictions as to how rapidly their devices will degrade in this hostile environment. Thus any information which we can glean from well-calibrated cells such as those on NTS-2 is very welcome, especially since, on this flight, two experimental, remote-reading radiation dosimeters were also flown (Evans *et al* *IEEE Trans. Nucl. Sci.* 25, 1619; 1978). Certain p-n diode structures degrade smoothly and predictably under irradiation. High-energy particles produce recombination centres in the silicon by atomic displacement and these centres cause a reduction in minority-carrier diffusion length, L . Photovoltaic efficiency is proportional to L^2 . Thus, some solar cells constitute good, if limited, integrating radiation detectors. Results such as those tabulated here can be used to reduce the uncertainty inherent in the current models of particle fluxes in the Van Allen Belt and predictions of the effects of these fluxes on other electronic devices. The NTS-2 experiment has helped to increase our confidence in the fluxes of electrons to be encountered in this orbit (an annual damage-equivalent flux of 2×10^{14} 1-MeV electrons cm^{-2} per year). This figure is six times higher than some of the estimates used previously, which were based on the crude belt models already mentioned.

The NTS experiments, though modest, demonstrate that it is advisable to test theoretical predictions of performance in space by flying a 'test-bed' vehicle. The experiments also show that useful information can be obtained by employing, as detectors,

certain semiconductor device structures which are not yet widely used in radiation physics. □

A. Holmes-Siedle is a consultant to the Physics Department of the Falmer Research Institute, Slough, UK

Have quarks been found?

from P. I. P. Kalmus

MORE evidence for the existence of fractional electric charge has been claimed in a recent paper by G. S. LaRue, W. M. Fairbank and J. D. Phillips of Stanford University (*Phys. Rev. Lett.* 42, 142; 1979). Results from an improved version of their superconducting magnetic levitation experiment, indicate residual charges of $+\frac{1}{3}e$ on two small niobium spheres. These together with results presented two years ago by LaRue, Fairbank and A. F. Hebard (*Phys. Rev. Lett.* 38, 1011; 1977), if accepted at face value, show a total of four fractional charges in a sample of 7×10^{-7} kg of niobium. If these results are verified they represent a major discovery in physics, the detection of free quarks.

In classic experiments starting in 1910, R. A. Millikan sprayed microscopic oil droplets into an air gap between two parallel horizontal plates. The drops were charged by friction or by X-ray ionisation. They normally fell under the influence of gravity, but their motion could be changed and even reversed by the application of an electric potential difference across the plates. The viscous drag of the air caused drops to acquire terminal velocities which were functions of their electric charge. Individual drops were observed for long periods and their velocities determined. From many such observations Millikan showed that electric charge appeared only in integer multiples of a fundamental unit, e , whose modern value is approximately 1.602×10^{-19} coulomb.

Every elementary particle discovered so far has a definite charge ne where n is an integer, usually +1, 0 or -1. Many particles have been discovered in the past 25 years, most of them belonging to the class known as hadrons which experience the strong nuclear force. The study of hadrons revealed an order and symmetry which was interpreted by M. Gell-Mann and by G. Zweig as being caused by a substructure of constituents, named

quarks, having fractional charges of values $-\frac{1}{3}e$ and $+\frac{2}{3}e$. Antiquarks would have opposite-sign charges to these. Various quark models, including some which do not involve fractional charge, have had considerable success in explaining features of high energy physics, and the quark hypothesis is generally accepted today. Many searches for free quarks have been performed, but apart from an occasional false alarm have all proved negative. Indeed there are theories in which interquark forces increase with distance and quarks therefore are necessarily confined within hadrons. A comprehensive review of the search for quarks was written in 1977 by L. W. Jones (*Rev. Mod. Phys.* 49, 717; 1977).

Millikan's droplets had masses of around 10^{-14} kg and the absence of fractional charge on around 100 droplets gives an upper limit of about 10^{-13} for the number of free quarks per nucleon. It is possible to obtain a significant improvement in sensitivity by using much more massive objects, but then a stronger force is needed to balance them against gravity, and magnetic levitation can be used. In 1977, Fairbank and his colleagues published results from experiments which made use of the magnetic repulsion experienced by spheres of superconducting material. In a cryogenic apparatus, niobium spheres of mass 9×10^{-8} kg were levitated in a shaped magnetic field in which they had a natural vertical oscillation period of 0.8 Hz. An alternative vertical electric field of the same frequency was applied. The amplitudes of oscillations were measured and from these the net charges on the spheres were determined. The charge could be changed by $\pm e$ by bombarding the sphere with positrons or electrons from radioactive sources, so that as in the Millikan experiment, a series of charge values, differing by unit steps was obtained for each sphere. The experiment was performed with eight spheres, six of which showed zero residual charge. However sphere No 6 gave a residual charge of $+\frac{1}{3}e$ (to an accuracy of a few percent) on the two

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occasions on which it was measured. Sphere No 3 gave a residual charge of $-1/2e$ on its first measurement, but after removal from the apparatus, cleaning in organic solvents and reinsertion gave zero charge. The authors went to considerable effort to understand and eliminate possible spurious effects which could mimic fractional charges. The main background forces are caused by induced and permanent electric dipole moments and by trapped magnetic flux in the spheres. All were claimed to be too small to account for the results. The samples were all accurately spherical and had been prepared carefully before use. To improve their oscillations the spheres had been heated to high temperature before being used. Five were heated on a niobium sheet and three on tungsten. Both fractional charges were found on the latter spheres and the authors suggest that fractional charges had transferred from tungsten to niobium.

In the same month in 1977, G Galilano, M. Marinelli and G. Morpurgo, from the University of Genoa, published results from their magnetic levitation experiment (*Phys. Rev. Lett.* 38, 1255; 1977). Like Fairbank, Morpurgo and his colleagues had been investigating levitometer techniques for quark searches ever since Gell-Mann's original hypothesis, and indeed had been the first to publish the principles of the method. In their latest experiment, they used small iron cylinders, of mass 2×10^{-7} kg as their samples. The iron is attracted from above by an electromagnet, and a dynamic equilibrium against gravity requires an electronic feedback to the magnet current. However the method has the advantage of operating at room temperature. Vertical electrical plates were used and the cylinders were oscillated horizontally by an alternating potential difference. Again the amplitude is a measure of the residual charge on the samples. Five samples were investigated and no fractional charges were found. The total mass of material in the Stanford and Genoa experiments was quite comparable, about 1 mg or nearly 10^{21} nucleons each. Of course if the Genoa group had also obtained evidence for fractional charges of the required value, the case for free quarks would have been strengthened greatly. Their negative result, however, was not incompatible with the Stanford claims, both on statistical grounds, and because different materials were used. It is conceivable that quarks may have greater affinity to heavier nuclei, in which case niobium (nuclear charge $Z=41$) would be better than iron ($Z=26$), and tungsten ($Z=74$) would be better still. Nevertheless it was clearly possible that one of these difficult experiments was wrong, and in the exchange of

several letters in *Physics Today* during 1977 and 1978, Morpurgo and Fairbank each pointed out the advantages of his own method.

In addition, during 1977 R. Bland, D. Bocobo, M. Eubank and J. Royer at San Francisco State University (*Phys. Rev. Lett.* 39, 369; 1977) examined tungsten directly in a Millikan apparatus using particles formed in an arc discharge between tungsten electrodes. Good measurements were taken on 69 particles. All were found to have zero residual charge to within a standard deviation of just over $0.01e$. However the total tungsten mass was only 3×10^{13} kg or about 2×10^{14} nucleons, and we can only conclude that tungsten is not several million times richer in free quarks than are niobium or iron.

The recent results published by the Stanford group, used an improved version of their apparatus. All three components of possible permanent electric dipoles could now be measured explicitly by moving a relatively large grounded copper sphere to various positions around 1 cm from the niobium sphere, and applying the alternating electric field symmetrically with respect to ground. The possibility of electric field gradients was further reduced by using optically flat and parallel quartz plates. Sphere No 6 which had charge $+1/2e$ in the previous experiment was used again and measured four times with an electric discharge occurring between levitations. Zero residual charges were found on the first, third and fourth measurements and $+1/2e$ on the second measurement. Sphere No 7 which had zero charge previously was used as a calibration during the same cooldown. During a second cooldown in the new experiment, sphere No 7 was used again, together with a new sphere, No 9. This latest sphere showed a charge of $+1/2e$ on each of the two occasions on which its residual charge was measured.

It is difficult to reach a satisfactory verdict on these experiments, and it is likely that most physicists will reserve judgment pending further results. It seems strange that the quarks in the Stanford experiment, if real, should behave in such a volatile manner. On the other hand, if the effect is spurious it is remarkable that the fractional values reported are just what are required for quarks. The experiments are obviously very difficult, but it is not clear from the published letters why so few measurements have been made. It would be useful to have more measurements, and preferably on samples of different sizes in the Stanford apparatus, and in addition, to suspend niobium samples on the iron in the Genoa experiment. □

Fluctuations in abundance of tropical insects

from Robert M. May

ONE tenet of earlier ecological speculation, still to be found in many popular pamphlets about 'ecology' and the environment, is the notion that complex and diverse communities (with many species and a rich web of interconnections among them) are more stable (with individual populations fluctuating less). A corollary is that, in the biologically crowded and species-rich tropics, populations should be more stable. While it is true that many temperate zone animals, particularly insects, do exhibit large population fluctuations, there is not much analogous information about tropical populations. Thus these theoretical ideas have received little in the way of empirical testing.

Recent work has considerably advanced our theoretical understanding of the possible range of relations between diversity and population stability in ecosystems (see for example *Nature* 276, 117; 1978), undercutting any simple notion that complexity, of itself, promotes stability. But the empirical facts have tended to remain largely anecdotal. In Sierra Leone, Owen and Chanter (*J. Entomol.* 46A, 135; 1972) found that a few species of *Charaxes* butterflies had fairly constant abundance, but these authors were concerned to emphasise their experience that most populations of tropical insects fluctuated a lot. In tropical America, Smith (*Carib. J. Sci.* 12, 45; 1972) has shown a moth, *Urania fulgens*, to exhibit dramatic population explosions. Bigger (*Nature* 259, 207, 1976) has reviewed data on some tropical crop pests, suggesting they fluctuate as much as temperate ones. Pursuing "arguments and data to show that tropical populations normally fluctuate no less violently than their appropriate counterparts of higher latitude", Leigh (in *Ecology and Evolution of Communities* Cody and Diamond (eds), Harvard University Press, 1975) showed that the amplitudes of fluctuation of some common vertebrate herbivores (ranging from northern temperate Isle Royale moose and Mt McKinley dall sheep to Serengeti buffalo and wildebeest) showed no consistent tropical-temperate differences. The difficulty is that most of these studies concern one, or at best a few, species, which can be argued to be exceptions.

In this context, a recent paper by

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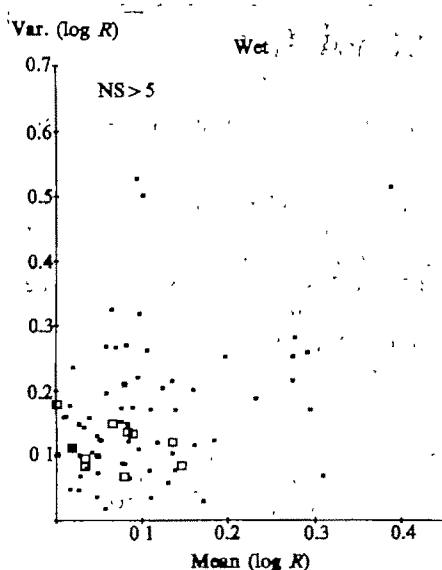


Fig. 1 The annual variability (AV = variance of $\log R$) plotted against the mean value of $\log R$, for various collections of data on insects. The open squares are for tropical insect communities, the solid squares for temperate ones, and the asterisks for (sub)arctic ones; in all cases, all populations are represented by at least five individuals ($NS > 5$).

Wolda (*Am. Nat.* 112, 1017; 1978) does two important things. First, he presents data, collected from light traps, that constitute the best study yet made on the year-to-year fluctuations in abundance of tropical insects. Second, Wolda gives a systematic compilation and analysis of some 138 studies providing information about the average level of year-to-year fluctuation exhibited by groups of tropical and temperate insects.

For his own study, Wolda used three sets of light traps, located at sites in Panama. From the lowland tropical monsoon forest, on Barro Colorado Island in the Panama Canal Zone, he obtained 32,494 individuals that could be classified into Homopteran species (for a total of 565 species) from a ridge site, and 1,538 such individuals (222 species) from a valley site where the light traps were operated less often. The third site was on the edge of a town near Panama City, overlooking second-growth forest, and produced 53,680 classifiable Homopteran individuals (507 species). A few species from orders other than the Homoptera were also included in the overall tropical-temperate comparison, as were unpublished data for Blattidae gathered by Fish. As discussed by Southwood (*Ecological Methods* (2nd ed), Chapman and Hall, 1978), collections from light traps contain biases that make them unreliable indicators of the total number of species in a region, or of the relative abundance of species. Light trap data should, however, pro-

vide a good estimate of the year-to-year fluctuations in abundance of any one given species; Wolda gives several lines of evidence (including independent estimates of abundance from netting) to suggest this is indeed the case for his study.

For each species, Wolda then calculates the ratio, R , that measures the change in abundance from one year to the next: $R = N_t/N_{t-1}$. For a group of species, one then obtains the frequency distribution of values of $\log R$, describing the year-to-year changes in abundance which occur within the group, the mean of this distribution measures the average population change for the group as a whole, and the variance measures the differences among the species. Note that Wolda's 'logR' is the standard 'k-factor' of the pest control literature (see for example, Varley, Gradwell & Hassell *Insect Population Ecology*, Blackwell, 1973). Wolda christens the variance of the $\log R$ distribution the "annual variability", AV, and proceeds to use it to characterise the range of increases and decreases in species abundance within the community under consideration. High values of AV correspond to a wide range of values of $\log R$, with some species showing marked increase in abundance, and others marked decrease, small values of AV correspond to the set of species showing little variation in abundance. Wolda's own data are then analysed in detail, to show *inter alia* that statistical fluctuations arising from small sample sizes introduce relatively little bias if species represented in any one year by less than five individuals are excluded (provided there is information either about many species in any one year, or about a few species for several years).

Wolda condenses the results of his experiments into the AV *versus* the mean value of $\log R$ for the set of Homopteran species, and for a miscellaneous set of other species, at each of his three sites. These results are compared with those of a synoptic compilation of other work. The collections of species in these 130-odd other studies were variously obtained by baiting, beating, emergence traps, light traps, direct observation, pitfall traps, quadrat counting, sweeping, sticky traps, windowpane traps, and other forms of traps. They come from physical environments that may be roughly grouped as (sub)arctic, wet temperate, dry temperate, wet tropical, dry tropical, and a special group for obviously unstable situations (such as migrating moths, or insects invading a newly available area).

Figure 1 summarises the results of AV *versus* mean ($\log R$) for the sites

classified as 'wet': the open squares are for tropical insect collections; solid squares are temperate insects; asterisks are (sub)arctic. Discussing the pattern that emerges, Wolda says "the tropical data in [this figure] are in the same part of the graph as the majority and the best of the temperate data. [The data points based on 100 or more individual values of $\log R$ tend to have AV less than 0.2.] The temperate data scatter more, but there are many more of them. Both the smallest and the largest values of AV are from temperate insects. The conclusion can thus only be that the data suggest rather strongly that tropical insects have, on the average, the same annual variability as have insects from the temperate zone. There is no indication of a greater stability of tropical insects." Wolda sensibly does not subject the data in Fig 1 to any statistical jiggery-pokery to buttress this assertion, and the reader is free to draw his own inferences from Fig. 1; I think Wolda's conclusion is reasonable.

For dry temperate, dry tropical, and 'unstable' collections of insect populations, there are fewer data points. These show more scatter (typically having higher values of AV and mean $\log R$) than the wet environments of Fig 1, and the tropical data are clearly mingled randomly amongst the temperate ones. As we might expect, the AV does show systematic increases in specific 'unstable' instances where the environment is disturbed or newly created. The AV for the community of insects and spiders that colonised the Lauwerszee polder, subsequent to its conversion to dry land in 1969, was 0.64, 0.49 and 0.37 for the years 1967-70, 1970-71 and 1971-72, respectively; this not only shows the predicted decrease as the community becomes more settled, but also is understandably larger than the corresponding AV of 0.10 for an older polder (Meijer *Oecologia* 16, 185; 1974). For aquatic invertebrates upstream of an industrial outlet of cooling water in Britain, AV was 0.18; downstream it was 0.25 (Langford *Hydrobiologia* 47, 91; 1975).

Given that tropical and temperate insect populations show similar levels of fluctuation in abundance under similar environmental conditions, Wolda goes further to claim that "tropical insects are not more to the K end of the r-K continuum of strategies" than their temperate counterparts". I think this goes too far. There are systematic trends in the life history strategies of many groups of animals, including insects, as one goes from the equator to the poles (see for example, Southwood *et al.*, *Amer. Nat.* 108, 791; 1974). Such trends toward more K-ish behaviour among tropical

insects are not inconsistent with tropical insect communities exhibiting levels of population fluctuation comparable with temperate ones; the stabilising tendencies of such K-ash life history strategies can easily be offset by the lower dynamical stability that is characteristic of more complex (species-rich) ecosystems. This view that tropical and temperate communities may manifest similar levels of AV, accomplished by tropical populations exhibiting special features that help offset the intrinsically greater dynamical fragility of a complex system, is clearly a personal one, but it deserves to be considered.

In short, the body of information collected and compiled by Wolda suggests that tropical insect populations, including those from tropical forests, do not show systematically lower fluctuations in abundance than their temperate cousins. Insect populations from relatively dry areas, where the rainfall is low and unpredictable, do tend to fluctuate more, this tendency is, however, equally true for tropical and temperate insects. □

What's special about actin?

from Michael Geisow

THERE is little doubt that actin enables cells literally to shape up to their role in an organised community. Even loners like the amoeba do not dispense with this ubiquitous protein. Although fibrous (F)-actin is most familiar as the protein component of the thin filaments of skeletal muscle, current interests centre on non-muscle actins which are probably present in the cytoplasm of all cells. In non-muscle cells, F-actin forms the microfilaments frequently observed in association with the plasma membrane. Representative structures are the fibrous bundles within cell microvilli and stress fibres apparent in fibroblasts spread on a solid surface. These structures are frequently labile—the major distinction between muscle and non-muscle cells is that the latter have mechanisms for maintaining actin in its non-polymerised state (G-actin). These general observations raise three key questions. In non-muscle cells, what regulates the intracellular distribution of actin filaments and the degree of actin polymerisation? What determinants govern the attachment of actin to the plasma

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Third age of quantum chemistry

from Graham Richards

THE earliest quantum mechanical calculations of molecular properties were very crude and hoped only for agreement with experiment within an order of magnitude. The interest in this pioneering work arose from the fact that approximate agreement did provide some insight as to the reasons for the values of measured properties.

A second age depended on the advent of increasingly powerful computers. *Ab initio* calculations of molecular orbital wave functions boomed and continue to fill the scientific literature in many areas. These more accurate calculations are in much closer agreement with experimentally measured parameters. The complexity of the calculation often precludes the use of simple pictorial explanations and the answers cannot be preferred to experiment. Apart from proving the methods, this type of work receives most justification when applied to systems where the experiment is not a viable alternative to computation; perhaps the molecule is unstable or cannot be synthesised.

Now steady improvements in theoretical calculations have led to the dawning of a third age: calculations which are more accurate than experiment or at very least sufficiently accurate to be indispensable for interpretation. An example of such a case is the elegant spectroscopic experiment of Hunziker and his colleagues at the IBM Research Laboratory San Jose (Wendt, Hippler & Hunziker *J. chem. Phys.* 70 in the press).

The experiment concerns the near-infrared electronic absorption spectrum of the *cis* isomer of electronically excited triplet acetylene, formed by mercury-photosensitised reactions of acetylene, ketene and diazomethane. Although aliphatic unsaturated hydrocarbons in their lowest triplet electronic states are important chemical intermediates they have not been studied spectroscopically, in contrast to the widely investigated aromatic triplets. In its lowest triplet excited state acetylene adopts a planar bent

geometry with both hydrogens on the same side of the carbon-carbon bond (*cis*).

The key to identifying the spectrum was the theoretical work of Wetmore and Schaefer (*J. chem. Phys.* 69, 1678; 1978) which showed that the observed features correspond closely with the predictions for the $^3A_1 - ^3B_2$ transition of acetylene. The spectroscopists were able to observe rotational and vibronic structure as well as deuterium isotope effects, again agreeing to a remarkable degree with the *ab initio* theoretical predictions.

An electric dipole transition between the triplet states is allowed for *cis* geometry ($^3A_1 - ^3B_2$) but parity forbidden in the *trans* case ($^3A_u - ^3B_u$) and the two forms are separated by a potential barrier of the order of 1 eV. The spectrum was observed using a photochemical modulation apparatus. With this technique small transient absorptions are observed by phase sensitive detection and reaction rates determined using phase shift measurements. These showed that the lifetime of the transient at 1.35 μm in the acetylene experiments was about 3×10^{-12} s. The principal feature of the spectrum is a single band at 1.35 μm supporting both the calculations and spectroscopic analysis which show that the geometries of the two electronic states are very similar. There is for instance a change in C-C-H bond angle of about 2° .

The formation of the excited *cis* acetylene is particularly efficient using Hg-photosensitised ketene and diazomethane. The suggested mechanism for this process involves the triplet state of methylene, CH_2 , in the reaction

The work represents perhaps a near perfect instance of theory being in harmony with experiment, each aspect vital to the other and the combination much more than the sum of the separate parts.

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membrane and to itself to form micro-filament bundles? And last, but most crucial, how are the assembled actin fibres used to generate the diverse types of motion (or tension) with which they are apparently associated?

Very often the most profitable lines of work in complex biological systems have tackled the molecular character of the protein responsible. It is therefore surprising that although many actins

are readily purified in quantity, structural information is sparse. The few amino acid sequences which are known indicate that actin is very highly conserved; there are only 17 changes out of 375 amino acids between mammalian cytoplasmic actin and that isolated from the lower eukaryote *Physarum polycephalum* (Vanderkerckhove & Weber *Nature* 276, 720, 1978). Five of these differences occur at the

amino terminus, which is highly variable in all actins.

Within this framework of close sequence homology, the non-muscle proteins (β and γ -isoactins) differ significantly from muscle proteins (α -actins). Non-muscle actins more closely resemble their counterparts in other species than muscle actins in their own species. There are 25 sequence differences between mammalian muscle and *Physarum* actin. Consistencies in the pattern of amino acid substitutions between the muscle and non-muscle classes of actin suggest that some of the differences reflect functional divergence rather than evolutionary 'drift'. For example, brain, platelet, *Acanthamoeba* and *Physarum* actins all have threonine (moderately polar) at position 129, while heart and skeletal muscle actins substitute valine (hydrophobic).

Slow evolution of amino acid sequence generally implies that a greater-than-average proportion of the polypeptide chain is determinant of biological function. For instance, in the cytochromes *c*, most of the numerous invariant residues are responsible for correct binding of the large, buried haem moiety. Sequence changes between species variants or even class variants of a given protein are usually accommodated at the molecular surface. Between the various actins, however, substitutions of charged (surface) amino acids are rare. In consequence, Vanderkerckhove and Weber point out that evolution of actin surface topography must be subject to unusually rigid constraints. In fact direct experimental support for this contention is provided by the weak immunogenicity of actin and the universal species specificity of some anti-actins.

Conservation of the surface structure of actin makes sense in terms of known functions of the protein. It can form extensive linear and lateral associations to produce F-actin and micro-filament bundles. In non-muscle cells these associations are readily reversible, but they are comparatively stable in muscle cells. Knight and Offer (*Biochem. J.* 175, 1023; 1978) describe a method of chemically cross-linking favourably-disposed cysteine and lysine residues in adjacent monomers of muscle F-actin. This ingenious modification not only offers the opportunity of identifying regions of the actin monomer involved in polymer formation, but may also allow stabilisation of F-actin under a variety of conditions in order to study interaction with other molecules.

Both G and F-actins also associate with unrelated proteins. It has been known for some time that G-actin tightly binds pancreatic DNase I with $K_{app} = 5 \times 10^8 \text{ M}^{-1}$ (Lazarides & Lind-

berg *Proc. natn. Acad. Sci. U.S.A.* 71, 4742; 1974). Rohr and Mannherz (*Eur. J. Biochem.* 89, 151; 1978) report the natural occurrence of this complex in rat pancreatic secretion. The same workers also demonstrate that 5'-nucleotidase (present in rat bile) acts in the formerly intractable G-actin-nuclease complex to produce filamentous actin with the release of active DNase I (*FEBS Lett.* 95, 284; 1978). The interaction between DNase I and actin led to the recognition that G-actin in spleen cells was bound to 'profilin'—a 16,000 molecular weight polypeptide. This complex was identical with a previously reported DNase I inhibitor from spleen. Because the profilin-actin interaction prevented the usual salt-induced polymerisation, this non-muscle actin could be crystallised as a 1:1 complex with profilin under conventional conditions of high ionic strength (Carlsson *et al. J. molec. Biol.* 105, 353; 1976). Similarly, the prospect of 3-D structural analysis of muscle actin was made more likely by the introduction of a non-ionic polymer (PEG) as a means of crystallising G-actin (Oriol *et al. FEBS Lett.* 73, 89; 1977). The reasons for the tight binding of G-actin to DNase I and profilin remain obscure, but may bear on the control of actin polymerisation which operates in non-muscle cells. G-actin also seems to form a polymeric complex with the unique molecule spectrin, a peripheral protein of the cytoplasmic face of erythrocyte membranes. It is not yet certain what the association state of actin is, but evidence favours the monomeric G-form.

F-actin appears to be catholic in its interactions with other cell proteins. Binding to α -actin, myosin, tropomyosin and troponin is established beyond doubt and is further indicative of the degree to which actin's molecular surface must be conserved. In non-muscle cells current reports frequently point to the importance of F-actin as a structure responsive to the distribution of membrane proteins. For example both actin and tubulin were found to co-cap with mouse B lymphocyte membrane immunoglobulin (Gabbiani *et al. Nature* 269, 697, 1977) and with membrane receptors cross-linked by concanavalin A (Toh & Hard *Nature* 269, 695; 1977). Flanagan and Koch (*Nature* 273, 278; 1978) demonstrate that the amount of IgG co-extracted with actin by a myosin-actin affinity technique was proportional to the extent of cross-linked membrane IgG. Murine mastocytoma (p815) cells spontaneously shed plasma membrane from microvilli. In this exfoliated membrane a strong association exists between the histocompatibility antigen H-2 and actin (Koch & Smith *Nature* 273, 274; 1978). It is significant

that the H-2 antigen is known to be a transmembrane protein and that both IgG and H-2 continue to associate with actin in the presence of detergents. Analogously, 5'-nucleotidase, freed from its membrane sites by the detergent action of bile salts, can also interact with actin in the form of its nuclease complex.

The strong implications of all these studies is that the extent of membrane attachment and possibly the degree of assembly of F-actin as a cytoskeletal element is sensitive to the state of association of a variety of membrane proteins. The stability of preformed membrane protein-actin associations after solubilisation from the membrane suggests direct binding of actin to membrane proteins by a transmembrane event. However there is as yet no unambiguous demonstration of direct interaction of actin with a transmembrane protein and most authors admit the possibility of intermediate components. The three questions posed initially have not been solved, but complete answers to some of them cannot be far off. It would be timely if the first answer to reach the press was the crystal structure of the source of all the problems; G-actin. □

Polymer mixtures

from Paul Calvert

A RULE which is taught in basic polymer courses is that polymers do not dissolve in one another because the entropy of mixing of large molecules is essentially zero. A review by Sonja Krause in 1972 (*J. macromolec. Sci., C*, 7, 251) listed several hundred pairs of polymers of which only about 10% were compatible and this is probably an overestimate. With the current stress on modifying existing synthetic polymers rather than manufacturing new ones, studies on 'polymer alloys' have become fashionable and compatible systems are of real interest.

The basic Flory-Huggins theory of polymer solution thermodynamics, extended by Flory to include free volume effects, has been applied to polymer mixtures by McMaster of Union Carbide and now more simply by Patterson and Robard of McGill (*Macromolecules* 6, 760; 1973; 11, 690; 1978). The essence of the argument is that the entropy of mixing in a solution is proportional to the number of molecules and so becomes very small for high molecular weight polymers whilst

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the free energy of interaction between chains depends only on the volume of the system and so dominates at high molecular weights. Free volume differences give rise to small increases in free energy when similar liquids are mixed, so mixtures of similar polymers, for instance two polyolefins, will be incompatible. In order for a homogeneous solution to form there must be some preferential bonding between the unlike chains, such as hydrogen bonding or dipole interactions. This is characterised by a negative interaction parameter χ . Unfortunately this does not help a great deal to explain why some polymers are compatible. Although it seems plausible that some specific 1:1 dipole interactions could take place between polyvinyl chloride (PVC) and polyesters such as polymethyl methacrylate (PMMA) or poly(ϵ -caprolactone), it is hard to see what interaction makes poly(2,6-dimethyl 1-4-phenylene oxide) (PPO) compatible with polystyrene (PS). Such things can always be explained but not in a way that has any predictive value.

The experimental basis for compatibility is also rather weak. Early studies were done on the basis that cloudy samples were phase-separated whereas transparent ones were compatible. In fact transparency may just represent fine scale phase-separation or a refractive index match between the phases. Electron microscopy of stained samples can be used but this is limited to a scale of 100 Å or more and depends on having a good stain. The other approach is to observe the glass transition temperature(s) of the sample. Two transitions close to those of the pure polymers implies separation whereas one intermediate transition is taken to show compatibility. However this only works if the two starting polymers have transitions 100 °C or more apart and is misleading if phase separation or dissolution takes place during heating. There is also considerable doubt as to whether two transitions will be seen if separation is on a fine scale. Thus we can demonstrate phase separation but cannot demonstrate compatibility. It is also unclear whether complete dissolution can take place or if all mixtures contain at least local heterogeneity.

Kwei, Wang and Nishi of Bell Telephone Laboratories have looked at polystyrene-poly vinyl methyl ether mixtures by microscopy, vapour sorption and diffusion, and NMR (*Macromolecules* 7, 667; 1974; 8, 227; 1975). They were able to outline a phase diagram with a lower critical temperature above which separation occurred and found a suitably negative χ factor at 30 °C although χ was dependent on the mixture composition which is rather unsatisfactory. These bulk

measurements suggest homogeneous mixing but two T_g values were observed by wide-line NMR implying that two types of environment are present and hence heterogeneity. One of these polymers performed the interesting trick of going from cloudy to transparent to cloudy again as it was heated to 150 °C.

The polystyrene-polyphenylene oxide system is of technical interest since PPO is usually modified by addition of PS which reduces the melt viscosity so that the polymer can be processed. Workers at General Electric and at University of Massachusetts have investigated mixtures of PPO with styrene-*p* chlorostyrene copolymers (*Macromolecules* 7, 902; 1974; 11, 151; 1978). Phase separation occurs when the copolymers contain less than 33% styrene. A single glass transition was seen in the compatible systems but this broadens significantly close to the critical composition, dielectric loss peaks broaden similarly, suggesting that there are large local composition variations before phase separation occurs.

Another interesting system is polyvinylidene fluoride (PVF2)-polymethyl methacrylate which has been studied at Bell Telephone Laboratories and at Groningen (*Polymer* 19, 173; 1978; *Macromolecules* 9, 603; 1976; 11, 766; 1978). PVF2 crystallises out of the

homogeneous mixture so that analysis of its melting temperature, allows a χ parameter to be determined. It is small and negative as expected. PVF2 is compatible with isotactic, syndiotactic and atactic PMMA whilst PVC is incompatible with isotactic PMMA but miscible with the other two up to a 1:1 ratio of PVC to PMMA monomer units (*Polymer* 16, 201; 1975). This indicates that some specific chain pairing is occurring which depends on the conformation of the PMMA and that these pairs are miscible with PVC but separate in excess PMMA.

Thus there is evidence in compatible systems for local segregation of each polymer and/or pairing of unlike chains. The structure of these materials must have much in common with that of block and graft copolymers where each chain is itself a mixture of two or more polymers. The 1977 ACS Symposium on Polymer Alloys (eds Klempner & Frisch, Plenum, 1977) shows the extent of industrial interest in these systems. As well as the familiar use of grafted elastomer phases to toughen brittle plastics it should be possible to improve solvent resistance, fire retardancy and oxidation resistance. However perhaps the most interesting possibility is the development of two phase or compatible polymers for selectively permeable membranes □

Complexities of vitamin D metabolism still increasing

from Alastair Hay

THERE is no disputing the activity of the kidney metabolite of vitamin D₃, 1,25(OH)₂D₃. It is an active hormonal form of the vitamin essential for calcium and phosphorus absorption from the gut, and mobilisation of calcium from bone. There is dispute, however, as to whether it is the only hormone of vitamin D. The second contender for hormonal status is the other kidney metabolite 24,25(OH)₂D₃. It has been suggested (Kanis *et al. Brit. Med. J.* 1, 1382; 1978) that this metabolite may also be a calcium-regulating hormone in man.

The arguments over the significance of these metabolites for calcium regulation were two of the themes considered at a recent workshop* on vitamin D.

To reinforce the point that 1,25(OH)₂D₃ is important, values for this metabolite were quoted for an array of clinical conditions. In contrast, 24,25(OH)₂D₃ measurements were less numerous but perhaps more contro-

versial. Serum measurements of 1,25(OH)₂D₃ were reported by B. Lund (Frederiksberg Hospital, Denmark) to be two to three times higher than normal in patients with hyperparathyroidism and acromegaly, and to be very high (~200 pg ml⁻¹) in children under 2 years of age. Values for 1,25(OH)₂D₃ in children fell progressively with increasing age but rose again during the adolescent growth spurt. Acromegaly is a syndrome in which there is an overproduction of growth hormone. This, together with the observations made in children, suggested to Lund that growth hormone is indeed involved in the regulation of 1,25(OH)₂D₃ production.

Lund reported low serum measurements of the metabolite in patients with senile osteoporosis and femoral neck fractures. Similarly in subjects with hypoparathyroidism he observed serum values of 20 pg ml⁻¹ compared with the 30-35 pg ml⁻¹ of control subjects. Serum values of 20 pg ml⁻¹ were also noted in subjects with kidney transplants M. R. Haussler (University of Arizona Medi-

*The 4th Workshop on Vitamin D was held in Berlin on 18-22 February, 1979.

cal College) also reported some low serum $1,25(\text{OH})_2\text{D}_3$ values. He measured 10 pg ml^{-1} in subjects with D-dependent rickets but in D-resistant rickets the measurements were 32 pg ml^{-1} and were similar to control subjects—a surprising finding, for the low serum phosphate levels in these subjects might have been expected to produce higher $1,25(\text{OH})_2\text{D}_3$ values. Nevertheless, Haussler reported that treatment with $1,25(\text{OH})_2\text{D}_3$ cured the rachitic lesions of D-resistant rickets and was similarly effective in the treatment of a patient with tumour-induced osteomalacia with a serum $1,25(\text{OH})_2\text{D}_3$ of $17\text{--}18 \text{ pg ml}^{-1}$.

One of the more curious twists to the $1,25(\text{OH})_2\text{D}_3$ story is Haussler's observation that female spawning trout have levels of the metabolite in excess of 75 pg ml^{-1} . The metabolite is said to be important for phosphate regulation in the eel (McIntyre *et al.* *J. clin. Endocr.* **5**(Supp.) 85S; 1976) yet neither D. R. Fraser (see *News & Views* **273**, 426; 1978) nor M. R. Holick (Massachusetts General Hospital) has been able to detect this or other dihydroxy metabolites of vitamin D_3 in fish receiving isotopically-labelled vitamin. In view of the specificity of Haussler's assay many would assume that it is indeed $1,25(\text{OH})_2\text{D}_3$ he is measuring. However, the observations of Fraser and Holick suggest that it may not be this metabolite, but perhaps one of a closely related structure.

Several authors stressed that $24,25(\text{OH})_2\text{D}_3$ may be important for calcium action in bone but not everyone is convinced that it is. S. Edelstein (Weizmann Institute, Israel) presented evidence that $24,25(\text{OH})_2\text{D}_3$ seems to be necessary for endochondral ossification of cartilage and bone. J. Kanis (University of Oxford) says the metabolite increases calcium retention in man and that this may be associated with increases in skeletal calcium accretion. And H. M. Malluche (University of Southern California School of Medicine) reported that in chicks neither $1,25(\text{OH})_2\text{D}_3$ nor $24,25(\text{OH})_2\text{D}_3$ would maintain bone integrity on their own, but that both metabolites were required for this purpose, a view put forward earlier by Ornoy *et al.* (*Nature* **276**, 517; 1978). S. W. Stanbury (University of Manchester) questions whether $24,25(\text{OH})_2\text{D}_3$ is so important for calcification of bone. In subjects treated for vitamin D deficiency with $1,000 \text{ IU}$ of $^3\text{H}\text{-D}_3$ Stanbury observed $1,25(\text{OH})_2\text{D}_3$ and $25,26(\text{OH})_2\text{D}_3$ in the serum in the first few days but no $24,25(\text{OH})_2\text{D}_3$. In some cases $24,25(\text{OH})_2\text{D}_3$ was not seen until 6 or 10 days after the start of vitamin D therapy. In view of the fact

that changes in the calcification front of bone are seen 3–5 days after the administration of vitamin D, Stanbury queried whether $24,25(\text{OH})_2\text{D}_3$ was the principal metabolite responsible for mineralisation. Could it be that $25,26(\text{OH})_2\text{D}_3$ is a more important metabolite for mineralisation he asked. Stanbury also reported that serum levels of $25,26(\text{OH})_2\text{D}_3$ were $0.5\text{--}0.8 \text{ ng ml}^{-1}$ and about a third of the $24,25(\text{OH})_2\text{D}_3$ values in D-replete subjects. J. L. H. O'Riordan (Middlesex Hospital) on the other hand found much lower $25,26(\text{OH})_2\text{D}_3$ values ($178\text{--}336 \text{ pg ml}^{-1}$ using a radioimmunoassay. The antibody used by O'Riordan has been developed for use in measuring $1,25(\text{OH})_2\text{D}_3$, however, in view of its high, and as yet unexplained, cross-reactivity with the other dihydroxymetabolites of vitamin D_3 it is being used to measure $25,26(\text{OH})_2\text{D}_3$.

Measurements of $24,25(\text{OH})_2\text{D}_3$ still tend to be controversial chiefly because of the twofold difference reported for UK and US subjects. C. M. Taylor (University of Manchester) says that $24,25(\text{OH})_2\text{D}_3$ values are about 8% of the $25(\text{OH})\text{D}_3$ values whereas Haddad *et al.* (*Arch. Biochem. Biophys.* **182**, 390; 1977) claim that in the US they are about 20%. Furthermore, Taylor says that there is no detectable $24,25(\text{OH})_2\text{D}_3$ in anephric subjects whereas Haddad, H. F. DeLuca (in *Proc. VII International Congress of Nephrology*, Montreal, 1978) and R. L. Horst (University of Wisconsin) say there is. However, it seems that these differences between the UK and US laboratories may not be irreconcilable. There are two possible explanations.

The first is one of three new metabolites of vitamin D_3 reported by H. F. DeLuca (University of Wisconsin) one of which comigrates with $24,25(\text{OH})_2\text{D}_3$ on Sephadex LH 20 chromatography, but which is separated from it on high performance liquid chromatography (HPLC). The metabolite is a lactone involving carbons 23 and 25 of the D_3 side chain. Although the lactone accounted for a sizeable portion of the $24,25(\text{OH})_2\text{D}_3$ peak on Sephadex when chick serum was used, Horst said that he had not seen it in human plasma. The other alternative is the $25,26$ -dihydroxymetabolite of vitamin D_2 which we (Hay & Jones *Clin. Chem.* **25**, 473; 1979) have reported also to comigrate with $24,25(\text{OH})_2\text{D}_3$ on Sephadex LH 20. Taylor confirmed this observation, but reported that this comigration also occurs on HPLC. It seems, therefore, that where vitamin D_2 is consumed in appreciable quantities in the diet—as it is in the US—that $25,26(\text{OH})_2\text{D}_2$ may be assayed together with $24,25(\text{OH})_2\text{D}_3$. This D_2 metabolite may also account for the $24,25(\text{OH})_2\text{D}_3$ values recorded in anephric subjects in the US.

Measurements of both $25,26(\text{OH})_2\text{D}_2$ and $24,25(\text{OH})_2\text{D}_3$ will be required before this can be answered definitively. Perhaps when these results are available the UK and US results will be seen to agree and investigators can turn once more to elucidate the actual functions of the $24,25$ and $25,26$ dihydroxy metabolites of vitamin D, and indeed that of all the other metabolites discovered over the past year, for it is quite clear that many doubts exist about some of the theories put forward so far. □



A hundred years ago

A STRIKING and highly promising line of research has been recently adopted by Mr. Muybridge, of San Francisco, at the instance of Governor Stanford, viz., the instantaneous photography of animals in motion. Some of his earlier photographs of a fast-trotting horse presented attitudes wholly unexpected, and they were even thought absurd. The method latterly adopted seems to have been making the horse trot or gallop past twelve cameras, arranged in series, and by breaking threads stretched across its path, release an electric current, which effected exposure of the plates. Thus the flying steed was obtained in every position. Mr. Muybridge gave public exhibitions of what had been done. The small negatives were magnified into life size, and projected on a screen, so that every motion was visible. These exhibitions do not seem to have been appreciated by the San Franciscans. The *Scientific American*, however, and afterwards *La Nature*, have published cuts taken from the photographs, and much general interest has been awakened in these researches. Among those specially interested is Prof. Marey, who desired to be put in communication with Mr. Muybridge, as he wanted to ask his aid in solving certain physiological problems, so difficult to solve otherwise; e.g., questions connected with the flight of birds. He had been dreaming of a kind of photographic gun, to seize the bird in an attitude or series of attitudes of flight. What beautiful zootropes, too, might be had! Mr. Muybridge's cartoons representing the fast gallop give a key to the breaking down of so many horses. It appears as though one foreleg had to sustain the whole weight of horse and rider while the body is moved along five feet. And just before the foot is raised, a perpendicular from it would strike the back of the saddle; so that there is immense leverage, the centre of gravity being thrown so far forward of its support, and the tendons must have a terrible tension. These inquiries are being further developed by the liberality of Governor Stanford and the skill of Mr. Muybridge, and valuable results may doubtless be looked for.

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articles

Ancient crustal metamorphism at low p_{H_2O} : charnockite formation at Kabbaldurga, south India

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Arrested charnockitic conversion of amphibolitic gneiss at Kabbaldurga, Karnataka State, south India, was studied mineralogically. Iron-rich pyroxenes were generated from amphibole in patches and stringers without melting. The dark colour of charnockite arises from numerous tiny veins of chlorite and manganese-bearing calcite, particularly in feldspars. The metamorphism was effected by very local, mainly grain-boundary, migration of volatiles low in H_2O , and probably dominantly CO_2 . This was followed by vein alteration at lower temperatures from volatiles richer in H_2O . The volatiles are ascribed to massive liberation from the mantle in upwelling areas, and this may have been an important process in the evolution of the deep continental crust.

CHARNOCKITE is a widespread orthopyroxene-bearing granitic rock in the Precambrian shield of southern India¹. It commonly has dark green to brown coloured fresh surfaces². Closely related intermediate to basic rocks at the Madras type locality were called the 'charnockite suite'². The abundant global distribution of the charnockite suite in Precambrian terrains is a key issue in discussions of crustal evolution³.

Charnockite apparently derives from both igneous and sedimentary rocks⁴⁻⁶ by a particular kind of metamorphism. The associated pore fluids must have been low in H_2O because the association orthopyroxene-potassium feldspar is limited to H_2O pressures below 700 bar, being replaced by either biotite-quartz or melting at higher H_2O pressure⁹. Carbon dioxide is a probable diluent: primary CO_2 -rich inclusions are characteristic of granulites¹⁰ and charnockites¹¹. The present mineralogical study of arrested charnockite development at a southern India locality shows that conversion of amphibolitic gneiss to charnockite was effected without melting by grain-boundary migration of low p_{H_2O} volatiles, presumably mainly CO_2 . A copious, possibly primitive, source of volatiles, such as a mantle plume or diapir, must be invoked.

Partial conversion of Archaean (~2,700 Myr) amphibolitic gneiss to charnockite at a quarry in Kabbaldurga, Karnataka^{12,13}, presents the opportunity for microanalytical study of amphibolite to granulite transition in both acid gneisses and basic enclaves.

The Kabbaldurga and similar occurrences in southern Karnataka¹⁴ may be the northernmost 'front' of charnockite

development which culminates in the great massifs of the Biligirirangan and Nilgiri hills. All features of the cratonic portion of Karnataka, including the Dharwar schists and younger granites, as well as the dominant Peninsular gneiss, can be traced southward as relics into the charnockite massifs.

Such large-scale conversion to granulite would culminate in a stable, refractory, and inert continental mass low in the incompatible and heat-producing elements¹⁵.

Mineralogical changes in charnockitisation at Kabbaldurga

The altered gneiss has the characteristic dark colour of charnockite and contains hypersthene in the most reconstituted specimens. Diffuse patches and stringers of charnockite, from a few centimetres to metres across, partially or completely obliterate the gneissic foliation and strongly suggest very local action of volatiles, whose access is controlled by shearing or other deformation. All stages of the amphibolite to granulite conversion occur in both the dominant acid gneiss country rock (Peninsular gneiss) and basic enclaves which are undoubtedly dykes of more than one generation, variously contorted and boudinaged. Rock analyses¹⁴ of unaltered gneiss and completely charnockitised gneiss (Table 1), show little change in bulk composition during the process. A slight depletion in K_2O may be suggested. That some basic dykes have conspicuous charnockite selvages might indicate metasomatic interchange between acid and basic rocks as a primary cause of the conversion, except that many dykes, although partially converted to granulite, have no such marginal alteration and some have it only on one side. Channelling of volatiles by less pervious basic horizons seems more probable.

Various degrees of conversion of amphibolitic to granulitic mineralogy are identifiable macroscopically and in thin section in both acid gneisses and their basic inclusions. A few specimens show some metasomatic changes related to charnockite development.

The least-altered acid gneisses are quartz-feldspar rocks with thin subsidiary mafic bands, mostly biotite-rich, but some with both green-brown hornblende and biotite. Magnetite and apatite are ubiquitous accessories. The potassium feldspar is deformed microcline with mottled and strained cross-hatched twinning and exsolution. Antiperthitic oligoclase shows strain-induced albite twinning. Undulatory quartz tends to be lenticular. Oligoclase-quartz myrmekite occasionally marks triple junctions of feldspars and quartz.

The first stages of alteration are signalled in hand-specimen by a dark greenish-brown discoloration of the larger, normally white, feldspar grains. In more advanced stages, the rock as a whole assumes a dark mottling in feldspar-rich portions with some interruption of gneissic banding. In thin section, the minerals are normal except for enhancement of strain effects. Feldspar grains are conspicuously laced with very thin greenish veinlets, which sometimes cut across grains but as often follow grain boundaries. Little change in mineralogy is evident.

At extreme alteration, the hand-specimen is typical dark-brown charnockite. All gneissic texture is gone, and hornblendes and biotites are smaller and fewer. Orthopyroxene appears abruptly in large bent grains whose margins are indeterminate because of late alteration to a dark-red, fine-grained material. Therefore, no clear-cut evidence of the relationship of orthopyroxene to biotite and hornblende exists, although these minerals presumably were its precursors. The orthopyroxene commonly has fine exsolution lamellae and about 10° of inclined extinction and is pleochroic in pale pink and green. Orthopyroxene is most abundant in mafic bands associated with large magnetite grains with quartz inclusions and intergrown ilmenite, but occasional large isolated grains are surrounded by quartz and potassium feldspar.

The thin greenish veinlets are more abundant in the most charnockitic specimens. Many feldspar grains are laced with them, and some cut across quartz grains. Many grains are enveloped by veinlets (Fig. 1), which occasionally open to tiny pods of birefringent material. The veinlets are accompanied by innumerable very brightly birefringent specks.

The dark colour of Madras charnockites was attributed¹⁶ to many apparently similar greenish veinlets cutting and enveloping feldspars. Several hours' contact with warm HCl bleached the charnockite to the appearance of a granite, undoubtedly due to removal of vein material. Chlorite was suggested as the vein filler¹⁶, and some iron loss has been observed after leaching. Qualitative microanalysis of vein material has shown major iron content¹⁷.

The basic inclusions show a more gradual progression to granulite mineralogy. In little-altered specimens the assemblage is green-brown hornblende, oligoclase or andesine and quartz, with smaller amounts of biotite, some of which is probably retrogressive. Diopside first develops in fine-grained symplectites with plagioclase marginal to large hornblende grains.

Hornblende relics in symplectite patches are optically continuous. Various stages of symplectite coarsening culminate in equigranular diopside-hornblende-plagioclase rocks. Veining of plagioclase is common, and large hornblende crystals are frequently cut by coarse biotite-calcite veins.

A few samples show strong metasomatic interaction. Gneissic contacts with granulitised metabasites may be converted to plagioclase-hornblende-diopside-quartz rocks with interstitial potassium-feldspar and typical dark charnockitic appearance. A biotite-rich altered dyke-rock has abundant large potassium-feldspar porphyroblasts associated with diopside. No hornblende is present. This dyke of ~1 m thickness has a 1 m-wide charnockite sledge on one side only. The metamorphic fluids were apparently able to transport potassium.

Microanalytic methods and results

Minerals were analysed with a ARL electron microprobe with a solid-state energy-dispersive system. Beam spot-size was 1 µm and beam current was 60 nA at 15 kV accelerating voltage.

The mafic minerals (Table 1) are richer in iron than those of the Madras type locality^{4-6,18}. The Fe/Mg ratio is higher for the minerals of the gneisses than for the basic rocks. The orthopyroxene (Fs₃₃) has a greater Fe/Mg ratio than any of the other mafic minerals, as was found for the Madras charnockites¹⁸. Note the high CaO and MnO contents of orthopyroxene. Biotite and hornblende become slightly but definitely more magnesian when orthopyroxene occurs in the acid rocks. No such change is evident as diopside appears in the basic rocks. Symplectitic diopside is not different from coarser, recrystallised diopside. The plagioclases do not change from An 27-35 as pyroxene develops in the acid rocks. Primary plagioclase in the basic rocks has a similar range with some slight normal zoning of large crystals. The symplectitic plagioclase in As 33 (Table 1) is An 24, compared to An 31 for the coarse primary plagioclase. Other than the slight zoning of some plagioclase, no significant zoning of minerals was found, thereby indicating thorough attainment of local equilibrium.

The thin greenish veins were scrutinised closely. The traces of extremely thin veins on polished feldspar surfaces gave significant iron and magnesium analyses, although the dominant contributor was wall-rock feldspar. Occasionally, a tiny speck of carbonate or chlorite in a vein gave practically uncontaminated

Table 1 Analyses of rocks and minerals from Kabbaldurga Quarry

	Whole rock		Biotite		Hornblende			Pyroxene		Plagioclase		Vein minerals		
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
SrO ₂	69.44	70.64	37.7	36.9	43.5	40.8	44.6	51.0	51.8	62.6	64.0	61.2	32.9	—
Al ₂ O ₃	13.79	13.82	13.6	13.6	9.7	12.9	9.5	0.9	1.6	23.4	23.8	25.2	11.3	—
TiO ₂	0.59	0.53	4.0	4.2	1.8	1.5	0.8	0.17	0.0	0.0	0.0	0.0	0.0	—
Fe ₂ O ₃	1.72	1.50	18.8	19.6	19.2	19.3	16.1	31.0	11.6	0.0	0.0	0.0	32.2	—
FeO	2.54	2.18												
MnO	0.02	0.07	0.0	0.0	0.3	0.5	0.17	1.6	0.3	0.0	0.0	0.0	0.2	4.8
MgO	1.01	0.82	11.9	11.2	9.7	8.3	11.6	14.5	11.6	0.0	0.0	0.0	11.6	—
CaO	2.84	2.74	0.0	0.0	10.8	11.0	11.9	1.0	21.9	4.0	3.9	6.3	0.4	61.2
Na ₂ O	3.85	4.11	0.0	0.0	2.0	1.9	1.8	0.17	0.4	9.4	9.8	8.1	0.0	—
K ₂ O	3.73	2.91	9.7	9.7	1.5	1.5	1.4	0.17	0.0	0.2	0.2	0.2	0.0	—
P ₂ O ₅	0.24	0.20	—	—	—	—	—	—	—	—	—	—	—	—
H ₂ O+	0.28	0.34	—	—	—	—	—	—	—	—	—	—	—	—
	100.05	99.86	95.7	95.6	98.5	97.7	97.8	100.3	99.2	99.6	101.6	101.0	88.7	66.0

- (1) Acid gneiss, average of two analyses, Herdman, analyst⁹
- (2) Charnockitised acid gneiss, average of three analyses, Herdman, analyst⁹
- (3) Biotite in completely charnockitised acid gneiss N3
- (4) Biotite in little-altered acid gneiss N1
- (5) Hornblende in completely charnockitised acid gneiss N3
- (6) Hornblende in little-altered acid gneiss N1
- (7) Hornblende intergrown with diopside in altered mafic dyke AS 33
- (8) Orthopyroxene in completely charnockitised acid gneiss N3
- (9) Clinopyroxene in altered mafic dyke AS 33
- (10) Oligoclase in completely charnockitised acid gneiss N3
- (11) Oligoclase in little-altered acid gneiss N1
- (12) Oligoclase in altered mafic dyke AS 33
- (13) Chlorite pod in vein, completely charnockitised acid gneiss N3
- (14) Carbonate grain in vein, completely charnockitised acid gneiss N3



Fig. 1 Grain-margin veinlet, separating twinned plagioclase from quartz. Note tiny, brightly birefringent specks in veinlet, and bending of plagioclase twin lamellae. Most charnockitised gneiss (N3). Width of photo: 0.7 mm. Partially crossed polars.

analyses. Rare pods of chlorite in thicker veins gave good analyses. The Fe/Mg ratios of heavily contaminated analyses were the same as for the pod analyses, and the veins must contain the same kind of chlorite as the pods, plus calcite. The chlorite is an iron-rich, low-aluminium variety and the calcite is manganese-bearing (Table 1). Plagioclase immediately adjacent to a vein is moderately to highly albitised, but without recrystallisation. Potassium feldspar undergoes an antipathetic reduction in sodium within a few mm of a vein to a nearly sodium-free variety. Unusual mineral specks in the veins were rich in phosphorus and vanadium.

Conclusions and discussion

Several conclusions were drawn about the charnockite-forming event seen arrested in progress at Kabbaldurga.

(1) The granulite metamorphism involved localised action of volatiles low in H_2O and, by implication, rich in CO_2 . No concomitant large changes in temperature or pressure were required. The pyroxenes were generated from amphibole without any visible evidence of partial melting. Myrmekitic intergrowths at quartz and feldspar junctures are virtually devoid of K_2O and cannot be a partial melting product¹⁹. Compositions of co-existing mafic minerals are essentially unchanged when pyroxene appears, suggesting no major change of physical conditions. Slow migration of H_2O -poor volatiles could well have been controlled by shearing, or the locations of fold hinges or other deformational features, but strong temperature differences over spaces of a few metres could not have been produced by the migrating gases. Such action of reduced H_2O activity in metamorphic fluids at more or less constant temperature and pressure was invoked to explain the regional amphibolite to granulite transition in southern Norway²⁰. In the south Norway granulite terrain, as in many others, the primary fluid inclusions of minerals are dominantly CO_2 -rich and very low in H_2O (ref. 10).

(2) The event was at relatively high level. Unfortunately, mineral assemblages in the acid or basic rocks place few controls on temperature and pressure. However, absence of garnet gives

an upper limit of ~9 kbar for acid compositions if temperatures in the upper end of the amphibolite range, 600–700 °C, are appropriate²¹. The orthopyroxene is not rich enough in iron to be useful in orthopyroxene–olivine–quartz barometry²². The ratio of tetrahedral aluminium to total aluminium in hornblende is 0.79 in metabasite AS 33 and 0.31 in acid charnockite N3 (Table 1), compared with an average of 0.80 for hornblendes co-existing with biotites in the low-pressure granulites of the southern Yilgarn Block in Western Australia²³. Aluminium is low in pyroxenes. Three to five kbar may be appropriate for the Kabbaldurga granulites.

(3) The waning phases of the metamorphism were characterised by cooler solutions, richer in H_2O , confined to the same localised channelways. Chlorite and manganese-calcite in the veins indicate lower-grade mineralisation than the pyroxene-forming event. Qualitatively, the assemblage is lower-temperature than amphibole + epidote + vapour, and has an intermediate CO_2/H_2O ratio, bounded by ankerite and epidote for CO_2 greater than about 50% of the vapour²⁴.

The veinlets must be related intimately to the pyroxene-producing metamorphism because of the strict correspondence between abundance of veins and the appearance of pyroxene, both in acid and basic rocks. Unaltered gneisses a few yards away from heavily charnockitised gneisses do not have the veins. Even partially charnockitised gneisses in which feldspars are macroscopically discoloured have relatively few veins.

From these conclusions, the picture emerges of copious streaming of low- H_2O , presumably CO_2 -rich solutions, especially along pathways created by deformation. Water is purged and, with it, the minor incompatible elements normally resident in amphiboles, micas and epidote¹⁵. Temperatures may be elevated over broad areas, but gas-streaming is unlikely to raise temperatures significantly on a very local scale. Deeper portions of the crust are converted to refractory granulite. Perhaps there is a crustal level above which, for reasons of low temperatures and readily available H_2O , the process is not operative, and Kabbaldurga is a critical horizon. Such a picture accords with the depth-zone relationship of metamorphism proposed for southern Karnataka¹⁴, culminating in the extreme south with the deep-crustal charnockite massifs.

What is the origin of the postulated CO_2 -rich solutions? Calc-silicate rocks are locally abundant in association with charnockite terrains in the southern Indian shield, and may represent former carbonate rocks. Some may be linked to charnockite development, although it is difficult to see how occasional carbonate horizons could account for charnockite development on such a vast scale as the Nilgiri massif, for instance. No calc-silicate rocks crop out in the Kabbaldurga area. Graphite-bearing rocks are common in the Precambrian, but reaction of graphite with a pore water would produce a vapour which is at least 50% H_2O (ref. 25); amphiboles could not dehydrate in such a water-rich vapour without widespread melting. Primitive sources of CO_2 are increasingly invoked to convert large portions of the continental crust to granulite^{26,27}; ascending mantle convection 'plumes' may be the answer. If these were active in the Precambrian^{28,29}, a volatile phase of initially primitive CO_2/H_2O ratio, believed to be about 3:1³⁰, would be expelled by mantle dehydration and decarbonation. During invasion of the lower continental crust the H_2O component should cause widespread melting and be filtered out, leaving CO_2 -rich ascending solutions. If geothermal gradients were quite high, as expected over the plume, a P - T field of scapolite mineralisation may be avoided³¹ and the CO_2 could continue upward to drive out water from the overlying crust. During the cooling phase of the process, H_2O might again be released by crystallisation of granitic melt at deep levels. The H_2O might mix with CO_2 still coming off the mantle plume or liberated by decarbonation or scapolite conversion to zoisite. These more H_2O -rich solutions would then follow the earlier H_2O -poor solutions, quite plausibly along the same channelways, and could have deposited the chlorite–calcite vein assemblage. Alternatively, heavy CO_2 input might 'swamp' H_2O

resident in the crust during the height of the activity, but be insufficient to lower the H_2O fraction to less than about 40% of the total during the waning stages. Whatever the exact mechanisms controlling vapour composition, combined petrographical and geophysical field work, as, gravity or magnetic surveys, should be useful in implicating or exonerating a mantle diapir as a source of the vapours.

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The symposium on the Evolution of the Archaean Crust, held in Hyderabad in November, 1977, under the auspices of IGUS-UNESCO-IGCP, and the associated field trips to Tamil Nadu and Karnataka, helped US-India collaboration in geochemistry. Electron probe analysis was supported by NSF grant no. EAR-74-22851 (RCN). Grant-in-aid NASA 14-001-171 is acknowledged by J.V.S.. We thank R. A. Howie for helpful comments.

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High magnesia liquids as the parental magma for ocean floor basalts

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The primary melt segregating from the upper mantle beneath an oceanic ridge is shown to contain ~18% MgO. Whereas these primary liquids are capable of generating the petrological features of the oceanic crust, this is not possible for tholeiitic basalts with 9–11% MgO which are highly fractionated. Compositions of liquids ranging from primary melts to high calcium picrites from the Tortuga ophiolite complex, Chile, are reported.

FOR several decades, the origin and diversity of basaltic rocks has been a foremost problem in igneous petrology. After Bowen¹ designated basalt as a primary magma which has risen to the Earth's surface chemically unmodified from its origin in the upper mantle, the predominant opinion among petrologists has been that basalts are primary mantle melts or have undergone only minor degrees of chemical modification during their ascent to the Earth's surface. In contrast to this hypothesis, O'Hara's^{2,3} review of pertinent phase equilibria in natural and synthetic basaltic systems led to the conclusion that basaltic magmas must be the result of advanced degrees of fractional crystallisation, especially at low pressures.

A major obstacle to reconciliation of these two opposing views on basalt genesis is that while phase equilibria indicate that basalts are highly fractionated, the overwhelming abundance of basalts stands in stark contrast to the complete absence, in the oceanic basins, of the proposed picritic magma of O'Hara. As a result, the concept of ocean floor tholeiitic basalt as a primary magma remains as a well accepted, albeit *ad hoc*, hypothesis.

The recent discovery of MgO-rich dykes representing liquid compositions in the Tortuga ophiolite complex, Chile, necessitates a re-evaluation of the nature of the genetic link between 'primitive' ocean floor tholeiites (9–11% MgO) and the upper

mantle. From several independent lines of reasoning, it is possible to demonstrate that magmas with ~18% MgO are primary mantle melts and the parental magmas for relatively evolved basalts with 9–11% MgO.

Composition of the oceanic crust

Ophiolites, as remnant sections of oceanic or marginal basin crust, can provide numerous constraints on the nature of magmatic evolution at a spreading ridge. A complete ophiolite sequence consists of a wide variety of igneous lithologies whose internal relationships have been described elsewhere^{4–6}.

From published data in the Vourinos ophiolite⁷, the Quebec ophiolites⁸, and the Newfoundland ophiolites⁹ as well as my unpublished data from the Tortuga ophiolite, Chile, a petrological columnar section of the oceanic crust has been constructed (Fig. 1). The thicknesses of the various petrological divisions have been constrained from geophysical data¹⁰ and measured sections in ophiolites^{7,8,11}.

An important chemical feature of the oceanic crust is that the crust becomes more enriched in ferromagnesian minerals, with increasing depth. The fractional crystallisation of ferromagnesian minerals from the magma results in the accumulation of olivine, pyroxene, plagioclase, and spinel in the magma chamber, with the extrusion of evolved basaltic liquids onto the floor of the ocean. This results in a mineralogical and chemical layering of the oceanic crust for which, excluding volumetrically minor trondhjemites and other silicic liquids, the most differentiated portion (with highest FeO/MgO) is the sheeted dykes and pillow lavas. Thus, even those sheeted dykes and pillow lavas with 9–11% MgO have undergone significant fractional crystallisation, as predicted by experimental work², and their compositions have been chemically modified since separation from the upper mantle.

Indirectly, the composition of the parental magma separating from the upper mantle can be estimated from Fig. 1 and analyses

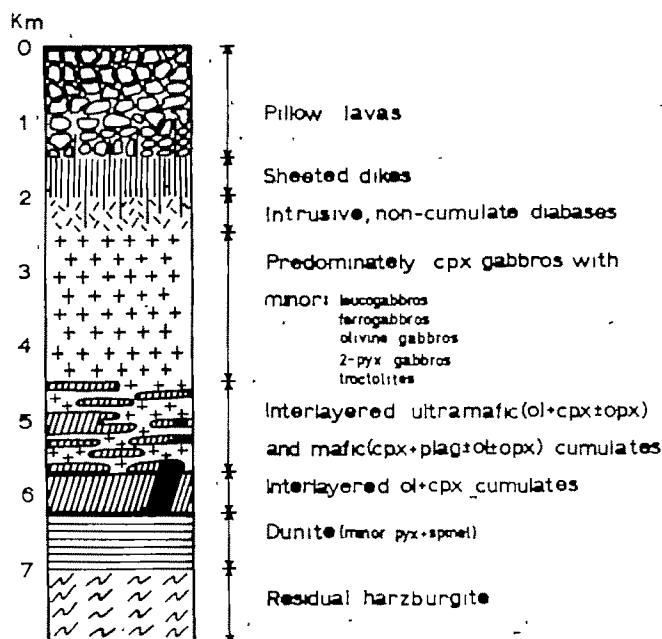


Fig. 1 A columnar section of the oceanic crust constructed from lithologies observed in ophiolites. The thicknesses of various lithological units have been constrained from measured sections in ophiolites and geophysical data. See text for details.

of rocks from the Tortuga ophiolite (Table 1). (The term parental rather than primary magma is used here to include the possibility of sub-Moho fractionation which may occur locally.) The bulk composition of the oceanic crust, used here to include all of the igneous rocks above the 'residual' harzburgite, must be equal to the composition of the parental magma as it enters the magma chamber before its extended history of fractional crystallisation and magma mixing, which result in the chemical and mineralogical layering observed in ophiolites (Fig. 1). It is critical while estimating the composition of the oceanic crust to include not only the fractionated magmas which erupt onto the ocean floor in the form of pillow lavas, but also the crystals which have accumulated within the magma chamber.

For these calculations, the oceanic crust can be divided into three sections: (1) pillow lavas, sheeted dykes, and intrusive diabases representing liquid compositions extracted from the upper portions of the magma chamber; (2) cumulate clinopyroxene-bearing gabbros with minor, associated leucogabbros, ferrogabbros, olivine gabbros, two-pyroxene gabbros, and troctolites; and (3) cumulate ultramafic and transition-zone lithologies. The composition of the average sheeted dyke in the Tortuga complex (D. E., unpublished data) is used as the representative composition for the pillow lava-sheeted dyke-intrusive diabase section of the oceanic crust. A comparison of the average sheeted dyke in Tortuga with the average Atlantic Ocean tholeiite glass¹² and the average FAMOUS glass¹³ demonstrate the chemical similarity of the Tortuga ophiolite with oceanic crust (see Table 1). As a model composition for the clinopyroxene-bearing gabbros (with the minor constituents described earlier) the average composition of the 'gabbros' from the Tortuga complex are used (D.E., unpublished data). These are genetically related to the dyke compositions and are chemically and mineralogically similar to gabbros from the ocean floor. Whereas chemical analyses for transition zone and cumulate ultramafic rocks are not available in sufficient quantity to reconstruct the chemical nature of this lowest region of cumulate rocks, the relative proportions of minerals can be estimated from Vourinos⁷ and Quebec⁸ with the mineral chemistry from these sources supplemented by the data of Church and Riccio⁹ and England and Davis¹⁴. The total cumulate ultramafic and

transition zone rocks are estimated to be 60% olivine(Fo_{85}) + 30% clinopyroxene($\text{En}_{50}\text{Fs}_{7}\text{Wo}_{43}$) + 10% plagioclase(An_{80}). From these chemical constraints, the bulk composition of the oceanic crust is estimated in Table 1. (The MgO contents can be expected to vary locally, probably from ~16 to ~20% MgO, due to the variable histories and parameters of partial melting and melt extraction.) This very magnesian composition (~18% MgO) of the oceanic crust requires that the parental magma for ocean floor basalts be equally very magnesian.

Magma generation in the upper mantle

It is widely accepted that the massive residual harzburgites found at the base of many ophiolites are the result of partial melting and extraction of magma from the upper mantle^{15,16}. As such, these harzburgites must have been in equilibrium with the parental magma of ocean floor basalts and place important constraints on the chemistry of this parental magma.

Petrologically, the harzburgites have a relatively constant olivine to orthopyroxene ratio of 80:20 with trace amounts of spinel and clinopyroxene^{15,16}. The clinopyroxene in the harzburgite has exsolved from orthopyroxene during re-equilibration in the upper mantle or has crystallised from trapped interstitial melt which was not extracted from the upper mantle^{15,16}. As such, clinopyroxene was eliminated as a residual phase during the melting event in the upper mantle and the primary melt must have been in equilibrium with olivine(Fo_{91}) and orthopyroxene(En_{91}), as these are typical compositions of residual minerals in upper mantle harzburgites^{15,16}.

The residual harzburgites from several ophiolite and alpine peridotite terrains are remarkably similar in their modal proportions, mineral chemistry, and bulk compositions^{15,16}, indicating that the process of magma generation in ophiolites and the oceanic crust is relatively uniform, with melting continuing until the magma has $(\text{Mg}/\text{Mg} + \text{Fe}^{2+}) \sim 0.77$ and is multiply saturated with olivine and orthopyroxene as liquidus phases at the pressure of final equilibration, that is, 5–10 kbar.

Melting of lherzolite compositions must progress to high melt increments to eliminate clinopyroxene and spinel resulting in melt–solid equilibria involving only olivine, orthopyroxene, and a MgO-rich liquid. Mysen and Kushiro¹⁷ have shown that such melts contain 19.6% MgO, 9.7% FeO^* , and 49.6% SiO_2 at 20 kbar with 28.5% partial melting. Boettcher *et al.*¹⁸ have produced a similar liquid by melting a spinel lherzolite at 20 kbar in the presence of CO_2 and H_2O . Similarly, high-pressure melting experiments on peridotitic komatiites¹⁹ suggest that at 10 kbar pressure, liquids in equilibrium with olivine and orthopyroxene contain 18–19% MgO.

Table 1 Lithologies used to calculate the composition of the oceanic crust

	Average FAMOUS glass	Average oceanic tholeiite glass	Average Tortuga sheeted dyke	Average Tortuga 'gabbro'	Computed TZ ultramafic rocks	Calculated composition for oceanic crust
SiO_2	50.23‡	50.68†	49.00‡	49.74‡	45.19	47.76
TiO_2	1.02	1.49	1.44	0.47	—	0.59
Al_2O_3	15.52	15.60	16.01	19.08	3.26	12.06
Cr_2O_3	0.05	—	0.05	0.10	—	—
FeO^*	9.17	9.85	11.12	5.50	9.99	8.96
MnO	0.15	—	—	0.10	—	0.12
MgO	9.11	7.69	7.81	8.89	32.91	17.78
CaO	11.89	11.44	11.77	14.36	8.21	11.20
Na_2O	2.31	2.66	1.91	1.79	0.44	1.31
K_2O	0.14	0.17	0.08	0.02	—	0.03
Total	99.59	99.58	99.19	100.05	100.00	99.81

Note the similarity between FAMOUS, average oceanic tholeiite, and Tortuga compositions. The cumulate transition zone (TZ) and ultramafic assemblages are calculated assuming 60% (Fo_{85}) + 30% ($\text{En}_{50}\text{Fs}_{7}\text{Wo}_{43}$) + 10% (An_{80}). See text for details.

‡ Ref. 13.

† Ref. 12.

‡ D. E., unpublished

Can the fractionation of basalts (~10% MgO) generate the oceanic crust?

It is generally accepted that the pseudostratigraphy of oceanic crust is a consequence of igneous processes operating at mid-oceanic ridges. The major operating processes are magma intrusion and eruption at shallow crustal levels and gravitational crystal accumulation in large magma chambers at deeper crustal levels. Hence, a prerequisite of melt ascending from the mantle is that it be capable of reproducing the observed crustal lithologies and the cryptic variations within them. The basaltic glasses recovered in the FAMOUS area are among the most magnesian glass samples recovered from the ocean floor^{13,20}. Their high *mg* values (0.66–0.72) and high nickel contents (100–260 p.p.m.) led Langmuir *et al.*²⁰ to suggest that they represent primary partial melts of the upper mantle. If this interpretation is correct, then, with reference to the discussion above, fractional crystallisation of these basalts should be capable of generating the petrochemical characteristics of the oceanic crust as shown in Fig. 1.

If we use this ophiolite constrained petrological model (Fig. 1), one constraint placed on the primary magma is that it must be capable of fractionating 10% Fo_{90} (representing the cumulate dunites) followed by fractionation of cumulate pyroxene bearing and transition zone lithologies and still be capable of extruding an ocean floor basalt from the upper portions of the crustal level magma chamber. FAMOUS glass 527-1-1 is an example of a basalt proposed as a primary mantle melt^{20,21}. Extraction of

10% olivine (Fo_{90}) from a melt of this composition (Liq_1) produces a residual liquid (Liq_2) (Fig. 2). The daughter melt (Liq_2) has a very low MgO content and *mg* value and is incapable of fractionating further to give the remaining ultramafic and mafic cumulate sequence. Thus it is concluded that basaltic melts with 9–11% MgO are incapable of producing the observed crustal lithologies and cannot be the ultimate parent to more evolved ocean floor basalts. Additionally, the recent data of Bender *et al.*²² demonstrate that 527-1-1 has undergone at least 10% fractionation of olivine and that the composition of 527-1-1 has been modified to the extent that <2% (by weight) of olivine fractionation will mark the onset of plagioclase crystallisation.

The above discussion suggests that a substantially more magnesian liquid may satisfy the petrological constraints. Hence, a similar calculation has been performed for NT-23, an MgO-rich dyke from the Tortuga complex with the results tabulated in Fig. 2. In this case, the daughter liquid remains highly magnesian (MgO = 14%, *mg* = 72) after 10% olivine (Fo_{90}) removal. Cumulate pyroxenite and transition zone minerals indicate a further removal of about 20% of olivine, clinopyroxene, and plagioclase in the ratio 40:45:15 occurs. The residual liquid (Liq_3) still has a slightly higher MgO content than FAMOUS glass 527-1-1, but is close in composition to the latter. The observed crustal lithologies and cryptic chemical variations can be best modelled by fractionation of highly magnesian melt with ~18% MgO. During this process, residual liquids approach the composition of ocean floor tholeiites,

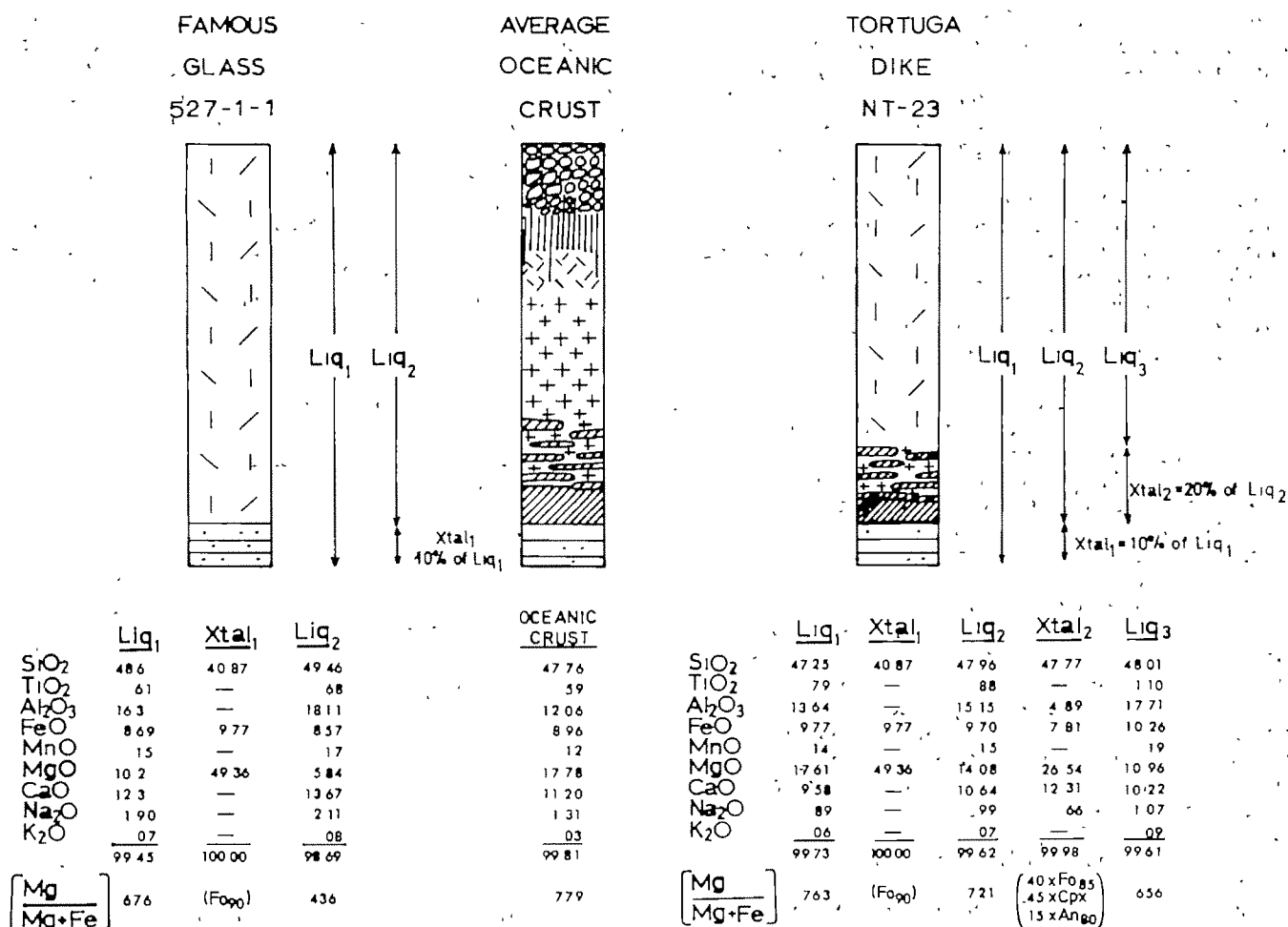


Fig. 2 Diagram demonstrating the results of fractional crystallisation of an ocean-floor tholeiite (FAMOUS glass) versus a high magnesian basalt (NT-23). The schematic columnar sections demonstrate that the FAMOUS glass is incapable of generating the petrological features of the oceanic crust, while fractionation of NT-23 is capable of reproducing these chemical features. See text for details.

including the most magnesian FAMOUS basalts, so that magmas erupted along mid-oceanic ridges are strongly evolved, secondary liquids which have been derived by almost 30% fractional crystallisation from the primary magma.

Flux of magma through the oceanic crust

Most models for the structure of oceanic crust at mid-oceanic ridges incorporate a large magma reservoir within the crust. Although direct evidence is lacking, thick cumulate sequences in ophiolites are ubiquitous and must represent the sites of former, volumetrically large magma chambers. These cumulate lithologies imply reservoirs ~5 km in vertical thickness situated just above the residual harzburgite, an estimate consistent with geophysical measurements¹⁰. The width of sea floor over which eruptions occur reflects a reasonable value for the width of the magma chamber. Measurements are scarce, but in the FAMOUS area basalts were erupted over the entire 6 km width of the median valley¹³. Therefore, a magma reservoir beneath an active ridge may have a volume of about 30 km³ (3×10^{10} m³) per km of ridge length.

If the magma chamber maintains a steady state, the volumetric addition of melt to the magma reservoir from the mantle is the product of spreading rate (~2 cm yr⁻¹) and crustal thickness (7 km), that is 1.4×10^5 m³ per yr per km of ridge length. This volume is balanced by the extrusion of $\sim 0.4 \times 10^6$ m³ yr⁻¹ of magma from the top of the reservoir per km of ridge length. However, the important consideration is that the flux of magma into and out of the magma reservoir is extremely small compared with the volume of the reservoir. (Nisbet and Fowler²³ have reinterpreted the data from the FAMOUS area to exclude any volumetrically large magma chamber from operating in the area at present. However, even if the estimate presented here is two orders of magnitude too high, the conclusions presented here remain the same.) Consequently, the addition of melt from the mantle has no measurable effect on the overall chemistry of the magma chamber. Any variation in the composition of mantle-derived melts entering the chamber will also be undetectable and it seems highly unlikely that such variations could be observed in surface eruptions. More likely, the compositional variations observed in erupted basalts²⁰ reflect fractional crystallisation and magma mixing within the crustal magma reservoir and are the result of crustal processes rather than dynamic mantle melting.

However, highly magnesian, aphyric lavas have been described from Baffin Island^{24,25} and Svartenhuk^{24,25}, and have recently been discovered as dykes in the Tortuga ophiolite

complex, Chile²⁶, indicating that occasionally such primary melts do reach the near-surface environment. As it seems unlikely that such magmas can ascend uninterrupted once a steady-state magma chamber has developed they must erupt before the reservoir development or in the dying stages of activity when the reservoir has largely solidified. The former situation may arise during the earliest stages of continental rifting and would fit the tectonic position of the Baffin Bay lavas²⁴. The latter situation may be the instance for the Tortuga dykes.

High magnesia dykes from Tortuga

Table 2 shows the compositions of four MgO-rich dykes which intrude the cumulate rocks in the Tortuga ophiolite complex. Textural and petrological evidence indicates that these dykes were intruded as liquids²⁶. The similarity of NT-23 to the Group II olivine basalts of Baffin Island and Svartenhuk²⁴ as well as the average oceanic crust (Table 1) indicates that this is a primary mantle melt and the parental magma for ocean floor basalts.

In conjunction with the data and observations of Donaldson and Brown²⁷ and Rhodes and Dungan²⁸, it is critical to evaluate the role of high calcium picritic magmas in the fractionation scheme producing ocean floor basalts. The similarities of NM-5 with Donaldson and Brown's²⁷ melt inclusions and Rhodes and Dungan's²⁸ calculated parental magma is surprising in view of the diverse methods from which these values have been derived. The gradual build-up of calcium in the magma during fractional crystallisation of the primary melt culminates in high calcium picritic liquids with ~12–14% MgO. Thus, a liquid line of descent from primary mantle melts (18% MgO) through high-calcium picrites (for example, NM-5) towards oceanic tholeiites is indicated by the compositions of these Tortuga dykes.

Conclusions

The present discussion has demonstrated the significance of highly magnesian basalts in the formation of the oceanic crust. Correlatively, ocean-floor tholeiites (9–11% MgO) cannot be accepted as primary mantle melts. High-temperature crystallisation experiments clearly indicate that oceanic tholeiites are multiply saturated with combinations of plagioclase, olivine, and clinopyroxene at low pressures^{2,3} as a consequence of low pressure fractionation. O'Hara^{2,3} has repeatedly used this powerful argument against the primary nature of oceanic tholeiites.

As a result of the steady-state magma chamber operating in the oceanic crust, O'Hara²⁹ conceptualised several characteristics of magmas in the oceanic crust which are controlled and modified by the magma chamber itself. Consequently, there remains a distinctive compositional gap between the magma segregating from the upper mantle and oceanic tholeiites with 9–11% MgO. Because this gap represents the effects of extensive fractional crystallisation and magma mixing, caution must be exercised when evaluating mantle chemistry and processes using evolved compositions.

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Table 2 Compositions of high magnesia Tortuga dykes

	NT-23	NT-20	PII*	NM-5	Melt inclusions†	Rhodes-Dungan parental magma‡
SrO ₂	47.25	47.70	49.13	50.83	50.26	—
TiO ₂	0.79	0.58	0.66	1.06	0.61	0.6–0.9
Al ₂ O ₃	13.64	14.36	16.38	14.64	14.21	—
Cr ₂ O ₃	0.20	0.12	—	0.05	0.10	—
FeO*	9.77	11.59	8.79	7.89	6.82	6.5–7.0
MnO	0.14	0.17	0.15	0.14	0.10	—
MgO	17.61	14.78	11.85	10.97	11.89	10
CaO	9.58	10.76	11.54	13.11	13.46	12.0–13.5
Na ₂ O	0.89	1.41	1.60	1.41	1.42	2.0
K ₂ O	0.06	0.14	0.04	0.14	0.07	—
P ₂ O ₅	0.07	0.02	0.06	0.07	—	—
Total	100.00	100.63	100.47	100.31	98.66	—

Note the gradual increase in calcium in the magma during fractionation of the primary melt (NT-23) resulting in high calcium picrites similar to the estimates of the parental magma for ocean floor tholeiites by Donaldson and Brown²⁷ and Rhodes and Dungan²⁸. All of the Tortuga samples are fine to very fine grain and have been altered to variable extents. Cpx (~En₄₄Di₄₂Pl₁₄), Plag (~An₇₀) ilmenite, and Picotite (Al₂O₃ = 30–40%, FeO = 24–26%, Fe₂O₃ = 1–2%, Cr₂O₃ = 23–31%, MgO = 4–9%) are observed in subophitic to interstitial textures. NT-23 contains a few per cent of plagioclase megacrysts.

* Most magnesian dyke reported by Stern³⁰.

† Average of 13 melt inclusions in spinels²⁷.

‡ Estimated composition of the parental magma for ocean floor basalts²⁸.

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Palaeoclimatic inferences from former glaciers in Scotland and the Lake District

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Firm line altitudes of Loch Lomond Advance glaciers in Scotland and the Lake District imply that snowfall was associated mainly with south to southeasterly air streams preceding fronts. Precipitation was high in the south-west Grampians and very low in the north-west Cairngorms and Speyside. The junction of polar and relatively warm ocean waters at the latitude of south-west Ireland was associated with many depressions following more southerly tracks than at present. It is suggested that during ice-sheet growth the zone of maximum snowfall moved southwards over the British Isles.

THE last ice-sheet that existed in the British Isles is generally believed to have attained its greatest extent ~17,000 BP, when its southern margin was located in the north-west Midlands. Radiocarbon dating of the basal organic material in present or former lake sites shows, if the dates are valid, that extensive areas of the Scottish Highlands had become ice-free by ~13,000 BP¹. By this time the polar water that had surrounded the British Isles had been replaced by much warmer water². This suggests that the remains of the ice-sheet would have melted rapidly, the British Isles becoming ice-free probably between 13,000 and 12,500 yr BP.

However, the polar waters subsequently spread southwards again, extending as far as the latitude of south-west Ireland³. This was accompanied by a return to cold conditions. This cold period—the Loch Lomond Stadial (equivalent to the Younger Dryas of Scandinavia)—occurred around 11,000–10,000 yr BP (more exact dating is being discussed). This paper makes climatic inferences from the glaciers that developed at this time.

Scotland

Figure 1 shows the limit of the Loch Lomond Advance in Scotland, although evidence is lacking in some areas, most notably in parts of the western Highlands, where a broken line is shown. Figure 2 shows generalised equilibrium firm lines for the former glaciers in the Highlands and Inner Hebrides based on 226 data points. Firm lines were calculated from altitude/area distributions of the glacier surfaces, using a method described elsewhere⁴.

By far the largest mass of ice was in the western Highlands southwards from the latitude of Skye (Fig. 1). This seems to suggest that snowfall was associated mainly with air streams from the west or south-west. However, superimposed on this pattern is another one: this is the contrast between north- and south-facing glaciers in many individual upland areas. For

example, on the external slopes of the Cuillin Hills of Skye (A in Figs 1 and 2) there is a continuous line of cirques facing directions that change from south-southwest through west to north-east. One might expect, from the influence of direct insolation, north-facing glaciers to have been larger and to have extended to lower altitudes than south-facing ones. However, the converse applied: the combined volume of the two glaciers that faced north and north-east was only one-third of that of the two glaciers that faced south-southwest, and the former had firm lines 300 m higher than the latter⁵.

In the central Grampians on the high ground on both sides of the Pass of Drumochter (through which run the Perth-Inverness railway and main road (A9)) ice built up to form an ice-cap, whose outlet glaciers flowed into the pass and down many other valleys (B in Figs 1, and 2). The ice-cap was asymmetrical in form, the bulk of it lying south of the east-west axis of the high ground. Equilibrium firm lines rose northwards, from below 700 m on the southernmost parts of the ice-cap to 800 m or more on its northern parts.

In the western Cairngorms (C in Figs 1 and 2) the largest glacier flowed southwards. In contrast, in the northern part of the area only three north-facing cirques contained glaciers, all of which were very small. Firm lines rose from below 800 m in the south to over 1,000 m in the north⁶.

The regional firm line in north-west Scotland increased in altitude from the coast to the interior (Fig. 2). This overall trend masks the detailed pattern in some individual upland areas. For example, in the Ben More Assynt Range, in the Fannich mountains, and in the mountain mass culminating in Ben Dearg, southward-flowing glaciers were larger, sometimes very much larger, than those that faced northwards⁷.

All the specific areas mentioned above have a north-south extent of no more than 25 km. Hence regional temperature differences cannot be invoked to explain the north-south contrasts. In fact, temperature differences related to altitude would have favoured the higher, north-facing glaciers in that here a higher proportion of the annual precipitation would have fallen as snow. Furthermore, the influence of direct insolation opposes the pattern described above. Hence some major factor must have been over-riding these influences. I suggest the only possible explanation is much higher precipitation on the southern sides of individual upland areas than on their northern sides. This in turn implies that the principal snow-bearing air streams were from southerly directions.

The firm lines in the south-east part of the Grampian Highlands, corrected for the influence of direct insolation (unpublished data), trend almost exactly parallel with the Highland Fault, rising northwestwards from 500 m to over 800 m (D in Fig. 2). Along the fault there is an abrupt increase of

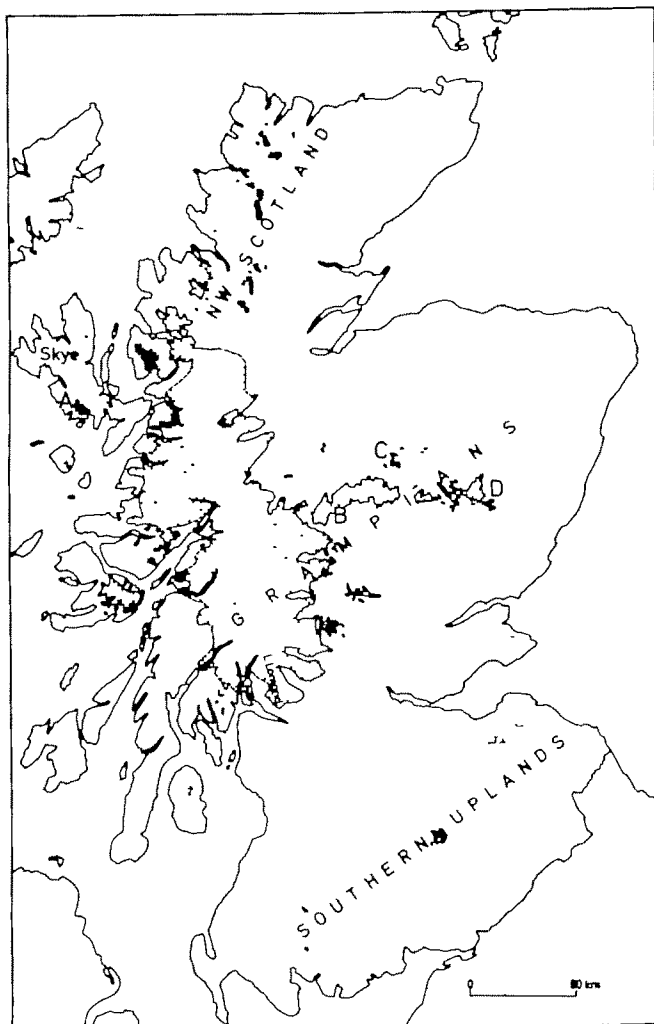


Fig. 1 Limit of the Loch Lomond Advance in Scotland. The map includes unpublished information provided by C. K. Ballantyne, R. Cornish, M. Robinson, D. G. Sutherland, P. Thorpe and T. Wain-Hobson. For reference letters see text.

several hundred metres in ground altitude. The firn lines imply that, at any given altitude, snowfall was heaviest along the Highland Edge and diminished thence northwards into the interior of the Highlands. This indicates uplift of air streams as they crossed the Highland Edge. Such uplift could not have occurred with southwesterly air streams, for they would have flowed parallel with the Highland Edge: with snow-bearing winds from southerly directions, it must have been south to southeasterly air streams that brought the principal snowfalls to the south-east Grampians.

Lake District

The Lake District differs from the preceding examples in that there is no south to north rise in firn line altitudes (unpublished data). In fact there is no significant trend in any direction. A major factor here was snow transfer to glaciers from surrounding higher ground by wind. Potential snow-blowing areas were measured on Ordnance Survey maps for each glacier and each quadrant, using rules specified elsewhere⁸. Each snow-blowing area was divided by its associated glacier area to give a snow-blowing ratio. Regression of firn line altitudes on snow-blowing ratios for the north-west quadrant for all glaciers indicated no significant relationship. This applied also to the north-east quadrant. For the south-west and south-east quadrants, however, there are highly significant inverse relationships. Thus some glaciers were relatively low because of snow blown from

the two latter directions and this distorts the distributional pattern of firn line altitudes.

Snow-blowing would have been most important during and immediately after snowfall, so that the above relationships again point to the importance of snowfall with southerly winds. The synoptic situation implied is one in which warm or occluded fronts approached from the west or south-west, the major snowfalls occurring with south to southeasterly winds before fronts arrived, succeeded by further snowfalls with moist south to southwesterly air streams after fronts had passed. Frequent repetition of this situation necessitates many depressions following more southerly tracks than are normal at the present day, this in turn being related to the junction of polar and relatively warm ocean waters at the latitude of south-west Ireland.

The largest glaciers in the Lake District were along the east-west mountain axis, especially in the central mountain area. This reflects the strong influence of relief on precipitation, as at present. However, despite much high ground, glaciers were very small in the north-west (north and north-east of Ennerdale): and size alone exaggerates their importance, for all of them occupied locations relatively sheltered from direct insolation and many have high snow-blowing ratios for the south-east and/or south-west quadrants. This implies relatively low precipitation in the north-west Lake District and points to southerly air streams, especially south to southeasterly ones, as having been associated with the principal snowfalls, the north-west being in the precipitation shadow of the central mountain area.

Snowfalls and glacier growth

Although south to southeasterly air streams were associated with the major snowfalls in the Highlands, moist southwesterly air streams would have been increasingly important providers of

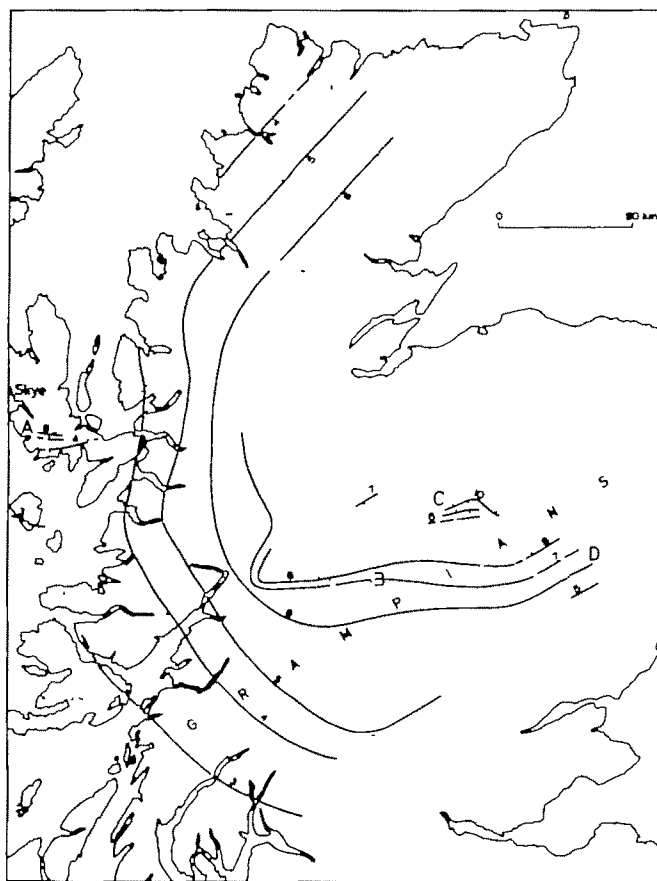


Fig. 2 Equilibrium firn line altitudes for Loch Lomond Advance glaciers in the Highlands and Inner Hebrides. Altitudes in hundreds of metres. For reference letters see text.

precipitation towards the west. Hence the largest ice mass developed in the west (favoured also by relief) and the regional firn line was lowest (300 m) in the south-west Grampians, which was most exposed to air streams from both directions. Present precipitation in the former glacier accumulation areas of the south-west Grampians is 3,000–4,000 mm. In stadial times it would have tended to be less due to lower air temperatures, but more due to the much more vigorous interaction of air masses⁸. Assuming these two tendencies roughly cancelled each other, we may obtain an indication of former summer temperatures using Liestøl's graph (Fig. 3). Allowing for 20–25% summer rain, this graph indicates that with a summer temperature of $3.5 \pm 0.5^\circ\text{C}$ the range of annual precipitation covered is 2,700–4,000 mm. I suggest this considerable range includes all reasonable possibilities. Using the 400 m firn line this is equivalent to a temperature at sea level (May–September) of 6.0°C , or a July temperature of 7.5°C . As these temperatures are derived from firn lines for glaciers in equilibrium, temperatures during the stadial would have been slightly lower.

In the Grampians the firn line rose 700 m (Fig. 2). That this rise was due mainly to precipitation differences rather than to temperature differences is indicated by the great contrasts in glacier dimensions. For example, in the south-west Grampians the Loch Lomond glacier alone had a volume of about 80 km^3 , whereas the combined volume of the 17 glaciers that existed in the Cairngorms, despite much higher altitudes, was only 1.7 km^3 . The glaciers in the north-facing cirques of the north-west Cairngorms were tiny and four cirques at 1,000 m altitude failed to nourish glaciers, suggesting extremely low precipitation here. An indication of the amount may be obtained as follows.

Assuming 750 yr for glacier growth, the average annual net accumulation on the Loch Lomond glacier above the equilibrium line was slightly over 600 mm (water equivalent). For the three north-facing Cairngorm glaciers the values are 100–150 mm. This suggests that precipitation on the Loch Lomond glacier was 4–6 times that of the three Cairngorm ones. Even taking the maximal value of 4,000 mm annual precipitation for the south-west Grampians proposed above, this implies only about 800 mm in the north-west Cairngorms. However, even this low value seems to be too high, for the following reasons. (1) During much of the time it was advancing the Loch Lomond glacier would have been subject to loss through calving in the ablation season. (2) This glacier flowed southwards whereas the three Cairngorm glaciers flowed northwards. (3) The latter were backed by plateaus from which snow would have been blown onto them by southerly winds. (4) The above estimate includes an allowance of 20–25% for summer rain, which seems reasonable for the south-west Grampians but is almost certainly too high for the high-altitude glaciers of the north-west Cairngorms. Taking these four factors into account, I suggest that annual precipitation at an altitude of 1,000 m in the north-west Cairngorms may well have been only 500–600 mm. At the much lower altitude of the floor of the Spey Valley 200–300 mm seems a reasonable estimate. Birks and Mathewes⁹ concluded from pollen analyses that the Spey Valley had "a rather arid climate" during the Loch Lomond Stadial.

Figure 1 also shows the very small area covered by glaciers in north-west Scotland. This partly reflects the much smaller area of high ground compared with further south in the Highlands. This is not apparently the sole cause, however. The average firn line altitude for 68 glaciers in north-west Scotland is 520 m. For 64 glaciers in the Lake District it is 540 m. As these two areas differ in latitude by 400 km, one might reasonably expect a much lower average firn line in north-west Scotland due to the influence of temperature. The small difference therefore suggests that precipitation was considerably lower in north-west Scotland than in the Lake District. The former probably received much of its precipitation from occasional depressions whose centres normally passed to the north-west of the area as they tracked northeastwards. But the majority of depressions took more southerly tracks (related to the junction of cold and relatively warm ocean waters) and this seems to have been the

basic cause of the precipitation deficiency in north-west Scotland.

Ice-sheet accumulation

This interpretation seems relevant to ice-sheet accumulation. It is known that when the last ice-sheet existed, the Southern Uplands of Scotland were a major centre of ice accumulation. In the west the Southern Uplands ice at first flowed northwards, despite the opposition of Highland ice, before the latter deflected it. The Southern Uplands ice extended nearly to the site of Edinburgh and it produced the major assemblage of ice-moulded features in the Tweed basin. However, during the Loch Lomond Stadial there were only paltry glaciers in the Southern Uplands. Some of these glaciers are not shown in Fig. 1 because details are not available, but the mapping is complete in the west (Loch Doon Basin and vicinity), which had previously been a major centre of ice-sheet radiation.

The question thus arises: why were ice-sheet conditions so different from those in the Loch Lomond Stadial? A lower firn line during ice-sheet build-up than during the stadial would have allowed glaciers to accumulate more widely in the Southern Uplands. However, a lower firn line would also have benefited the Highland ice. If we regard Fig. 1 as portraying a stage in ice-sheet growth, the lowering of the firn line would probably have caused the Highland ice to overwhelm the Southern Uplands before they could develop a large ice mass.

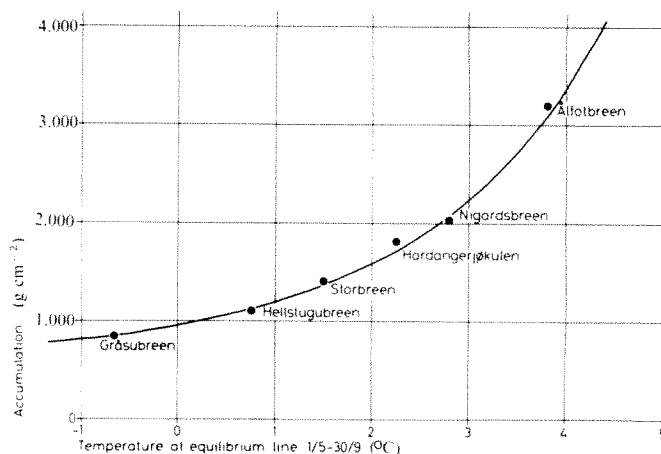


Fig. 3 The relationship between accumulation (winter balance) and summer temperature at the firn line for certain Norwegian glaciers. Compiled by O. Liestøl.

Conclusions

As the oceanic polar front moved southwards during ice-sheet growth, and with it the polar front in the atmosphere, I suggest that the zone of maximum precipitation also moved southwards. During the Loch Lomond Stadial, precipitation seems to have been deficient in the northern mainland of Scotland. We may envisage the next stage as a decrease in precipitation over the main Highland ice mass and an increase over the Southern Uplands. This idea can also explain why the Lake District, with only 3.3 km^3 of ice during the Loch Lomond Stadial, was an important centre of ice radiation in ice-sheet conditions. Continued southwards movement of the zone of maximum precipitation can explain why the Welsh uplands, where stadial glaciers were tiny, had an extensive plateau ice-cap feeding into the ice-sheet.

If this idea is valid one consequence is as follows: Many maps of the glaciation of the British Isles have shown ice from the Scottish Highlands flowing down the Firth of Clyde and through the Irish Sea to reach south-west Wales and the English Midlands. Among the erratics that contribute to this picture are

the famous ones of Ailsa Craig. However, if the zone of maximum precipitation moved southwards as an ice-sheet built up, thus affecting the relative importance of different parts of the ice-sheet, one may envisage erratics having been transferred from one area of local ice dominance to the next, without the occurrence of the continuous flow apparently suggested by erratics.

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Transmission of allosteric effects in DNA

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Binding of distamycin induces a cooperative transition of calf thymus DNA to a new form with higher affinity for the drug and altered structural properties.

A PROTEIN or other ligand bound to one section of DNA can affect the expression of genetic information in a different DNA segment by two general mechanisms. The first of these is direct physical interaction between one bound protein and another. For example, promoter and repressor binding sites may overlap, as in the *Escherichia coli lac* operon¹, so that binding of repressor interferes sterically with polymerase binding, producing negative control. Positive control by direct interaction could result from favourable contacts between polymerase and a bound activator.

The alternative possibility for exertion of control-at-a-distance is indirect influence of one bound protein on binding of another because of structural alteration of the DNA that intervenes between the two sites. We refer to this as transmission of allosteric effects through DNA, a mechanism which though purely conjectural is supported by enough indirect evidence to require its serious consideration. For example, two arabinose promoters whose polymerase binding sites are separated by 100 base pairs clearly interact². At the level of model studies the size of the DNA region excluded by binding of drugs such as actinomycin³ or ethidium⁴ is larger than the size of the drug molecule as measured along the helix axis. Therefore, the neighbour exclusion effect⁵ must be propagated through the DNA helix; a specific stereochemical mechanism has been proposed by Sobell *et al.*⁶. The clearest example of transmission of long-range allosteric effects in a polynucleotide is the demonstration by Pohl *et al.*⁷ that ethidium binding affects the balance between the high and low salt double-helical forms of poly d(GC). Drug binding at high salt concentration was markedly cooperative, implying interaction over hundreds of base pairs. Furthermore, Wells *et al.*^{8,9} have provided evidence for transmission of structural effects along DNA.

Our purpose here is to show that a naturally occurring drug, distamycin, alters cooperatively the properties of a natural double-helical DNA. The results are interpreted as an allosteric conversion of DNA to a form that exhibits increased distamycin binding, and imply that a length of hundreds of base pairs of the average DNA is altered by drug binding.

Cooperative binding of distamycin to calf thymus DNA

Distamycin is a basic oligopeptide which exhibits strong binding to DNA¹⁰. The interaction is only weakly ionic¹¹ and does not involve intercalation¹²; attachment probably occurs in the small groove^{12–14}. Circular dichroic spectral changes imply a substan-

tial DNA conformational change^{11,15}; viscosity measurements reveal a length increase and perhaps an increase in persistence length¹⁶. Binding occurs preferentially to AT-rich regions^{16,17}.

We used a phase partition technique to measure the equilibrium binding of distamycin to calf thymus and *E. coli* DNA. This method was chosen because aqueous solutions of distamycin are too unstable to allow equilibrium dialysis, and optical titrations require the questionable assumption of a linear relationship between optical change and extent of binding. Figure 1 shows the marked contrast in binding properties observed for the two DNA samples. Cooperative binding of distamycin to calf thymus DNA is indicated by the pronounced maximum which occurs at about one drug molecule bound per 30 base pairs. Much weaker binding (except in the limit as the extent of binding approaches zero) characterises *E. coli* DNA, the monotonically decreasing curve indicating that cooperative interactions are absent. A cooperative binding isotherm was also found for distamycin with calf thymus DNA in 1 M salt concentration (data not shown).

DNA length changes on binding

We used the method of transient electric dichroism of rod-like DNA molecules¹⁸ to measure apparent length changes that occur in DNA on distamycin binding. Figure 2a and d show such data for calf thymus and *E. coli* DNAs, again revealing a distinct difference between the two samples. The apparent length of calf thymus DNA molecules increases by about 13% at a ratio of one drug molecule bound per 30 base pairs, and decreases again as more distamycin is added. At the maximum of the curve the calculated length increase is about 14 Å per drug molecule, much greater than we observed for intercalating drugs (2–4 Å) by the same technique¹⁹. *E. coli* DNA, on the other hand, showed no detectable length increase in the same conditions (Fig. 2d). Other DNA samples, such as those from *Clostridium perfringens*, *Micrococcus luteus* or human placenta changed in length by only a few per cent (data not shown) without evidence for the local maximum observed for calf thymus DNA. The antibiotic netropsin produced length changes in *E. coli* and calf thymus DNAs closely similar to those found for distamycin (manuscript in preparation).

Ethidium as a probe of altered DNA structure

To obtain further evidence for an alteration of DNA structure by distamycin, we studied ethidium binding in the presence of distamycin. We found a striking change in the orientation of the bound ethidium chromophore (at $r_{\text{ethidium}} = 0.1$) in the presence of distamycin at $r_{\text{distamycin}} = 0.03$ (this r value corresponds to the maxima of both the distamycin binding isotherm and the distamycin-induced length increase).

The reduced dichroism, ρ , of a molecule orientated in an electric field is given by $\rho = \frac{1}{2}(3 \cos^2 \alpha - 1)\Phi$, where α is the angle between the transition moment and the orientation direction, and Φ is the fractional orientation of the sample^{20,21}. In the limit of infinite electric field, Φ approaches 1, and α can be calculated from the extrapolated value of ρ , ρ_∞ . The maximum negative dichroism occurs when $\alpha = 90^\circ$, yielding $\rho_\infty = -\frac{1}{2}$. Figure 3 shows the field dependence of the reduced dichroism of bound ethidium in the presence of distamycin at $r_{\text{distamycin}} = 0.03$. The much larger dichroism values observed in the presence of distamycin extrapolate to -1.5 , indicating that the ethidium chromophore is perpendicular to the helix axis. In the absence of distamycin, as we have reported¹⁹, the ethidium dichroism is considerably smaller, implying tilting of the intercalated chromophore out of the plane perpendicular to the helix axis.

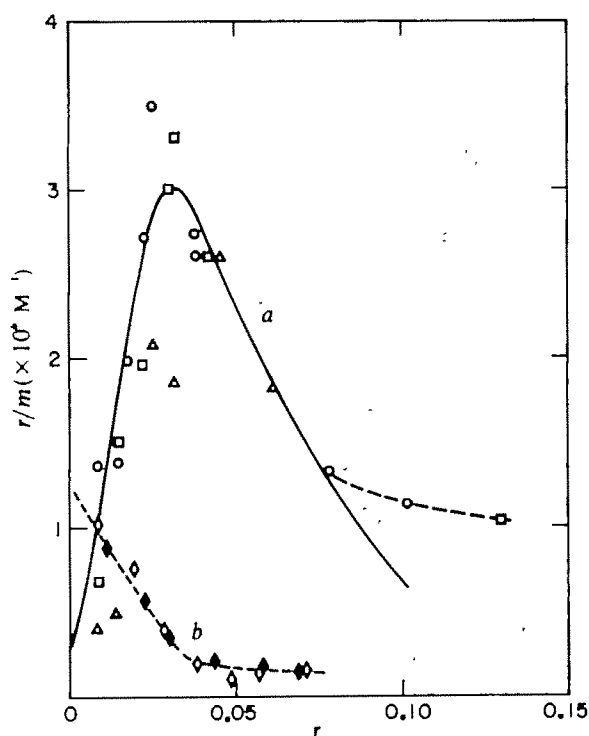


Fig. 1 Scatchard plot²⁴ of the binding equilibria of calf thymus (a) and *E. coli* (b) DNAs with distamycin at 11°C in PES buffer (50 mM NaCl, 6 mM Na₂HPO₄, 2 mM NaH₂PO₄, 1 mM EDTA, pH 6.5). Duplicate calf thymus (●, ○, □, △) and *E. coli* (◆, ◇) DNA samples were prepared by dissolving commercial (Sigma) DNA sample in 6 mM Na₂HPO₄, 2 mM NaH₂PO₄, 1 mM EDTA, 1 mM Na cacodylate buffer and sonicating for 30 min at ice bath temperature with a Branson sonicator operated at full power. Following deproteinisation by repeated phenol extraction, samples were dialysed into 8 mM Na₂HPO₄, 2 mM NaH₂PO₄, 180 mM NaCl, 1 mM EDTA and fractionated on a Sepharose 4B column. The centre portion of the elution profile was pooled and dialysed into measurement buffer. Transient electric dichroism¹⁸ and gel electrophoresis against markers of *Hae*III restriction fragments of ColEI DNA²⁵ yielded 150 base pairs as the size of the calf thymus and *E. coli* samples used for measurement. Binding isotherms were measured by partitioning the drug between *n*-pentanol and an aqueous solution of DNA. 2 ml of *n*-pentanol was added to 2 ml of PES buffer containing DNA and distamycin A (Boehringer, Sigma, purified by LH 20 (Pharmacia) chromatography in CH₃OH/CHCl₃, 9/1 volume ratio) at known total concentrations. The mixture was shaken at 11°C for 2 h, and the phases were allowed to separate. The free distamycin concentration (m) was determined from the absorbance of the organic phase ($\epsilon_{306} = 42,400 \text{ M}^{-1} \text{ cm}^{-1}$), using the partition coefficient $K = 19.5$ (organic/aqueous) determined in the absence of DNA. The total distamycin concentration (free+bound) was measured by dissociating the aqueous complex through addition of an equal volume of dimethyl sulphoxide (DMSO) (ϵ_{306} in PES/DMSO, 1:1 volume ratio = $32,800 \text{ M}^{-1} \text{ cm}^{-1}$). The bound drug concentration was calculated from the difference of free and total concentrations, and divided by the DNA base pair concentration to obtain r , the number of drug molecules bound per base pair. The solid curve (a) was calculated from a statistical mechanical theory (see text) of cooperative allosteric transition of calf thymus DNA from its initial form to another structure that has higher affinity for distamycin (manuscript in preparation).

Figure 2b, c, e, f show the results obtained from experiments similar to that in Fig. 3 but at different $r_{\text{distamycin}}$ values ($r_{\text{ethidium}} = 0.1$). The ethidium orientation angle (b) reaches a maximum at nearly the same $r_{\text{distamycin}}$ value for which the length increase is maximal. The calf thymus DNA length increase due to distamycin in the presence of constant ethidium (c) is only slightly smaller than observed in absence of ethidium (a). The control sample of *E. coli* DNA showed no variation in length or ethidium orientation angle as a function of distamycin concentration (e, f). The dichroism, ρ_∞ , of ethidium bound to *E. coli* DNA was very similar to the value found for ethidium bound to calf thymus DNA in the absence of distamycin.

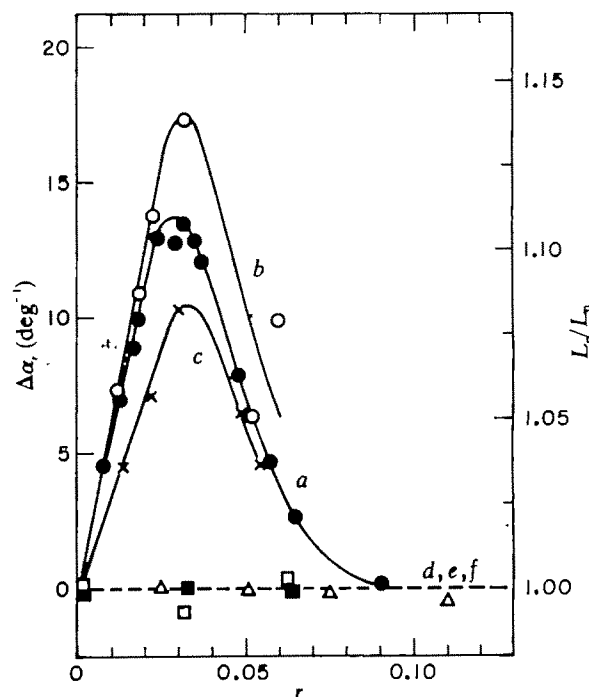


Fig. 2 Apparent fractional length (L) changes (a, c, d, f) and ethidium orientation angle (α) changes (b, e) as a function of degree of distamycin binding for calf thymus (a, b, c) and *E. coli* (d, e, f) DNA samples, prepared as described in the legend to Fig. 1 and dissolved in dichroism buffer (0.5 mM Na₂HPO₄, 1 mM NaH₂PO₄, 0.25 mM Na₂EDTA, pH 7.0) at 11°C. Additional samples of calf thymus DNA were obtained by protease K (Merck) digestion and phenol extraction of mononucleosomes isolated from HI-depleted chromatin²⁶. These samples gave results identical to those obtained with sonicated samples. Samples a and d contain no ethidium, whereas samples b, c, e and f contain a constant amount of bound ethidium, $r_{\text{ethidium}} = 0.1$. Lengths were calculated from the dichroism rise time τ and Broerema's equation²⁷ for the rotational diffusion constant of a cylinder, which we earlier verified for rod-like DNA molecules¹⁸. We assumed no change in helix diameter on drug binding, an assumption justified by the logarithmic dependence of r on diameter, compared with variation with the third power of helix length. Length values are normalised to 1 at zero distamycin concentration. Ethidium orientation angles were measured at 520 nm by extrapolation of the dichroism to infinite field as described in the text. a (●), Length change for calf thymus DNA in absence of ethidium; b (○), angle change for calf thymus DNA in presence of ethidium; c (×), length change for calf thymus DNA in presence of ethidium; d (△), length change of *E. coli* DNA without ethidium; e (□), length change of *E. coli* DNA with ethidium; f (■), angle change of *E. coli* DNA with ethidium present.

We also investigated the dichroism of ethidium at constant $r_{\text{distamycin}} = 0.03$ and varying r_{ethidium} values (Fig. 4). Up to about $r_{\text{ethidium}} = 0.1$, the dichroism extrapolated to infinite field was equal to -1.5 , but larger r_{ethidium} values led to a progressive decrease in the dichroism.

Finally, we examined the influence of bound distamycin ($r_{\text{distamycin}} = 0.03$) on the binding isotherm of ethidium, and

observed that ethidium binding becomes cooperative. We were not able to use the partition technique at the low salt concentrations used for Fig. 1, because the ethidium partition coefficient was concentration dependent in those conditions. Therefore, the data shown in Fig. 5 were measured at 1 M salt concentration. However, we verified that the distamycin-calf thymus DNA binding isotherm was cooperative in these salt conditions, with the distamycin binding affinity estimated from r/m at its peak reduced by about threefold from the value in Fig. 1. As seen in Fig. 5, r_{ethidium} values less than about 0.06 are characterised by lower r/m values than found in the absence of distamycin (open circles). Therefore, distamycin at $r_{\text{distamycin}} = 0.03$ substantially reduces the affinity of calf thymus DNA for ethidium, in the limit as r_{ethidium} approaches zero.

Interpretation as an allosteric transition of DNA

There are two general mechanisms which can lead to cooperative binding of drugs to nucleic acids, as found in Fig. 1; (1) the drugs can interact directly on the DNA lattice, as, for example, in the stacking of acridines on the DNA helix, or (2) the drug molecules can interact indirectly by altering the state of the nucleic acid. Examples of the latter are the ethidium-induced transformation of poly d(GC) from one form to another⁷, and conversion of poly dA·2poly rU from triple to double helix²². Mechanism (2) requires that the drug bind more tightly to the alternative form; for example, ethidium binds more tightly to double-helical poly dA·poly rU than to triple-helical poly dA·2poly rU. The process is analogous to allosteric conversion of a protein from a low to a high affinity form by an effector molecule.

Our results strongly support alternative (2), namely, that distamycin serves as an allosteric effector to convert calf thymus DNA from one form to another. The results with the ethidium probe are essential for this conclusion. The distamycin molecule is long enough to cover only about five DNA base pairs. Therefore, at $r_{\text{distamycin}} = 0.03$ only about 15% of the base pairs could be in contact with distamycin, leaving about 85% of the DNA molecule free. Thus, if the only effect were clustering of distamycin molecules, 85% of the DNA should show unaltered ethidium binding properties. However, the measured ethidium dichroism can reach the theoretical limiting value of -1.5 (Fig. 3) only if virtually all the ethidium molecules are bound perpendicular to the helix axis. Therefore, the 85% of the DNA that is distamycin-free cannot be binding ethidium in the same way as in the absence of distamycin, and we conclude that bound

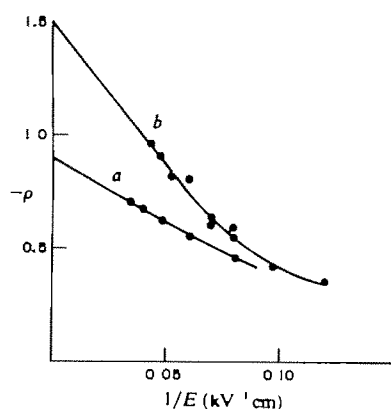


Fig. 3 Extrapolation of the observed dichroism of ethidium ($r_{\text{ethidium}} = 0.1$) to infinite field in the absence (a) and presence (b) of distamycin, $r_{\text{distamycin}} = 0.03$. Calf thymus DNA samples, dissolved in dichroism buffer, 11 °C.

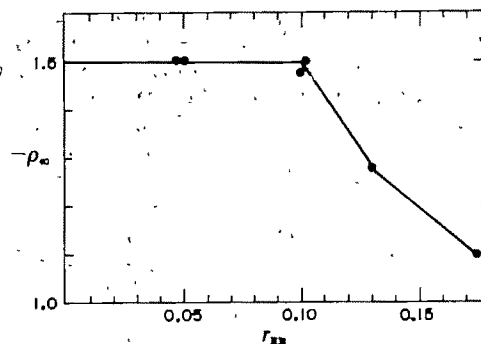


Fig. 4 Variation with extent of ethidium (E3) binding of the extrapolated dichroism of ethidium bound to calf thymus DNA in the presence of distamycin, $r_{\text{distamycin}} = 0.03$. Dichroism buffer, 11 °C.

distamycin alters the form of virtually all the DNA. This conclusion is also supported by the ethidium binding isotherm (Fig. 5), as distamycin at $r_{\text{distamycin}} = 0.03$ reduces the affinity for ethidium by at least a factor of two in the limit as r_{ethidium} approaches zero. This would not be possible if 85% of the DNA were unaltered in structure and ethidium affinity.

Comparison with theory

To determine quantitative features of the observed allosteric transition, we developed a general statistical mechanical theory of the process (manuscript in preparation). The model allows the DNA double helix to be in one of two forms: form (1) has a lower affinity for distamycin than form (2), but (1) has slightly lower free energy in the absence of distamycin and so is generally the

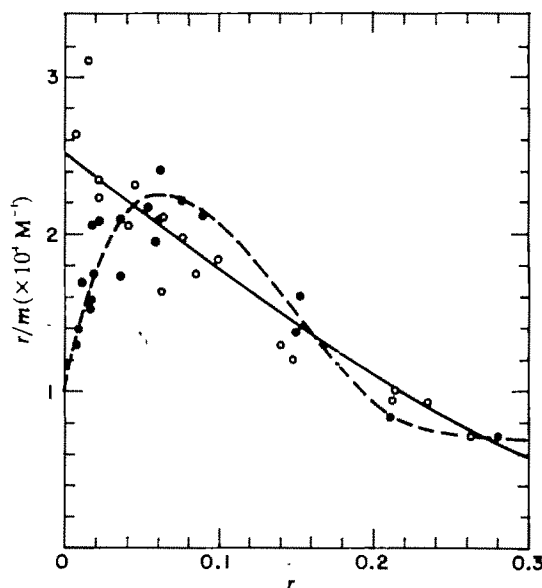


Fig. 5 Binding isotherm of ethidium to calf thymus in absence (O) and presence (●) of distamycin, $r_{\text{distamycin}} = 0.03$ r and m were measured by the phase partition technique as described in the legend to Fig. 1, except that samples were dissolved in PES buffer made up to a total concentration of 1 M in NaCl

preferred structure. A positive interfacial free energy characterises each boundary between forms (1) and (2). Figure 1 shows a comparison of the experimental binding isotherm with a theoretical curve obtained by adjusting the parameters of the theory for best fit. The calculation reveals that the rising portion of the binding isotherm is associated with the cooperative conversion of the double helix from form (1) to (2). After the

maximum is reached, the transition is essentially complete and the curve follows the binding isotherm expected for pure form (2). The intrinsic distamycin binding constants⁵ are $0.3 \times 10^6 \text{ M}^{-1}$ for form (1) and $4.8 \times 10^6 \text{ M}^{-1}$ for form (2). A neighbour exclusion range⁵ of six was assumed for form (2) so as to fit the data beyond the maximum in r/m ; a more accurate theory would have to take account of the (unknown) base sequence preference of distamycin when binding to form (2) (ref. 5). The interfacial free energy necessary for the observed cooperative transition is 3–4 kcal mol⁻¹. Only a very small free energy difference, about 0.02 kcal per mol of base pairs, is found between forms (1) and (2). However, because of the cooperative character of the transition, less than 1% of form (2) is present in the absence of distamycin.

An important result of the calculation is that the average length of form (1) and form (2) helices is hundreds of base pairs in the transition region. This conclusion has two significant implications: first, allosteric effects in DNA can be transmitted over hundreds of base pairs, and, second the theory is only approximately applicable to the measurements, as the latter were done with DNA fragments about 150 base pairs long.

Characteristics of the altered form

Thus, three features of the DNA structure induced in calf thymus DNA by distamycin binding are (1) ethidium binds to form (2) with the long wavelength transition perpendicular to the DNA helix axis; (2) the apparent length of form (2) is substantially larger than that of form (1), by at least 13%, which

is the apparent length increase at the point where the cooperative transition to form (2) is essentially complete; (3) form (2) becomes distinctly shorter in apparent length as more distamycin is bound.

Why only calf thymus DNA?

Perhaps the most surprising feature of our results is the failure to obtain the effect with any DNA except that from calf thymus, out of three bacterial and two mammalian DNA tested. We believe that this is due to the requirement for near-equality of the free energies of the two forms, and the sensitivity of this free-energy balance to the details of base sequence or base modification, or both. If the average free energy difference of 20 cal per mol of base pairs estimated between forms (1) and (2) in calf thymus DNA were increased to 200 cal per mol of base pairs (a change per base pair considerably less than the thermal energy), the transition would not be observed. Hence, we conclude that the details of DNA base modification and sequence are extremely important for the long-range transmission of allosteric effects. Thus, negative results found in particular cases cannot be taken as general evidence against propagation of structural effects along DNA²³. The finding that distamycin acts as effector of an allosteric conversion in calf thymus DNA, when considered with previous evidence⁷⁻⁹, shows that DNA can transmit structural influences. It remains to be seen, however, whether such phenomena are of general importance in genetic regulation.

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Construction of a site-specific, deletion-frameshift mutation in an essential gene of bacteriophage ΦX174

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By in vitro methods, we have deleted 80 nucleotides from a preselected site in the coding sequence of gene G of bacteriophage ΦX174. This mutant (GV/FS ZH1) also carries a –2 frameshift. The mutation is lethal, but mutant virus can be stably maintained on host strains bearing plasmids carrying ΦX gene G.

To explore molecular mechanisms of mutation by carcinogens, we have developed a system for examining the *in vivo* expression of well characterised, site-specific, covalent modifications of biologically active DNA^{1,2}. Because of the extensive chemical and biological information available^{3–5}, we have chosen an

essential gene (gene G) of the virulent phage, ΦX174, for our initial studies. As the nature of the mutation produced by a site-specific modification cannot be predicted, we need a host that is permissive for all types of mutation in gene G. To deal with this problem in the most general way, we have developed cell lines that carry ΦX gene G on a plasmid¹. These pΦXG-bearing hosts produce functional ΦX gene G protein and provide a means of rescuing ΦX174 carrying mutations in this gene.

We have demonstrated that cells carrying pΦXG are permissive for conditionally lethal mutants (both nonsense and missense) in gene G (refs 1, 2 and R.W.C., unpublished results). A crucial test of our system is to show that pΦXG-bearing host cells are also permissive for lethal mutations in gene G (frameshifts, large deletions and rearrangements).

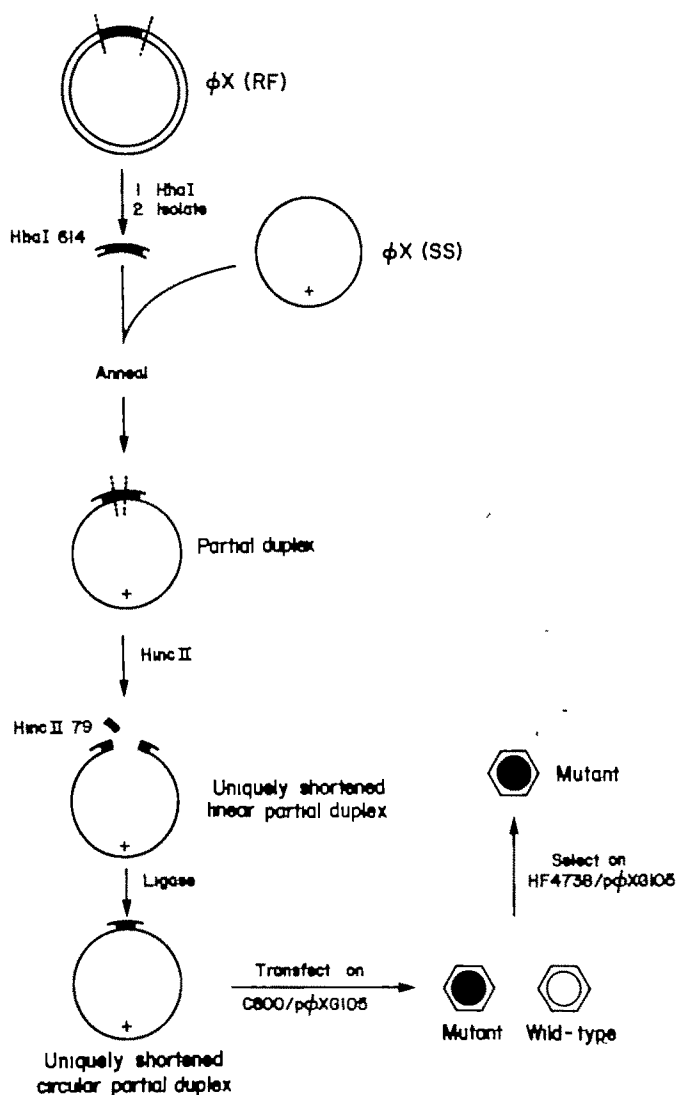


Fig. 1 Strategy for the construction of specific ΦX deletion mutants. The gene G coding sequence is indicated by the heavy black line. *HhaI* 614 (fragment H3) was isolated by procedures similar to those described elsewhere¹⁶. Wild-type ΦX viral DNA was prepared by phenol extraction of a purified phage preparation. Viral DNA (30 μ g) and *HhaI* (5 μ g) were annealed in 200 μ l of buffer (90 mM NaCl, 10 mM $MgCl_2$, 7.5 mM Tris-HCl, pH 8, and 0.75 mM EDTA) by boiling for 3 min, chilling rapidly to 0 $^{\circ}C$ and incubating at 67 $^{\circ}C$ for 2 h. The mixture (50 μ l) was made up to 100 μ l by adding buffers and enzymes to the following final concentrations: 45 mM NaCl, 12.5 mM Tris-HCl, pH 8, 5 mM 2-mercaptoethanol; 0.38 mM EDTA, 100 units ml^{-1} *HincII* (an isoschizomer of *HindII*, Bethesda Research Laboratories). Restriction was carried out for 2 h at 37 $^{\circ}C$ before termination by a phenol extract followed by three ether extractions and dialysis of the DNA against cold 10 mM Tris-HCl and 1 mM EDTA (pH 8). This DNA preparation was ligated at 20 $^{\circ}C$ for 5 h in 400 μ l with buffers and enzyme at the following concentrations: 50 mM Tris-HCl, pH 7.5; 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 0.08 mM ATP; 0.25 mM EDTA, 50 units ml^{-1} T4-DNA ligase. The reaction was terminated by one phenol and three ether extractions. The DNA was redialysed against cold 10 mM Tris-HCl and 1 mM EDTA (pH 8). Spheroplasts were prepared as described elsewhere¹⁷ from C600/p $\Phi XG105$ constructed by transforming the K12 strain C600 (Su⁻, ΦX^+) with p $\Phi XG105$ DNA by methods described elsewhere¹. The above ligated DNA (40 μ l) solution was diluted to 400 μ l with 50 mM Tris-HCl (pH 8) and used to transfect 400 μ l of the spheroplast preparation by published procedures¹⁷. Spheroplast lysates plated on the *E. coli* C/K12 strain, HF4738 ΦX^+ Su⁺ recA/p $\Phi XG105$, gave about 10⁷ plaque-forming units per μ l of the ligated DNA preparation. The lysate was screened for lethal mutants by plating on double-layer lawns of HF4738/p $\Phi XG105$ over HF4738. After 7 h at 37 $^{\circ}C$ the plates revealed that 2–5% of the plaques were small and turbid, and the remaining plaques had wild-type morphology (large and clear). Thirty turbid plaques were punched out at random with a capillary. Each plaque was suspended in buffer (50 mM sodium borate, 1 mM EDTA, pH 9). Each suspension was titrated at suitable dilutions on C, HF4738 and HF4738/p $\Phi XG105$, and nine were found to plate on C and HF4738 at a plating efficiency lower than 10⁻⁴ compared with that on HF4738/p $\Phi XG105$. One of these suspensions constituted the seed stock for ΦX GV/FS ZH1.

Here, we report the *in vitro* construction of a site-specific deletion-frameshift mutant, GV/FS ZH1, in which residues 2,513–2,592 (80 bases) of the wild-type sequence have been deleted. This also produces a –2 frameshift in which 14 in-frame, nonsense codons are generated at, and downstream from, the site of the deletion. We will show that this mutation is lethal, but the mutant virus grows in host cells carrying p ΦXG . The procedure we have used for constructing this deletion frameshift is simple and specific. It should be applicable not only to other regions of $\Phi X174$ DNA but also to other interesting DNAs, provided that a system for growing the mutant is available. The results also demonstrate that it should be possible to rescue most $\Phi X174$ gene G mutants regardless of the origin or nature of the mutation.

Construction of $\Phi X174$ GV/FS ZH1

Figure 1 is a scheme of the procedures used for the construction and isolation of the deletion mutant. This involved the following steps. (1) ΦX wild-type replicative form DNA (RF DNA) was cleaved with the restriction endonuclease *HhaI*, and the fragment *HhaI* 614 (H3)³, was isolated. This fragment contains all the translated portion of gene G, 29 of the nucleotides preceding the translated portion of gene G and 49 residues from gene H (ref. 3). (2) Wild-type ΦX single-stranded viral DNA (SS DNA) was annealed with double-stranded restriction fragment *HhaI* 614. (3) The ΦX SS DNA–*HhaI* 614 partial duplex was cleaved with *HincII* (an isoschizomer of *HindII*) at the known *HindII* sites, R9/10 and R10/2 (ref. 3). These cut sites are 79 nucleotides apart and include residue 2,571, the preselected target for our projected modification studies. (4) Without isolation, the specifically shortened, linear, partial duplex was rejoined by blunt-end ligation with T4 DNA ligase to obtain a covalently closed, circular, partial duplex with a unique deletion. (5) Infectious virus particles were produced by transfection of C600/p $\Phi XG105$ spheroplasts with the mixture containing uniquely shortened circular partial duplex DNA. (6) Mutants were selected from the resulting phage population by plating on double-layer lawns of HF4738/p $\Phi XG105$ over HF4738.

In a typical experiment (see legend to Fig. 1), 2% of the phage population obtained after step (5) were mutant by the criterion of plaque formation on HF4738/p $\Phi XG105$ but not on the parent strain HF4738. Three such mutants were randomly selected for further analysis and were found to have similar properties. Most of the experiments described below refer to one of these mutants, named GV/FS ZH1.

Biological characterisation of GV/FS ZH1

Table 1 lists some of the ΦX -sensitive host strains that have been tested for plaque formation with ZH1. (The abbreviation GV/FS ZH1 means a deletion and frameshift mutation in gene G, isolate ZH1. For simplicity, we will mainly use the shortened designation ZH1.) The data show that the mutant will not grow on host strains without p $\Phi XG105$. These experiments demonstrate that the genetic defect in ZH1 is lethal, and is confined to gene G, as p $\Phi XG105$ is a well characterised plasmid known to complement for gene G product(s) alone (refs 1, 2; M.Z.H. and R.W.C., unpublished). Also, in a complementation experiment carried out by methods similar to those described by Zuccarelli *et al.*⁶, the gene H amber mutant, N1 (ref. 7), but not the gene G mutant, am9 (ref. 5) is rescued by ZH1. No infective particles are observed in artificial (freeze-thaw) lysates of the wild-type host, *Escherichia coli* C, infected with ZH1. Thus, a defect in lysis cannot be solely responsible for the lethality of the mutation. ZH1 does not grow on *E. coli* C at either 30 or 42 $^{\circ}C$.

Single-cell, single-burst experiments, as well as one-step growth experiments⁸, revealed that ZH1 has a burst size of about 20 on HF4738/p $\Phi XG105$. Wild-type ΦX has a burst size of 200–300 on a similar strain with or without p $\Phi XG105$ (M.Z.H., unpublished results). We have recently shown that the small burst size of *Gam9* on p ΦXG -bearing strains is attributable to its known polar effect on the expression of gene H (M.Z.H., unpublished results). The low burst size of ZH1 is

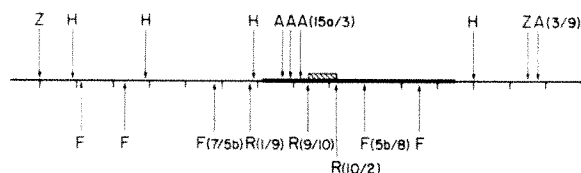


Fig. 2 A schematic representation of a part of the physical map of ΦX wild-type RF DNA to show the site of the deletion in *ZH1* (hatched rectangle). Each unit is approximately 100 base pairs. The cutting sites of some relevant restriction enzymes are indicated by vertical arrows identified by the letters A(*AluI*), F(*HinfI*), H(*HhaI*), R(*HindII*) and Z(*HaeIII*). Fragments separated by cleavage at some of these sites³ are shown in parentheses. The coding sequence for gene G principal product is indicated by a thick line.

probably a result of nonsense codons generated downstream from the deletion.

Recombination occurs between *ZH1* and p ΦX G105, although it is quantitatively less than recombination between *Gam9* and p ΦX G105 (ref. 1). The level of recombination is further reduced by at least two orders of magnitude in a *recA*⁻ background (such as HF4738) as compared to a *rec*⁺ background (H514). For example, in one experiment single plaques produced by *ZH1* on plasmid-bearing *rec*⁺ strains, picked after incubation for 5–8 h at 37 °C, showed 1–5% wild-type recombinants, whereas similar plaques formed by *Gam9* showed at least 50% wild type. On a *recA*⁻ lawn, *Gam9* and *ZH1* gave 10^{-4} and 10^{-5} wild-type levels, respectively, in plaques picked after incubation for about 5 or 6 h at 37 °C.

These data show that *ZH1* is a gene G mutant; it is not temperature sensitive, and does not grow on any of the nonsense suppressor strains tested (Table 1). It can be maintained in a stable, apparently normal, virulent cycle on host strains carrying p ΦX G, implying that it is capable of all phage functions except those performed by the gene G product(s). It has a low burst size on p ΦX G-bearing strains, and its recombination frequency with p ΦX G is lower than that of the point mutant, *Gam9*, with p ΦX G.

Characterisation of *G^v/FS ZH1* DNA

ZH1 RF, as well as SS DNA preparations, have electrophoretic mobilities very close to those exhibited by the corresponding

wild-type DNAs on 1% agarose gels. The predicted deletion (approximately 1.5% of the ΦX genome) is not expected to change the mobility of *ZH1* DNA appreciably compared with wild type in these conditions.

The known restriction sites in gene G of ΦX 174 RF DNA and the method of constructing *ZH1*, enable us to draw a new (expected) cleavage map for *ZH1* (Fig. 2). This prediction was examined with five restriction enzymes.

Analysis of cleavage products from *ZH1* RF DNA produced by four restriction endonucleases (*HaeIII*, *HhaI*, *AluI* and *HinfI*; Fig. 3) revealed that 80 ± 3 base pairs have been deleted from wild-type DNA, as expected. Digestion of *ZH1* with *HincII* revealed not only the loss of fragment R10 (79 base pairs), as expected, but also a fusion between fragment R9 (162 base pairs) and fragment R2 (770 base pairs) that flank fragment 10 in the wild type⁹. This generates a new fragment about 930 base pairs long. Our construction strategy was expected to regenerate a single *HincII* site by fusing the left half of the site separating R9 and R10 and the right half of the site separating R10 and R2 in the wild-type DNA⁹. Our results with *HincII* (Fig. 3) show that this expected site was not formed. All the restriction fragments seem to be sharp and, with the exceptions noted above, identical to the corresponding wild-type fragments (Fig. 3). This suggests that except for the deletion, the wild-type sequence has been conserved in *ZH1* to the extent definable by restriction.

To characterise the mutation precisely, we determined the DNA sequence of *ZH1* around the expected site of the deletion as well as around other selected parts of the gene G coding sequence³. The results (Figs 4, 5) unambiguously show the loss of an 80 nucleotide-long sequence extending from base number 2,513 to 2,592 of the revised sequence by Sanger *et al.*⁹. Of these deleted 80 nucleotides, 79 are present in the *HincII* fragment 10. The loss of a single, additional base pair (CG) at position 2,513 eliminates the *HincII* site that would otherwise be regenerated by ligation (Fig. 1). Although we cannot rule out an *in vivo* event for the loss of this base pair, we believe that *ZH1* has been derived from a parent molecule in which this additional loss occurred during *in vitro* manipulations. Some of our original mutant isolates may have the expected deletion of only 79 nucleotides. Except for the deletion, we observe no deviation in the *ZH1* sequence so far determined, compared with the wild-type sequence in gene G (ref. 9).

Fig. 3 Fractionation of restriction endonuclease products from ΦX *G^v/FS ZH1* RF DNA by polyacrylamide gel electrophoresis. The DNA was prepared by infecting *E. coli* C grown in 3XD to 5×10^8 cells ml⁻¹ with ΦX *G^v/FS ZH1* ($<10^{-4}$ wild type) at a multiplicity of infection of 3. Ten min after infection, chloramphenicol was added to a concentration of 30 μ g ml⁻¹ and shaking was continued for 3 h at 37 °C. Lysis, removal of host DNA and ethanol precipitation were carried out exactly as described by Godson and Vapnek¹⁸. The DNA pellet from a 1-l culture after ethanol precipitation was suspended in 20 ml 50 mM Tris-HCl:5 mM EDTA (pH 8). The turbid suspension was extracted three times with phenol using centrifugation to separate the phases after each extraction. The aqueous layer was extracted three times with ether and the solution dialysed against the above Tris-EDTA buffer. The solution was adjusted to pH 12.11 at 25 °C with NaOH. The pH and ionic strength at this step are critical¹⁹. The pH was measured with a radiometer type B glass electrode with a negligible Na⁺ correction in these conditions. The electrodes were standardised with fresh Ca(OH)₂ just before use. The pH was maintained for 10 min at 25 °C before neutralising the solution with HCl. Pancreatic RNase A (20 μ g ml⁻¹; heat treated at 70 °C for 15 min) was added and the solution was incubated at 37 °C for 30 min before concentrating *in vacuo* to ~2 ml and fractionating the DNA on a Sephadex S200 column (2.5 $\times$ 35 cm), equilibrating and eluting with 0.3 M NaCl and 0.03 M sodium citrate, pH 7 (Sephadex G-100 gives similar results). Fractions containing the RF DNA eluted near the void volume as a discrete peak. These fractions were pooled and dialysed extensively against cold 10 mM Tris-HCl and 1 mM EDTA (pH 8). The average yield of this preparation is about 1 mg of RF DNA per l of culture. About 2 μ g of *ZH1* or wild-type RF DNA were digested in 100 μ l with *HaeIII*, *HhaI*, *AluI* or *HinfI* and the products were fractionated on 5% polyacrylamide slab gels¹⁶. The *HincII* digest was end-labelled with ³²P as described elsewhere¹⁶ and fractionated on 10% (a) and 5% (b) polyacrylamide gels. The wild-type fragments affected by the deletion are indicated by solid arrows; the fragment resulting from the deletion is shown by a broken arrow. The approximate size in base pairs is indicated and is based on electrophoretic mobility relative to the known wild-type fragments.

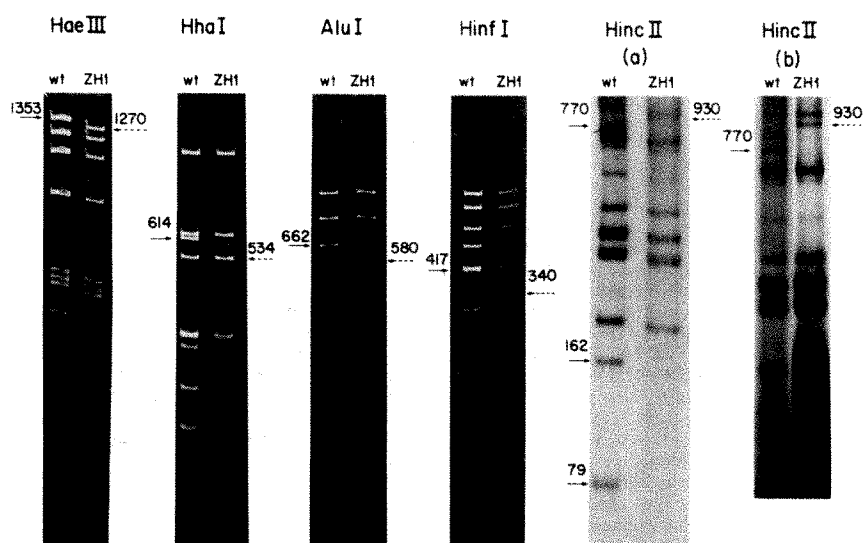


Table 1 Permissivity of Φ X174 *ZH1* on various host strains

Host strain and relevant characteristics	Plaque formation by Φ X174 <i>ZH1</i>	Efficiency of plating relative to HF4738/p Φ XG105
C (rec ⁺ , Su ⁻)	-	<10 ⁻⁴
H514 (rec ⁺ , Su ⁻)	-	<10 ⁻⁴
HF4704 (rec ⁺ , Su ⁻)	-	<10 ⁻⁴
HF4714 (rec ⁺ , Su ₁ ⁺)*	-	<10 ⁻⁴
HF4738 (rec ⁻ , Su ₁ ⁺)*	-	<10 ⁻⁴
HF4740 (rec ⁻ , Su ⁻)	-	<10 ⁻⁴
C1757 (rec ⁺ , Su ₁ ⁺)*	-	<10 ⁻⁴
C1792 (rec ⁺ , Su ₃ ⁺)*	-	<10 ⁻⁴
CR63.1 (rec ⁺ , Su ₁ ⁺)*	-	<10 ⁻⁴
SuVIII (rec ⁺ , Su ⁺)†	-	<10 ⁻⁴
CIT103 (rec ⁺ , Su ⁺)‡	-	<10 ⁻⁴
H514/pMB9	-	<10 ⁻⁴
H514/p Φ XG105	+	0.9
HF4704/p Φ XG105	+	0.8
HF4738/p Φ XG105	+	1.0
HF4740/p Φ XG105	+	1.2
C1757/p Φ XG105	+	0.6
C1792/p Φ XG105	+	0.4
C600 (Φ X ^r , Su ⁻) spheroplasts§	-	
C600 (Φ X, Su ₂ ⁺) spheroplasts*§	-	
C600 Su ⁻ /p Φ XG105 spheroplasts§	+	

* *amber* (UAG) suppressor; † *amber* (UAG) and *ochre* (UAA) suppressor;‡ *opal* (UGA) suppressor; § these Φ X^r strains were transfected with viral DNA.

Some consequences of the mutation in *GV/FS ZH1*

Examination of the expected (and partially confirmed) sequence of gene G from *ZH1* (Fig. 5) reveals that the deletion eliminates 27 out of 175 gene G codons. However, a total of 136 gene G codons are affected because of a -2 shift in the reading frame that occurs at the site of the deletion. Thus, the gene G sequence in *ZH1* can code for a polypeptide with a sequence identical to the first 39 amino acids from the amino terminus of the wild-type, gene G spike protein. The deletion creates a -2 frameshift in the 40th codon (residue 2,512, Fig. 5) converting a serine codon (UCA) to an *ochre* codon (UAA). (All the Φ X mRNAs are transcribed from the minus strand of Φ X RF DNA. Therefore, they have the same sense as the viral strand⁴.) Presumably, this leads to premature termination of translation. Downstream from the site of the deletion, there are 13 additional, in-frame, nonsense codons (7 UAA and 6 UGA), and several codons that might allow reinitiation, but translation from any of these possible sites encounters in-frame nonsense codons. Because of this nonsense codon barrier, it seems unlikely that any gene G product large enough to have gene G spike protein function will be coded from *ZH1* DNA. This would explain why this particular mutation is lethal and why complementation is required to produce infectious virus particles.

The generation of nonsense codons also seems to lead to abnormal termination of transcription. Our main evidence for this is the small burst size (~20) of *ZH1* grown in a p Φ XG105-bearing strain compared with that of wild-type Φ X (~300) on the same strain. We believe this is a result of the nonsense codons that are generated downstream from the mutation in *ZH1* and the polar effect of these nonsense codons on the adjacent gene, gene H.

Note that gene G and H products are both essential proteins^{4,5}. Both products are made from polycistronic mRNA(s)¹⁰⁻¹⁴. Cells bearing p Φ XG105 make large amounts of Φ X gene G protein even in the absence of viral infection (M.Z.H., unpublished results). The mutant virus must provide all other required proteins, including gene H product, for an infectious particle to be produced. With certain polar Φ X mutants, such as *Gam9*, production of gene H product is markedly reduced¹⁵. We have shown that this polar effect persists with *Gam9* grown in p Φ XG105-bearing cells, giving

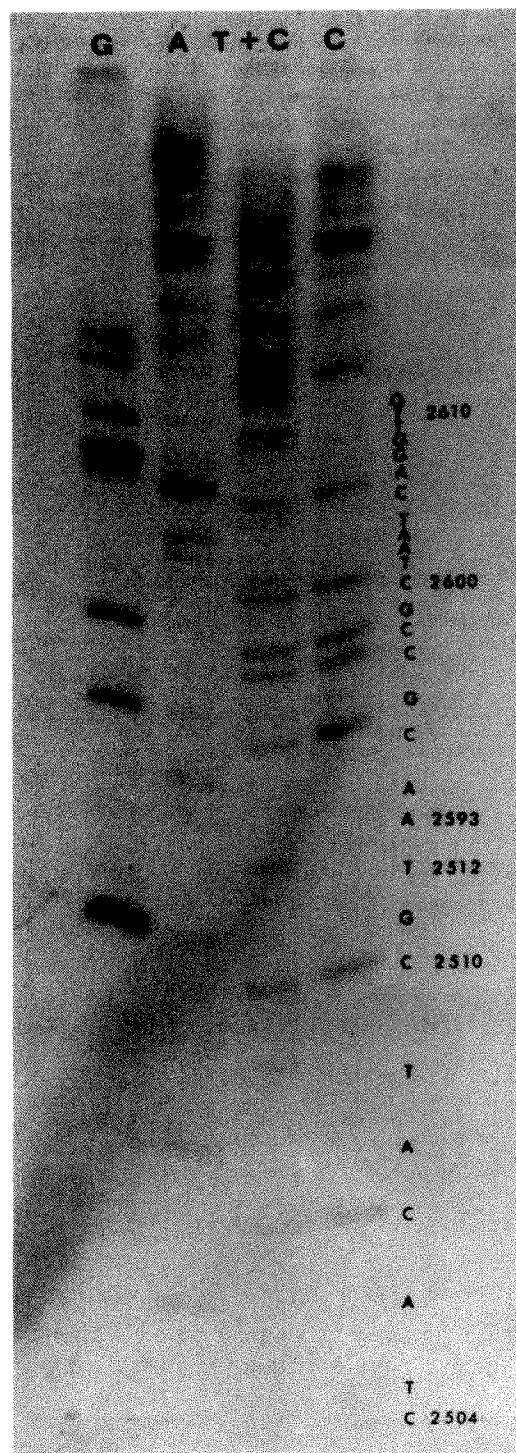


Fig. 4 Autoradiograph of a Maxam-Gilbert²⁰ sequence gel. For this sequence, the *ZH1* *AluI* fragment 3 (Fig. 3) was isolated, end-labelled with ³²P and redigested with *HaeIII* to generate *AluI/HaeIII* ~546 labelled at the *AluI* end by the methods described by Humayun *et al.*¹⁶. Partial chemical degradation products were fractionated on a 25% polyacrylamide-7 M urea gel. Nucleotides are numbered according to Sanger *et al.*⁹. Note the deletion of the wild-type sequence from 2,513 to 2,592. The ambiguity in the first two residues at the 5' end of A3 (bottom of figure) is resolved by the known specificity of *AluI*, AG/CT. Thus, the first two residues must be CT, in agreement with the published sequence.

rise to a small burst size (10–15) that is increased five- to seven-fold by an *amber* suppressor strain (non-permissive by itself) carrying pΦXG105.

The generation of 14 nonsense codons in *ZH1* brings this mutant close to a possible 'worst-case' mutation elicited by a site-specific modification of DNA in this region. Before the isolation of *ZH1*, we considered the possibility that the generation of a large number of nonsense codons would be so polar for gene H that the mutant would not be rescued by pΦXG. Our results indicate that polar effects do not seem to affect our studies as seriously as we had anticipated.

Concluding remarks

The isolation and characterisation of ΦX174 *GV/FS ZH1*, shown here to be a deletion-frameshift mutant at a preselected site in gene G, an essential gene of ΦX174, validates the biological part of the system developed in this laboratory for studying the molecular mechanisms of mutation by carcinogens. We have now shown that nonsense mutations^{1,2}, missense mutations (R.W.C., unpublished results), frameshift mutations (this work) and 'large' deletion mutations (this work) can be isolated using host cells carrying pΦXG. Thus, it should be

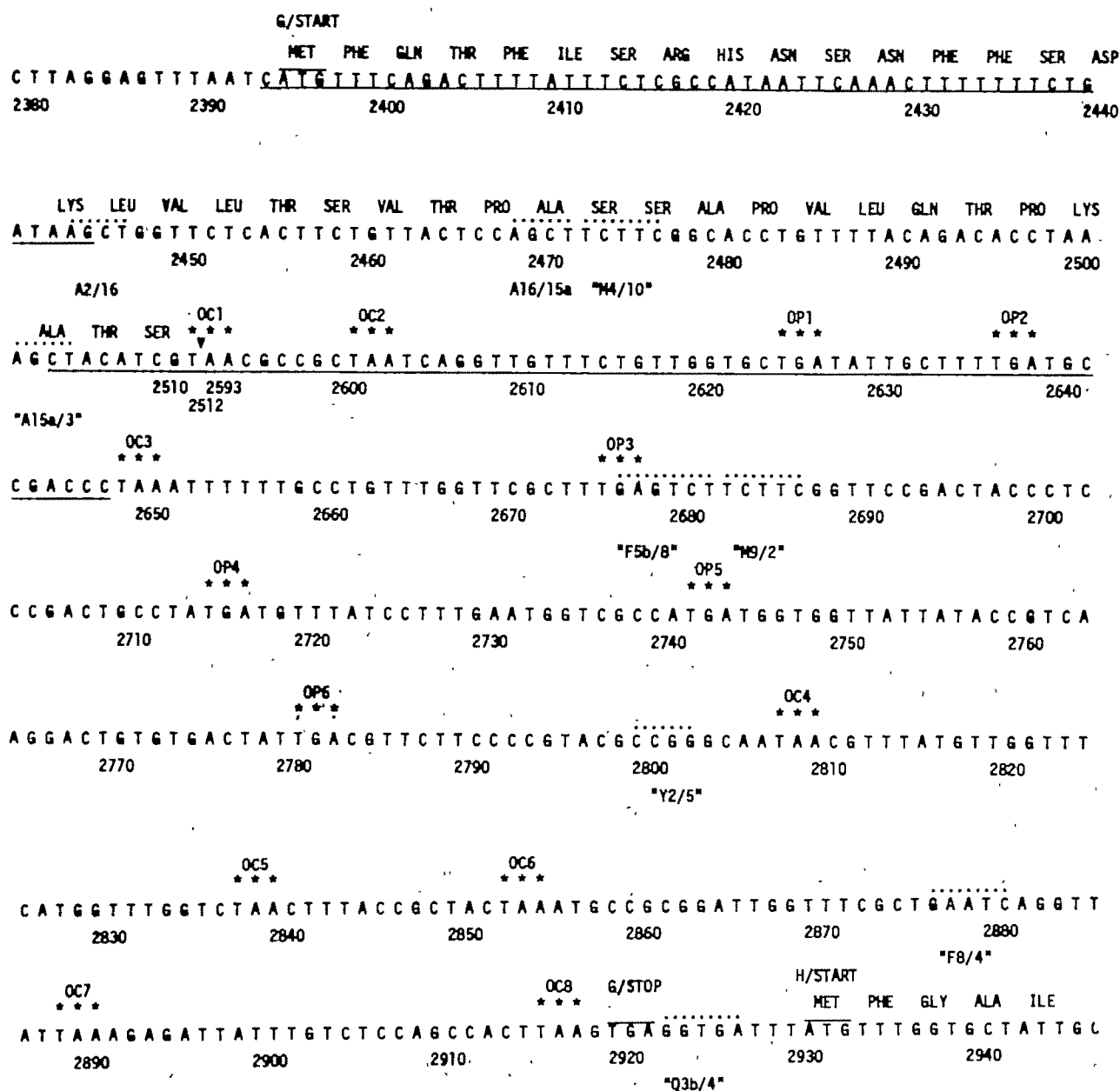


Fig. 5 Predicted (and partially confirmed) nucleotide sequence of gene G in *ZH1*. The sequence indicated by a continuous line was determined by us by the Maxam-Gilbert²⁰ technique. The remaining sequence and the nucleotide numbering were those of Sanger *et al.*⁵ In the nucleotide sequence, the sites of some restriction endonucleases are indicated by dotted overlining and are identified by the nomenclature used by Sanger *et al.* (A = *AluI*, F = *HaeIII*, M = *MboII*, Q = *HpaII*, R = *HindIII* and Y = *HpaIII*). For ease of comparison, we have also retained the cutting site identifications (fragments separated by each site) of Sanger *et al.*⁵, but note that the deletion in *ZH1* affects the sizes of some of the fragments produced by restriction. The site of the deletion and frameshift is indicated by an inverted solid triangle. The wild-type translational start signals for gene G (G/START at position 2,395) and gene H (H/START at position 2,931), as well as the wild-type G translational stop signal (G/STOP at position 2,920), which is now out of phase with the G start signal, are identified by solid overlining. Nonsense codons (for example, 8 UAA; OC1, 6 UGA; OP1 and so on) encountered in the same reading frame as the G start signal are identified by asterisks. The sequence of a 39 amino acid-long polypeptide possibly coded by the mutated gene G sequence in *ZH1* (identical to the first 39 amino acids from the N-terminus of the wild-type G product) is indicated.

possible to isolate any mutant that might be produced by a single, preselected, site-specific, covalent modification of ΦX gene G with a given carcinogen. The nature of the mutation(s) produced by the premutational lesion in the parental DNA can then be determined by sequencing the mutant, progeny DNA.

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letters

A search for X-ray pulsations from the Galactic Centre

AN intriguing problem in astrophysics is the determination of the conditions which prevail at the centre of the Galaxy. X-rays can penetrate the intervening dust and gas which obscure visible light from this region, and we show here that they may thus provide a sensitive probe of the hot gas and peculiar objects which may populate the Galactic Centre.

X-ray emission was first detected from an extended ($\sim 2^\circ$) region in the direction of the Galactic Centre by Kellogg *et al.*¹. With the spatial resolution of the Uhuru detector used for that observation, it was not possible to determine whether the emission was diffuse, or whether discrete sources contributed to at least part of the flux from this region (designated GCX). Discrete, albeit short-lived sources were later discovered there—two transients^{2,3} and at least three bursters⁴. Lewin *et al.*⁴ noted that two of the three burst sources might be the same as the above transients. They also pointed out that many burst sources have a 'steady' X-ray component which has a time-averaged luminosity ~ 100 times that of the bursts. It was therefore proposed that the GCX bursters also have steady components which would provide a major part, or perhaps even all of GCX flux. This idea was supported by the subsequent detection of several point sources in GCX^{5,6}, at which time it was also noted that the positions of some of the steady sources were in agreement with the positions of the bursters and transients.

The sky distribution of burst sources⁷ and the absence of pulsations, periodicities, and eclipses in the steady components of many other burst sources⁸ make it plausible that the bursters are Pop. II objects, similar to the 'galactic bulge' and the X-ray emitters in globular clusters⁹. However, there may be other sources in the GCX complex which are not associated with bursters. Some of these might be Pop. I objects which might produce pulsations. Radio observations testify to Pop. I activity near the Galactic Centre: that is, active regions of star formation and the presence of supergiant stars and ionising O and B stars. Therefore, it is not impossible that transient X-ray sources such

as the Be stars A1118-61 and A0535+26, which showed pulsations of 405 s and 104 s respectively^{10,11}, might also be detected at the centre of the Galaxy.

We aimed to determine the nature of the discrete sources in GCX by looking for periodic X-ray behaviour. To do this, we used data taken from long pointing observations of GCX (6.3 h in 1976 Feb and 18.9 h in 1977 Feb) by the SAS 3 X-ray observatory. We used the horizontal tube system¹² which had a 1.7° (FWHM) field of view and spanned the energy interval 2.5-35 keV. The data were analysed using the Cooley-Tukey fast Fourier transform (FFT) algorithm. A complete description of the data reduction techniques is given elsewhere¹³. The sampling length used in this analysis restricted our search to periods > 1.7 s, while the low-frequency noise introduced by spacecraft pointing jitter made the search meaningful only for periods ≤ 40 min.

No periodic X-ray behaviour was detected from GCX. In the energy range 2.5-10 keV the 90% confidence upper limits to pulsations range from $\sim 1.6 \times 10^{-3}$ ct cm⁻² s⁻¹ for periods from 2 to 20 s, to $\sim 1.0 \times 10^{-2}$ ct cm⁻² s⁻¹ for periods of the order of 30 min. These correspond to pulsed fractions of the total GCX flux of 1.1% and 7%, respectively. In the 8-35 keV band these limits range from $\sim 2.3 \times 10^{-3}$ ct cm⁻² s⁻¹ (3.3% of the total GCX flux) for periods $\leq 6 \times 10^3$ s, to 6.7×10^{-3} ct cm⁻² s⁻¹ (9.5% of GCX) for longer periods up to 40 min. (The calculation of the pulsed fractions applies only for a sinusoidal pulsation.)

If we assume, as seems likely, that part of the steady GCX flux is due to the steady contribution from bursters, we note the similarity between these and the other X-ray bursters. For the latter, Cominsky *et al.*⁸ have determined upper limits to the X-ray pulsed fraction of a few per cent (2-66 s) and 10% (66-1,000 s). Therefore, some of the discrete GCX sources may be similar to the other bursters which have been associated with Pop. II type X-ray sources. As noted by Lewin (ref. 14 and refs. therein) the absence of pulsations is not compelling evidence against the proposed binary-neutron star models for the burst sources. The magnetic fields of these objects may have decayed (ref. 15 and refs. therein) or, alternatively, the neutron stars in these systems may never have had strong magnetic fields.

In conclusion, we have not detected any pulsations in the range ~ 2 – $2,000$ s from GCX, a region which is known to contain several point sources. However, a search for periodicities < 2 s and > 30 min would be valuable.

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The angular size of the high-redshift quasar Q0000–398

THE optically selected quasar¹ Q0000–398 has a redshift² $z = 2.827$. This quasar has been identified with a faint double radio source whose angular extent is 134 ± 40 arc s. As all known high-redshift radio-selected quasars have much smaller angular sizes, it has been suggested that the upper envelope of the angular size–redshift diagram of radio quasars is depressed by an anticorrelation of linear size and radio luminosity³. We show here, first that the radio source associated with Q0000–398 is compact and centred on the optical quasar, and second that there is no evidence in our data on other quasars for the suggested anticorrelation.

We observed Q0000–398 at 1,465 MHz with the partially completed VLA during 16–18 June 1978 as part of a larger programme to study radio emission from optically selected quasars. Nine scans of Q0000–398 were made at uniform intervals within 2 h of transit. All 15 pairs of the six antennas were correlated; baselines from 3,500 to 52,000 wavelengths were available. The synthesised beam is shown in Fig. 1a. The 'clean' map of Q0000–398, restored with an 18 by 3 arc s elliptical gaussian beam, is shown in Fig. 1b.

The only radio source in the field is at 1950.0 position $\alpha = 00^h 00^m 30.29 \pm 0.02$, $\delta = -39^\circ 48' 47.6 \pm 0.8$. It is unresolved (< 6 by 1 arc s) and has a 1,465 MHz flux density of 14 ± 2 mJy. The optical position of Q0000–398, measured from the Whiteoak extension of the National Geographic–Palomar Observatory Sky Survey print, is $\alpha = 00^h 00^m 30.26 \pm 0.13$, $\delta = -39^\circ 48' 49.6 \pm 1.5$. Thus we identify Q0000–398 with a single compact radio source centred on the optical quasar. M. R.

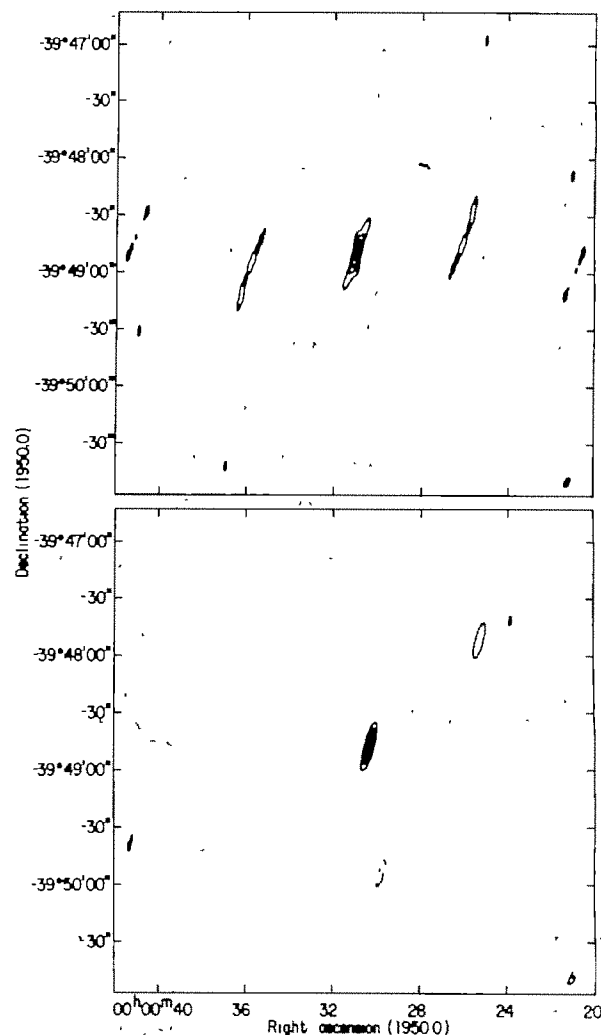


Fig. 1 a, Synthesised beam for the 1,465 MHz VLA observations of Q0000–398; b, The 1,465 MHz VLA map of Q0000–398. Negative contours are indicated by broken lines.

Gearhart (personal communication) has suggested that marginal observing weather coupled with the low elevation of the source may be responsible for the large angular size observed at 2,695 MHz with the NRAO three-element interferometer.

Our preliminary 1,465 MHz VLA maps of other high-redshift quasars indicate that the faint radio sources sometimes associated with optically selected quasars do not have larger angular sizes than the more luminous radio-selected sources.

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Martian extratropical cyclones

VIKING Orbiter 1 has recently photographed two cloud formations in the northern hemisphere of Mars which closely resemble satellite pictures of terrestrial extratropical cyclones. The pictures, (Figs 1 and 2) were acquired during the summer season and are the first evidence that baroclinic waves, prevalent during other seasons, also occur in summer. Physical properties of these systems deduced from the vidicon images and from the IR thermal mapping experiment (IRTM) are discussed here.

The north polar region of Mars has long been recognised as one of the most meteorologically active areas on the planet, particularly during winter when the polar cap is obscured by a diffuse hood of condensate clouds. Mariner 9 acquired several images of the region in late winter, $L_s = 342^\circ$ to $L_s = 354^\circ$, which have been analysed in detail by Briggs and Leovy¹. The development of the clouds observed in the region surrounding the seasonal polar cap indicated a circulation pattern consistent with baroclinic waves, which had been predicted previously by Mintz².

Viking Lander 2, situated at 48° latitude, just at the edge of the seasonal cap at its largest extent, has monitored local meteorology for more than one martian year; results of a large part of this mission have recently been reported by Ryan *et al.*³. During the autumn season periodic changes are observed in the pressures and wind vectors obtained by VL-2 (but not by VL-1, located in the equatorial region) which strongly suggest the passage of baroclinic systems to the north of the lander site. These variations were obscured by diurnal variations during the first summer, and the major winter dust storm strongly interfered with the activity.

Viking orbiters were not designed primarily as weather satellites; monitoring for meteorology could only be conducted when the spacecraft were near their respective apoapsides because of the relatively large focal lengths of the telescopes. Such monitoring was severely restricted by orbital constraints and by competition for a limited number of frames with several other activities. During the latter part of the Extended Mission and the



Fig. 1 The martian storm system observed by the Viking Orbiter 1 at (81, 160), $L_s = 105^\circ$.



Fig. 2 The martian storm system observed by the Viking Orbiter 1 at (66, 227), $L_s = 126^\circ$.

Continued Mission VO-1 has been able to perform this monitoring in the north polar region; these two pictures represent a small part of that data set, which characterises the spring and summer seasons in the martian northern hemisphere.

The basic parameters which characterise the storms are given in Table 1. These include the areocentric longitude of the Sun (L_s), the coordinates, the local time of day, and an estimate of the radius of curvature. IRTM data relevant to the storms are presented in Table 2 in the form of brightness temperatures in the five IRTM channels. The 7, 9, 11, and $20\ \mu\text{m}$ channels generally characterise the ground temperatures with small differences produced by dust and condensates in the atmosphere⁴; the $15\ \mu\text{m}$ temperatures are characteristic of the atmosphere at a pressure of 0.6 mbar, equivalent to an altitude of about 25 km. Only limited IRTM data are available for the second storm, owing to the geometry and spatial resolution of the scans.

The IRTM measurements show that a large temperature gradient existed just north of storm 1; the magnitude of this gradient was $\sim 0.2\ \text{K km}^{-1}$, and the change was confined to about 60 km. The area occupied by the spiral cloud is generally characterised by a ground temperature of $\sim 230\ \text{K}$, while temperatures of 215 K are found adjacent to this area to the north. To the south of the storm, between 75° and 80° latitude, there is a gradual rise in the temperature to 237 K. These are precisely the effects which are expected for such a system since the waves which give rise to these disturbances occur in the polar front which separates the frigid arctic air from the warmer mid-latitude atmosphere. The temperature gradient is confined to the lower atmosphere where the clouds are located since there is no structure in the $15\ \mu\text{m}$ data; this is generally expected as only the major dust storms seem to produce pronounced effects at the 0.6 mbar level⁶.

Table 1 Location and scales of the extratropical storms

Storm	L_s	Lat.	Long.	LT	R (km)
1	105°	81°	150°	10.00	100
2	126°	66°	227°	06.33	300

The observed brightness temperatures clearly show that the atmosphere is too warm for the observed cloud to be composed of CO₂ ice particles. The temperature difference between the 9 and 11 μm channels is sensitive to the cloud composition^{4,5}; the negative values of $T_9 - T_{11}$ seen over much of the region suggest a generally dusty background atmosphere, consistent with the appearance of the vidicon images. However, for observations which apparently include the cloud, values of $T_9 - T_{11}$ which are near zero or slightly positive are seen; this strongly suggests a water ice composition for the cloud. Since the interpretation depends on the vertical thermal structure of the atmosphere and as the IRTM resolution is large compared with the detail of the picture, this information is probably not definitive. However, there are several other facts which are consistent with condensate (water) rather than dust.

(1) The albedos associated with the clouds are about 0.3, higher than the surrounding ground and higher than expected for dust.

(2) Comparison of the image in Fig. 1 with a picture taken through the red filter at the same time shows a much larger contrast between cloud and ground in violet than in red; this generally suggests condensate as opposed to dust, as the clouds in the former case are expected to be much more spectrally flat than martian ground or dust.

(3) The water vapour content of the atmosphere is maximum over the north polar region during this season⁷. However, as mentioned, there seems to be dust generally mixed in the atmosphere; the dust may enhance the cloud formation by nucleating the condensation of ice particles to form clouds as air parcels are cooled. This mechanism, the stirring up of surface dust and subsequent nucleation, may be quite important in the seasonal recycling of atmospheric water vapour and in the ultimate formation of the hood.

A dusty atmosphere is also suggested by $T_9 - T_7$, although the 7 μm data points are not conveniently situated for computing this difference in the vicinity of the storm. There is a suppression of the magnitude of the brightness temperature for those IRTM points identified as cloud; this is anticipated, as the increased opacity implies a higher origin for radiation. Unfortunately, two colour coverage of the second storm is not available. However, the limited IRTM data suggest that this cloud is also composed of water ice. The geometry of the scans, and the proximity of the terminator, preclude observation north of this storm, so that there are no direct measurements of expected local temperature gradients.

Table 2 IRTM of the storms (K)

Storm 1	T_7	T_9	T_{11}	T_{15}	T_{20}
Storm area	236	233	233	177	232
Area to north	221	217	218	177	216
Storm 2					
Storm area	216	215	214	167	213

The geometry of the scans prevents observations of the region north of storm 2.

The wind velocity, the pressure gradient, and the radius of curvature determine the force balance for parcels in the storms. The resulting gradient wind, for which the pressure gradient force, the Coriolis force, and the centrifugal force are in balance, is given by

$$v = -fR/2 + ((fR/2)^2 + R|\nabla p|/\rho)^{1/2}$$

where R is the radius of curvature, ∇p the pressure difference, ρ is the density, and f is the Coriolis parameter, roughly the same for Mars as for Earth. The signs are chosen to represent the normal counter-clockwise circulation around a low pressure cell as seems to be indicated by visual interpretation. VL-2 data were used to estimate the appropriate values of mean pressure and of pressure fluctuations to be used

in the formula; a value of $\nabla p = 0.25$ for the amplitude of the pressure difference seems to be a reasonable upper limit which may be used to estimate an upper limit for v .

The resulting wind velocities and accelerations are given in Table 3. The resulting velocities, 31.5 m s^{-1} and 23.1 m s^{-1} for storms 1 and 2 respectively, lead to surface wind speeds of 12.6 m s^{-1} and 9.2 m s^{-1} if a scale factor of 0.4 is used to interpolate between surface winds and winds at the top of the boundary layer¹⁸. These are certainly reasonable when compared with the typical wind speeds observed by VL-2, and the consistency of the force balance supports the interpretation of these systems taken here. The more northerly storm 1, because of its small size, is approximately cyclostrophic while the larger storm 2 is approximately geostrophic¹¹. The predicted near surface wind speed of $\sim 12.6 \text{ m s}^{-1}$ for storm 1 is close to the saltation velocity (Greeley, personal communication), which is consistent with our interpretation that dust is mixed with the ice particles in the atmosphere.

Table 3 Meteorological properties of the storms

Storm	v (m s^{-1})	p/ρ (N T kg^{-1})	fv (N T kg^{-1})	v^2/R (N T kg^{-1})	Vorticity
1	31.5	0.0144	0.0044	0.0100	6.3×10^{-4}
2	23.1	0.0048	0.0030	0.0018	1.5×10^{-4}

In the case of storm 2, cloud shadows are seen which may be used to estimate the altitude of the clouds. This estimate leads to a height of $\sim 6\text{--}7 \text{ km}$ for this system which is similar to the altitudes deduced for many other martian clouds although some clouds have been seen which are 50 km above the surface^{9,10}. This altitude is quite compatible with the temperature suppression effect mentioned earlier: if a linear lapse rate is assumed, the brightness temperatures observed for cloud 1 are appropriate for an altitude of about 4 km, implying that the intervening material is at least that altitude.

In conclusion, two storm systems observed by Viking seem to be similar to terrestrial mid-latitude cyclonic storms in appearance. Water ice is the favoured composition for the clouds. The scales are somewhat different than for terrestrial storms, but this may be associated with the northerly location. Further work is underway to combine these data with other information acquired by the orbiters to prepare an integrated picture of meteorological phenomena in the Mars north polar region.

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Differential aeolian redistribution rates on Mars

THE surface of Mars gives abundant evidence for aeolian activity, including wind-laid dust and ice deposits at the poles that may be several kilometres thick, a vast dune field surrounding the north polar deposits, a dust mantle of varying thickness covering the high to mid-latitudes, and both depositional and deflationary aeolian landforms in the equatorial latitudes. Two major questions concerned with the martian aeolian activity are: first, what are the rates of erosion and redistribution of surface materials such as rock and soil deposits; and second, are most of the large-scale aeolian features seen on Mars products of the present aeolian environment, extrapolated back through geological time, or were most of the features produced in a different climatic regime? Presumably, a different climatic regime, either associated with an early, denser atmosphere, or with periodic variations in atmospheric conditions induced by obliquity changes, would strongly modulate the vigour of aeolian activity¹. The Viking Orbiters and Landers, which began acquiring data during the summer of 1976, have now monitored the aeolian environment for slightly more than one full Mars year. Data acquired during the mission have enabled us to investigate these questions for aeolian effects averaged over billions of years, tens of thousands of years, and during a single year.

The integral effect of aeolian activity can be sampled over geological time scales by examining the degree of modification of features large enough to be seen by the Viking Orbiters. Figure 1a shows an orbital view over the Viking Lander 1 (VL1) site. VL1 is sitting on terrain which from orbit seems similar to a lunar *mare* surface. Crater counts by Dial³ show that the crater population of this region is in production, at least for crater sizes extending down to 100 m. Craters smaller than that size are less abundant than would be predicted for a population in production. The deficiency of small craters is probably due to information loss, as the image ground resolution is 25 m per line-pair and a feature typically needs to be several times that size to be detectable. Thus, the crater count data constrain the integral effects of aeolian activity over the lifetime of the crater population to values small enough to have only obliterated craters smaller than 100 m in diameter.

Figure 1b is a VL1 frame showing a crater on the horizon. The crater can also be seen on the orbital mosaic. It is about 2 km from the Lander and it is about 420 m in diameter. The rim height to crater diameter ratio for this feature is about 0.12, which compares favourably with the same ratio for the lunar South Ray crater located at the Apollo 16 landing site. The ratio for South Ray crater, which is 640 m in diameter and 2 Myr old, is 0.09 (ref. 4). South Ray is young enough to be virtually untouched by lunar erosion. The similarity in ratios for the two features strongly implies that the crater in Fig. 1b is also preserved virtually intact. In addition, examination of the orbital mosaic shows that all other craters of similar size have raised rims and bowl-shaped floors. No spectrum of morphologies can be discerned, implying that erosion and deposition have been minimal over the lifetimes of these craters. The rim height of the crater in Fig. 1b is 25 m, so the extent of removal of material must have been some small fraction of that number. Such a conclusion is consistent with the crater count data, because the extent of rock breakdown removal has most probably been a few metres over the lifetime of the crater population.

The extent of preservation of the crater population provides excellent data for estimating that lifetime. From our own crater counts we find that there are $\sim 1.5 \pm 0.2 \times 10^3$ craters per 10^6 km^2 larger than 1 km in diameter within a radius of 100 km of the landing site. Neukum and Wise⁵ developed a cratering flux model for Mars by assuming that the flux of impacting objects is the same for Mars and the Moon. They also find after

scaling for impact velocity and gravity effects, that the resultant cratering rate would be 0.22 that of the Moon's rate. Such a model results in an age of about 3,600 Myr for the region surrounding VL1. Given the uncertainties of the martian cratering history the actual age could be a factor of 2 or 3 younger than this estimate⁶. However, for any reasonable cratering flux model, the computed erosion rate of metres per 10^9 yr is surprisingly small, some $10^{-3} \mu\text{m yr}^{-1}$, or $\sim 1 \text{ m}$ per 10^9 yr .

From both orbital and lander views, the VL2 landing site seems quite distinct from the VL1 site⁷. Extensive evidence for differential deflation can be seen from orbit (Fig. 2a,b). The more resistant areas consist of ejecta deposits from craters, while the deflated regions were probably once covered with fine-grained aeolian deposits that were part of the extensive mantle of deposits surrounding the north pole. Relatively rapid

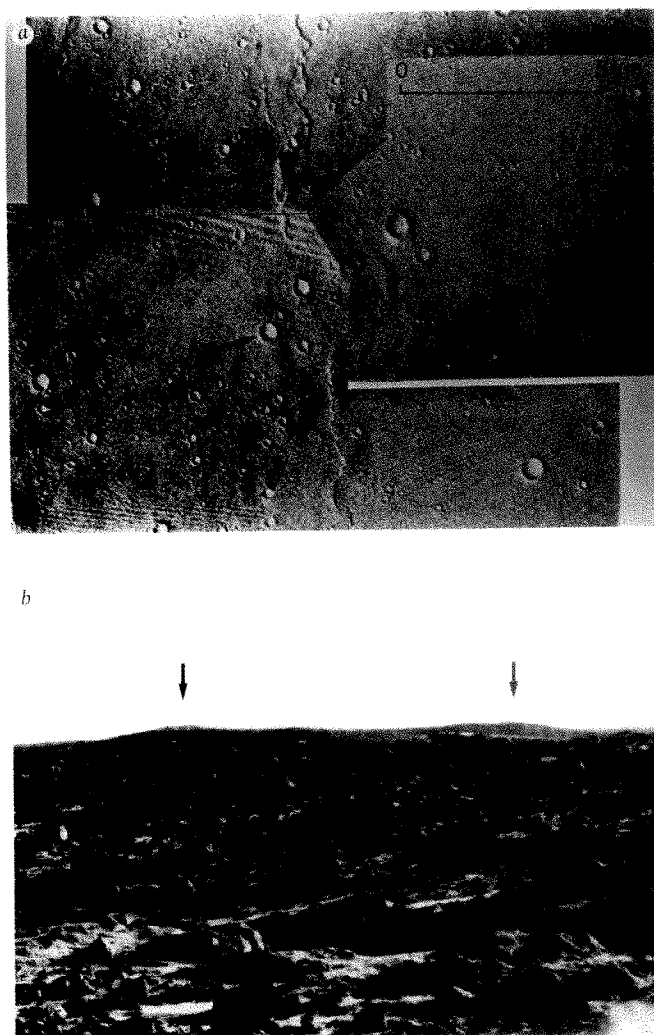


Fig. 1 a, Viking Orbiter 2 mosaic of high resolution frames showing the Viking 1 landing site in Chryse Planitia. The Lander location as derived by Morris *et al.*² and updated by Jones (personal communication). The arrow points to the crater that can be seen in the Lander view shown in b. Crater counts by Dial³ show that the crater population is in production at least down to the counting resolution limit of $\sim 100 \text{ m}$. This result, together with the fresh-looking appearance of the craters and wrinkle ridges suggests that not more than metres of material have been stripped away over the lifetime of this surface. For reference, VL1 is located at 22.5° N , 48.0° W (ref. 2). VO II frames 452B8–12. b, Viking Lander 1 view of the crater delineated by an arrow in a. The crater is about 2 km from the Lander and 420 m in diameter. The rim height to diameter ratio for this crater indicates that little to no erosion has occurred since its formation. For scale, the bottom of the frame covers 3.3 m. VL1 frame A235.

wind erosion of regions not covered by craters and ejecta have left the latter features standing above most of the terrain as plateaus or pedestals. Ejecta deposits may be more resistant to erosion because they are protected by a population of blocks. Blocky ejecta surfaces may form as a result of ejecta emplacement in a debris flow mode⁸. During debris flows, larger blocks are preferentially forced to the surface because of a vertical dispersive pressure within the flow that scales as the square of the block diameter⁹. Thus, a debris flow mode of ejecta emplacement may create a ready-made armoured surface that lasts for a considerable length of time, given the very low rate of rock removal computed from VL1 data.

The extent of deflation between ejecta and surrounding terrain in the vicinity of VL2 is several hundred metres, assuming a conservative estimate of 10° for the slope of the talus between

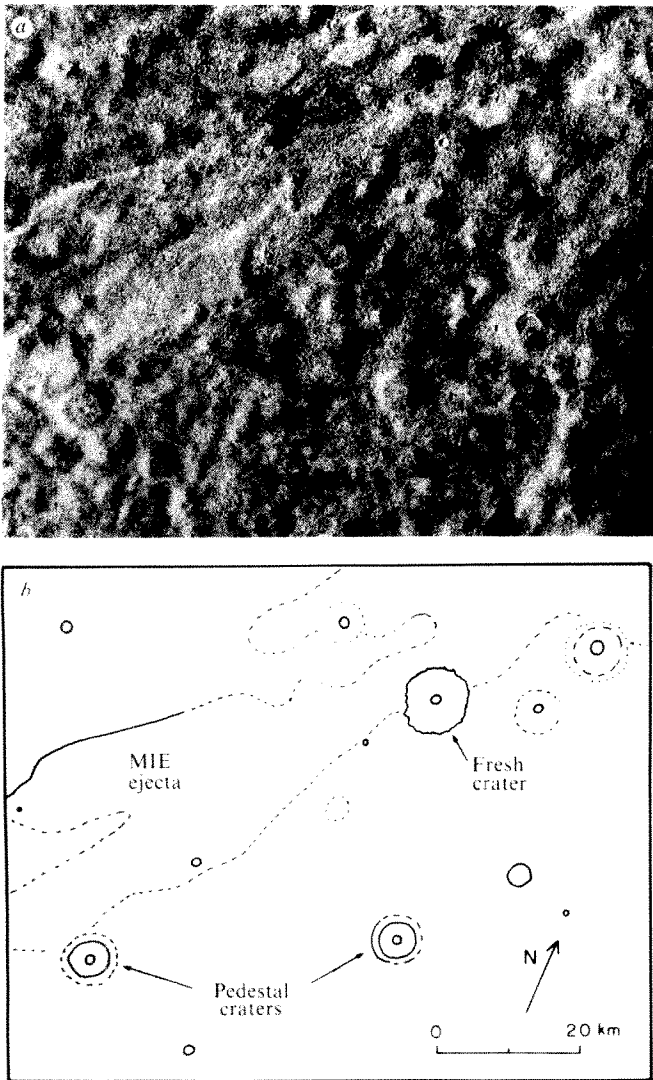


Fig. 2 *a, b*, Viking Orbiter 2 photograph and sketch map of part of Utopia Planitia. Viking Lander 2 touched down in an area that is just off the upper left edge of the frame. The frame is 90 km across. VL2 is located at 48.0°N , 225.6°W , within the northern mantle of aeolian debris surrounding the eroded polar cap deposits. Only one crater with a sharply raised rim, deep, bowl-shaped floor and extensive ejecta unit can be seen. It is shown on the sketch map as a fresh crater. Most other craters have a pedestal-like appearance. The tongue-shaped plateau extending across the upper part of the frame is a lobe of ejecta from the 100 km diameter crater, Mie, located ~ 200 km to the north-east. The topography of this region is controlled largely by differential deflation; high areas consist of craters and ejecta deposits that have resisted erosion. The deflated material probably consisted of fine-grained aeolian sediments associated with the debris mantle. The fresh crater must post-date any period of extensive deflation. VO II frame 9B16.

the edge of the pedestal craters shown in Fig. 2*a, b* and the surrounding deflation horizon. The antiquity of the terrain surrounding VL2 can be estimated, to a first approximation, from the superimposed crater abundances. An age estimate based on the crater population ($1.4 \pm 0.2 \times 10^3$ craters per 10^6 km^2 larger than 1 km) shown in Fig. 2*a* and surrounding areas would be about 3,400 Myr with the flux model used for the VL1 site. The average deflation rate would thus be very roughly 100 m per 10^9 yr, which is less than $1 \mu\text{m yr}^{-1}$. The estimate is several orders of magnitude greater than the rate of rock erosion derived for the VL1 site and, by implication, for the armoured ejecta deposits at the VL2 site.

Constraints can also be imposed on the integral effect of aeolian activity averaged over time scales of tens of thousands of years. Windblown drifts seen at both landing sites have morphologies indicative of accumulation during a prevailing north to south wind regime—the situation prevalent during the annual global dust storms occurring near the time of perihelion^{10,11}. Surface winds during the global perihelion storms seem to carry near surface dust in a net north to south direction in areas to the north of the subsolar latitude at perihelion (the SLP; presently located at $\sim 15^\circ\text{S lat.}$) and in a net south to north direction in areas more southerly than the SLP. A complex but generally west to east flow seems to dominate the SLP proper¹¹. Most of actual entrainment of dust that feeds the global dust clouds seems to be concentrated in a wide latitude belt centred about the SLP, giving rise to the speculation that the major dark areas of Mars, which roughly coincide with the SLP, have been stripped of a thin, bright dust cover¹². That dust cover would now reside in other regions in the form of deposits such as the drifts at VL1 and VL2. Due to precessional effects, the SLP migrates $\pm 25^\circ$ in latitude about the Equator over a 50,000 yr time span¹³. Because of the enhanced stripping associated with regions close to the SLP, it seems probable that the drifts at VL1, with their strictly north to south trend, must have accumulated since the SLP was last over the landing site latitude (24°N lat.), which would have been about 15,000 yr ago. Presumably drifts with west to east trends, or perhaps a stripped surface, would have dominated the surface at that time. If this line of reasoning is accepted, then a net accumulation rate of about 10 cm (mean drift thickness) per 15,000 yr, or several $\mu\text{m yr}^{-1}$, can be computed.

Figure 3 shows a pair of Viking Lander 2 (VL2) pictures acquired 476 martian days apart. The striking difference is related to the deposition of a thin layer of bright dust during the second major dust storm that occurred during the Viking mission. The dust is detectable only by its increased brightness; even then, it is only detectable in pictures acquired at high Sun angles (low phase angles). This constraint implies that the dust layer is probably only micrometres thick¹⁴. So far, no significant erosion of the new dust deposit has taken place, although such an accumulation, without subsequent erosion, cannot occur over more than a few tens of thousands of years without burying the boulders exposed at the landing site. Conditions at VL1 have been similarly quiet, with the accumulation of a bright dust layer, again probably only micrometres in thickness, being the dominant activity¹⁴. Thus, both landing sites have experienced an aeolian environment that, averaged over a yearly time scale, has resulted in the redistribution of several micrometres of dust. Such a conclusion is consistent with Lander camera observations of atmospheric opacity during the same time frame, which suggest dust transport rates of about $3 \times 10^{-4} \text{ g cm}^{-2} \text{ yr}^{-1}$ (ref. 15). Deposited evenly over the surface, such a rate equates to a dust layer of about $1 \mu\text{m}$ in thickness.

Our results for the Viking Landing sites indicate that little rock breakdown and removal by wind has occurred over approximately the past 3,000 Myr. On the other hand, relatively rapid redistribution of friable or loose, fine-grained materials has occurred. Many of the landforms seen in higher resolution Viking Orbiter frames covering other parts of Mars are consistent with such interpretations. Many volcanic flow fronts, well preserved crater populations, and other features which are

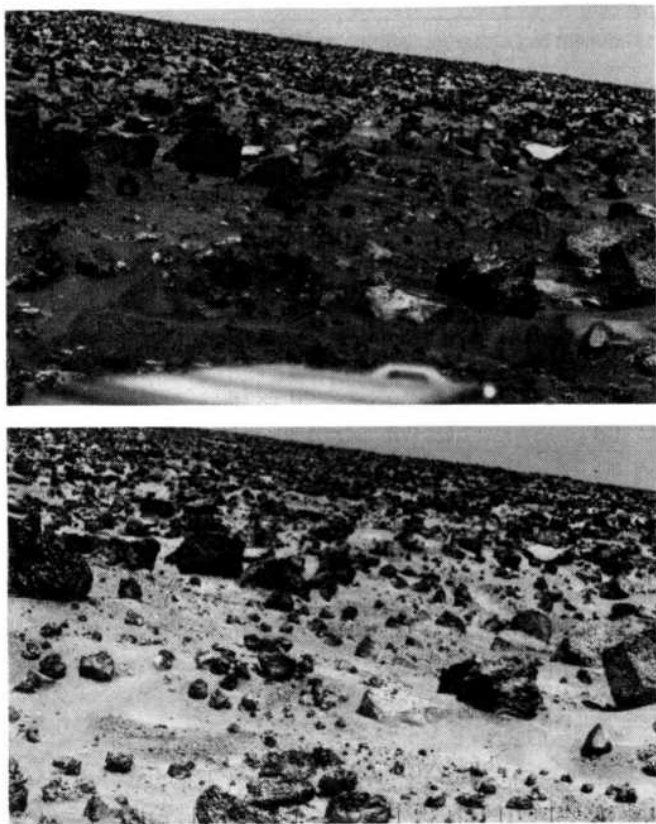


Fig. 3 The top VL2 frame was acquired 32 d after landing, during the Northern Hemisphere's summer season. The bottom frame was acquired 476 d later, with identical lighting. The brightness difference is related to an accumulation of several micrometres of bright dust during the second global dust storm that occurred during the martian year of Viking observations. The top frame is B013 and the bottom is G052.

indicative of little to no significant modification can be discerned. In other regions, the surface has been stripped, yardangs have formed, and in general the topography seems to have been largely configured by aeolian activity. Such regions must be composed of friable deposits, such as volcanic ash or older aeolian materials. Finally, the similarity of redistribution rates for aeolian deposits averaged over three vastly different time-scales implies that roughly the present aeolian environment has been operative for a significant fraction of geological time.

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Gravitational magnetism

BLACKETT¹ has pointed out that the ratios of magnetic moment to angular momentum for Earth, the Sun, and the star, 78 Virginis, are nearly equal and of a magnitude which suggests a gravitational origin for the magnetic fields of these bodies. After his laboratory experiment testing this hypothesis yielded negative results², gravitational magnetism became largely ignored. Recent magnetic field measurements of the moon³, Mercury⁴, and Jupiter⁵, however, yield ratios quite close to that considered by Blackett. Moreover, Russell⁶, who emphasises only the phenomenology of these ratios and eschews any physical model, adds Venus and Saturn to the list, although these data are tentative and will become firm only with the completion of probes to Venus and Saturn. His re-evaluation of Mars, however, puts this planet into an exceptional position as it seems to have no measurable magnetic field. I agree with his suggestion that to account for this we must ultimately consider the interiors of the planets. Hopefully more precise magnetic measurements will be made on Mars. Meanwhile a new data point strengthens the gravi-magnetic hypothesis: the magnetic field of a pulsar—Hercules X-1—has been directly measured^{7,8} and yields the Blackett ratio. Because the Hercules data is both fairly exact and represents an extremely high magnetic field (5×10^8 T) and angular velocity (5 rad s^{-1}), it is appropriate to reconsider Blackett's gravi-magnetic hypothesis, and I show here that the gravi-magnetic hypothesis has yet to be tested definitively in the laboratory.

The gravi-magnetic hypothesis proposes that a rotating mass, measured in gravitational units, has the same magnetic effect as that of a rotating charge, measured in electrical units. The respective force constants determine this relationship

$$G^{1/2}m = k^{-1/2}q \quad (1)$$

where G is the gravitational constant, m is mass, k is the Coulomb constant, and q is electric charge. Thus the ratio of magnetic moment to angular momentum for a sphere of mass, m , density factor, f , radius, r , angular velocity, ω , and magnetic field, B , is (in SI units)

$$P/U = 5/4 \pi B / \mu_0 r / \rho \omega m = G^{1/2} / 2k^{1/2} \quad (2)$$

Table 1 and Fig. 1 show the degree to which this expectation is met: the data points in Fig. 1 fit the $G^{1/2}/2k^{1/2}$ line remarkably well.

Note that the mere fact of a linear relationship between the magnetic moments and angular momenta is not what is surprising in these data. *A priori*, we should expect a correlation between P and U . In equation (2) we expect r to be correlated with m , and B to be correlated with ω ; and thus, we expect P to be correlated with U . The surprise is that this correlation ratio, P/U , should turn out to be close to $G^{1/2}/2k^{1/2}$. Without the gravi-magnetic hypothesis, there is no reason to expect the regression line for the data of Fig. 1 to be close to $P = G^{1/2}/2k^{1/2} U$. If we have only one datum point, and it has a P/U close to $G^{1/2}/2k^{1/2}$, we should be surprised enough to take notice of the gravi-magnetic hypothesis. However, that one datum point may not turn out to be typical. The value of Fig. 1 is in showing that the data points typically fall close to the gravi-magnetic hypothesis line. The only atypical point, thus far, is Mars. The gravi-magnetic hypothesis (stated in $\log_{10}$ form) predicts a P/U of -10.37 . The mean P/U for the data points plotted in Fig. 1 is -11.13 with a standard deviation of 0.42.

As we have no *a priori* way of knowing the likelihood of the near coincidence of P/U with $G^{1/2}/2k^{1/2}$, it may be useful to calculate how easily the data points can be pulled away from the gravi-magnetic hypothesis line. To do this, we must take into account the interdependence of the variables: r , m , B , and ω . Thus, if one changes r , the other variables would be expected to change in accordance with this dependence. There are two

Table 1 Maximum and minimum data for ratio of magnetic moment to angular momentum for massive bodies

Body	f	m Mass	r Radius	ω Angular velocity	B Magnetic field	U Angular momentum $\frac{2}{5} f \omega m r^2$	P Magnetic moment $\frac{1}{2} \frac{4\pi}{\mu_0} B r^3$	P/U $\times 10^{-11}$ $\frac{P}{U} \div \frac{G^{\frac{1}{2}}}{2k^{\frac{1}{2}}}$	$\beta^{\dagger}$
(refs)		(kg)	(m)	(rad s ⁻¹)	(T)	(J s)	(A m ²)	(C kg ⁻¹)	
Earth (1, 15-17)	0.88	5.98×10^{24}	6.378×10^6	7.272×10^{-5}	7.0×10^{-5} 2.5×10^{-5}	6.22×10^{33}	9.1×10^{22} 3.2×10^{22}	1.5 0.51	0.35 0.12
Sun (15, 16, 18, 19)	0.16	1.99×10^{30}	6.960×10^8	2.9×10^{-6} 2.7	27×10^{-4} 0.8×10^{-4}	1.79×10^{41} 1.67×10^{41}	4.6×10^{30} 0.14×10^{30}	2.7 0.078	0.63 0.02
78 Vir (1-3)	0.16	4.8×10^{30} 4.4×10^{30}	1.8×10^9 1.0×10^9	5×10^{-4} 0.5×10^{-4}	0.2 0.1	5.0×10^{44} 0.14×10^{44}	5.8×10^{33} 0.5×10^{33}	41 0.1	9.8 0.02
Moon (3, 15, 16)	0.88	7.35×10^{22}	1.738×10^6	2.66×10^{-6}	10.3×10^{-8} 3.8×10^{-8}	2.08×10^{29}	2.7×10^{18} 1.0×10^{18}	1.3 0.48	0.30 0.11
Mercury (4, 6, 15)	0.88	3.3×10^{23}	2.434×10^6	1.24×10^{-6}	4×10^{-7} 2×10^{-7}	8.5×10^{29}	2.8×10^{19} 1.4×10^{19}	3.3 1.6	0.77 0.37
Venus (6, 15)	0.88	4.87×10^{24}	6.052×10^6	2.99×10^{-7}	5.9×10^{-8} 2.7×10^{-8}	1.88×10^{31}	6.5×10^{19} 3×10^{19}	0.35 0.16	0.08 0.04
Jupiter (5, 6, 15)	0.66	1.90×10^{27}	7.143×10^7	1.773×10^{-4}	14×10^{-4} 3×10^{-4}	4.54×10^{38}	26×10^{26} 5.5×10^{26}	0.57 0.12	0.13 0.03
Saturn (6, 15, 20)	0.66	5.69×10^{26}	5.98×10^7	1.706×10^{-4}	3×10^{-4} 1×10^{-4}	9.16×10^{37}	3.2×10^{26} 1.2×10^{26}	0.35 0.13	0.08 0.03
Her X-1* (7, 8, 21, 22)	0.88	3.5×10^{30}	1×10^4 0.7×10^4	5.076	7×10^8 5×10^8	3.1×10^{38} 1.8×10^{38}	35×10^{25} 8.6×10^{26}	1.9 0.28	0.44 0.07

* For neutron stars, the larger mass is associated with the smaller radius²¹.

[†] $G^{1/2}/2k^{1/2} = 4.3 \times 10^{-11} \text{ C kg}^{-1}$.

measures of this dependence. First, each datum point has its own dependence relations. Second, there is a set of mean dependence relations for the data as a whole. Thus for all the data points (except 78 Vir and Her X-1)—in log₁₀ form, and in SI units—the mean of the ratio m/r is 3.57 (s.d. = 0.11); the mean B/r is -0.726 (s.d. = 0.35); the mean ω/r is -0.701 (s.d. = 0.21).

If one changes r for each datum point (except 78 Vir and Her X-1) by an order of magnitude, and changes m , B , and ω according to the individually calculated dependence relations, P/U for each datum point will change by more than two orders of magnitude. The mean P/U for these changed data points will be -13.95 (s.d. = 0.47), compared with the mean P/U of -11.18 for the unchanged data points.

A greater change than this will occur if one uses the mean dependence relations mentioned above. In this case the mean P/U for the changed data points will be -14.20 (s.d. = 2.21).

Since r in Table 1 ranges over five orders of magnitude, a change in r of one order of magnitude is relatively small, and thus we can say that the data points are sensitive to relatively small changes in the relevant variables.

A striking characteristic of the data in Fig. 1, is that the deviation from the gravi-magnetic hypothesis line is fairly systematic. It should be obvious, of course, that deviations from the $G^{1/2}/2k^{1/2}$ may well be due to electrical-magnetic effects. For example, equation (2) predicts a surface field about three times greater than that measured at the surface of the Earth. However, because of the offset between the magnetic and gravitational poles of Earth, electrical-magnetism must also be at work. Whatever accounts for this electrical component of the magnetic field of Earth must also account for the damping of the gravitational component.

The *modus operandi* of the electrical component may also help account for the deep mine measurements of Runcorn^{9,10}, which show the magnetic field increasing towards the centre of the Earth. Blackett² regarded these measurements as decisive evidence against his gravi-magnetic hypothesis, because the gravi-magnetic hypothesis, taken by itself, predicts that the magnetic field will decrease in this direction. This evidence, however, is not decisive. To see this, we must solve equation (2)

for the magnetic field B . The expected magnitude is thus seen to be

$$B = 4/5 \mu_0/4\pi G^{1/2}/2k^{1/2} f \omega m/r \quad (3)$$

For a given body, f and ω remain constant, yielding m and r as the only variables. M , for a uniformly dense sphere, is a linear function of volume— $4/3 \pi r^3$. However fast r falls off in the denominator of equation (3), m in the numerator falls off faster. B thus decreases as r decreases.

To counter the decisiveness of Runcorn's measurements, one must first note that the Earth is not a uniformly dense sphere, so that m is a function of density as well as of volume. As density increases towards the Earth's centre, the decrease in B expected from the gravi-magnetic equation (3) is not as precipitous as the above volume argument might lead one to expect. At the Earth's surface, for instance, equation (3) predicts a B of 2.1×10^{-4} T. This is not, however, a great deal more than the 1.4×10^{-4} T that equation (3) predicts for the surface of Earth's core. More importantly, this core magnetism predicted by the gravi-magnetic equation is greater than the magnetic field of 6×10^{-5} T measured at the Earth's surface. Runcorn^{9,10} measured an increase of about 7×10^{-9} T over Earth's surface field of 6×10^{-5} T, still approximating 6×10^{-5} T, and considerably less than the 2.1×10^{-4} T predicted by gravi-magnetism for Earth's core. This is what we expect if we suppose that gravitational magnetism is modified by an electrical-magnetic effect stronger at the Earth's surface than in the interior. Runcorn's measurements are thus not decisive.

A decisive test for gravitational magnetism must be with laboratory-sized samples. Although Blackett's experiment² gave the appearance of being such a test, I now argue that this and other experiments have not definitively tested the predictions of the gravi-magnetic hypothesis, but only various extra assumptions, which were necessary at the time to predict effects large enough to be seen in laboratory-sized samples.

Wilson¹¹ exploited an ambiguity in the most general form of the gravi-magnetic hypothesis, which states that a mass moving either translationally or rotationally generates a magnetic field, just as a charge moving either translationally or rotationally

generates a magnetic field. There is a great difference, however, between translational and rotational movement. The former is relative, in the sense of special relativity; the latter is not. For charge, a translational movement does set up a magnetic field relative to the motion of the observer, a fact which was the starting point for Einstein's theory of relativity. Thus, although the observational basis for gravitational magnetism is only that massive rotating bodies possess nearly proportional magnetic fields, Wilson wrongly assumed that equation (3), which predicts the magnitude of the gravi-magnetic effect, could be made completely parallel to the electrical case by writing it in velocity form and assuming this velocity to be relative. Thus he had

$$B = 4/5 \mu_0/4\pi G^{1/2}/2k^{1/2} m v/r^2 \quad (4)$$

Because linear velocity is relative, Wilson could swing an iron bar in the laboratory and with his assumptions, consider that it was the Earth with all its mass that was moving past the bar. He thus put the Earth's mass and radius into equation (4) and expected a measurable field of 10^{-7} T. Wilson found that no field of such magnitude exists for laboratory-sized bodies in linear motion, thus ruling out the linear version of the gravi-magnetic hypothesis.

Swann and Longacre¹² tried a different idea. Swann¹³ had shown that by rewriting equation (4) in density form (where ρ is density)

$$B = 8\pi/15 \mu_0/4\pi G^{1/2}/2k^{1/2} \rho \omega r^2 \quad (5)$$

and replacing $\rho \omega r^2$ in equation (5) with $\rho \omega^4 r^4$, magnetic field ratios of the correct magnitude for Earth and the Sun can be predicted as well as measurable magnetic fields for rotating bodies of small mass. The substitution of $\rho \omega^4 r^4$ for $\rho \omega r^2$, however, makes equation (5) dimensionally incorrect, Swann therefore had to add arbitrary parameters to yield correct magnetic moments. This *ad hoc* procedure yielded a field expectation of about 10^{-7} T for a 20 cm copper sphere spinning at 200 Hz. Swann and Longacre spun such a sphere and showed that no field existed, to the limits of their measuring equipment, which was on the order of 10^{-8} T. Swann's version of equation (5) was thus ruled out. The Swann-Longacre experiment,

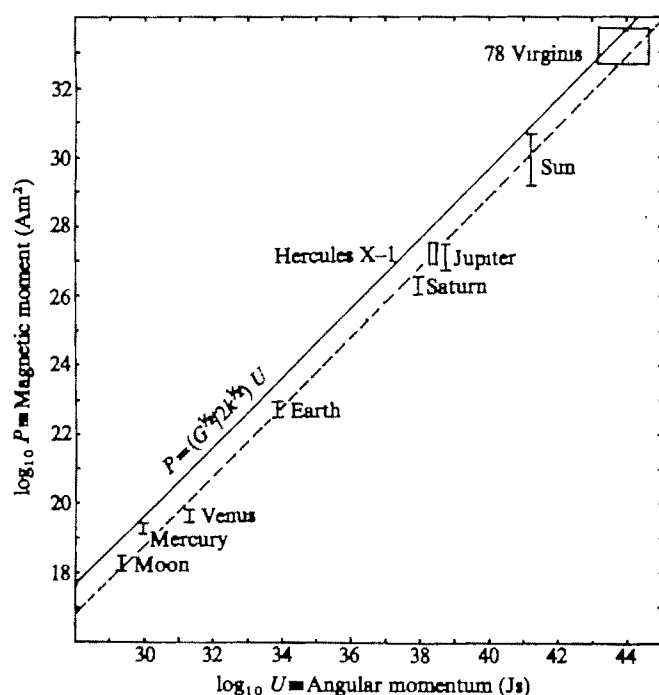


Fig. 1 Magnetic moment/angular momentum diagram. Comparison of gravi-magnetic hypothesis line, $P = (G^{1/2}/2k^{1/2}) U$, with P and U for massive rotating bodies. The dotted line is the regression line for the data. In $\log_{10}$ form, and in SI units: $G^{1/2}/2k^{1/2} = -10.37$; the mean P/U is -11.13 ± 0.42 .

Table 2 Laboratory experiments testing gravitational magnetism

Experiment (refs)	Assumption (equation number)	Expectation (T)*	Sensitivity (T)	Result
Wilson ¹¹ (1923)	Translational effect field due to mass and radius of Earth (4)	10^{-7}	10^{-8}	0
Swann & Longacre ¹² (1928)	Special rotational effect: $\rho \omega r^2$ becomes $\rho \omega^4 r^4$ (5)	10^{-7}	10^{-8}	0
Blackett ² (1952)	Virtual charge effect, static-body field due to rotation of Earth (4)	10^{-12}	10^{-13}	0
SQUID ¹⁴ (future)	Simple rotation effect, field due to rotating mass of laboratory-size body (3)	10^{-12}	10^{-13}	?

* 1 T = 1 tesla = 10^4 G (of B).

however, did not rule out the simple rotational version of the gravi-magnetic hypothesis, as this version of equation (5) yields a prediction of 10^{-13} T, which was five orders of magnitude below the sensitivity of their measuring apparatus.

After a gap of two decades, Blackett¹² took up the challenge of a laboratory test, encouraged by the new data from 78 Virginis which fit the gravi-magnetic hypothesis. However, Blackett, like Swann and Longacre before him, could not devise an instrument capable of measuring in the range of 10^{-13} T generated by a laboratory-sized spinning body. But he could measure the field of a static body, even if the field was only 10^{-12} T. Blackett therefore investigated a version of the gravi-magnetic hypothesis which predicted a static-body field on the order of 10^{-12} T. According to this version, a rotating body, such as the Earth, by reason of its mass, sets up a virtual electric current, so that a measuring instrument at rest on its surface registers a magnetic field on a small mass which is also at rest; both the small mass and the measuring instrument participate in the Earth's rotation, so that their velocity component, which is due to the Earth's rotation, can be put into equation (4). Thus Blackett calculated a field expectation of 10^{-12} T for a 15 kg mass at rest; and by clever experimental procedures he was able to rule out such a static-body field. He regarded this negative result as a confirmation of Runcorn's results which had been announced just as his own experiment was starting.

Such a catalogue of negative results has perhaps caused physicists to lose sight of the fact that a definitive test of the simple rotational version of the gravi-magnetic hypothesis has never been carried out in the laboratory. A definitive experiment may now be possible and should be attempted. Blackett² himself pointed out that a 1-m bronze sphere spun at 100 Hz (its maximum safe speed) should, on the simple rotational gravi-magnetic hypothesis, yield a field of 10^{-12} T. Such a field can be measured by a SQUID magnetometer¹⁴ limited by an internal field of only 8×10^{-14} T (r.m.s. per root cycle). Table 2 summarises this experimental situation.

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Low-level air flow over the western Indian Ocean as seen from METEOSAT

DURING the northern summer, the low-level air circulation over the Indian Ocean is characterised by cross-equatorial flow from the Southern to the Northern Hemisphere. The south-east trade winds of the Southern Hemisphere are sucked in by the zone of continental low pressure located over central India and are mainly deflected by the Coriolis force after crossing the Equator; they give rise to the south-west Indian monsoon and associated precipitation during the summer months¹. The cross-equatorial air flow is not uniform at all longitudes: it is weak over the eastern Indian Ocean and particularly strong along the East African coast, where it is concentrated into a low-level jet (maximum intensity 30 ms^{-1} at between 1 and 3 km) flowing to the Somalia coast from the northern tip of Madagascar². We now report the first results from a study of the low-level airflow circulation over the western Indian Ocean and more particularly the Somali low-level jet stream. Successive images taken by the European geostationary satellite METEOSAT were used to determine wind vectors.

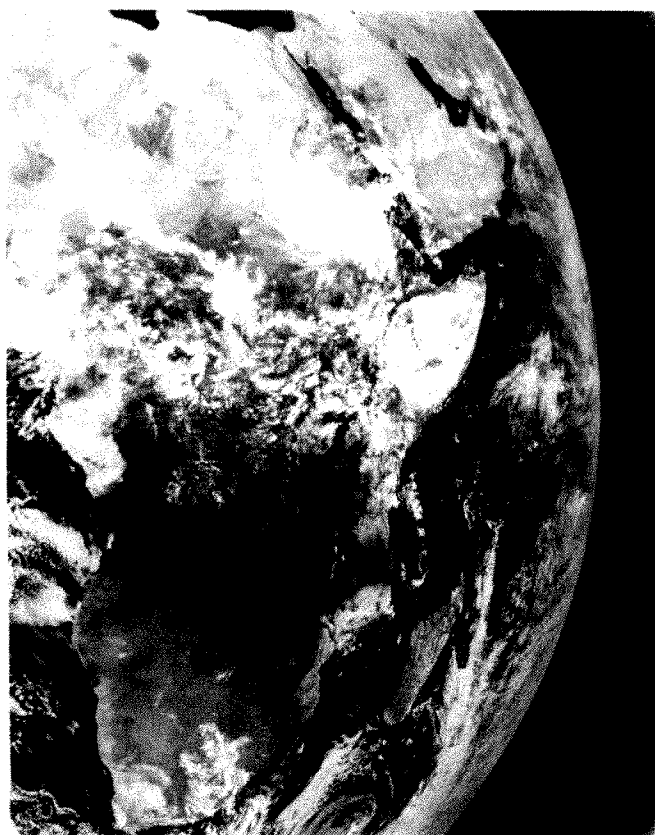


Fig. 1 METEOSAT view of the Indian Ocean on 16 July 1978, at 12.00 GMT.

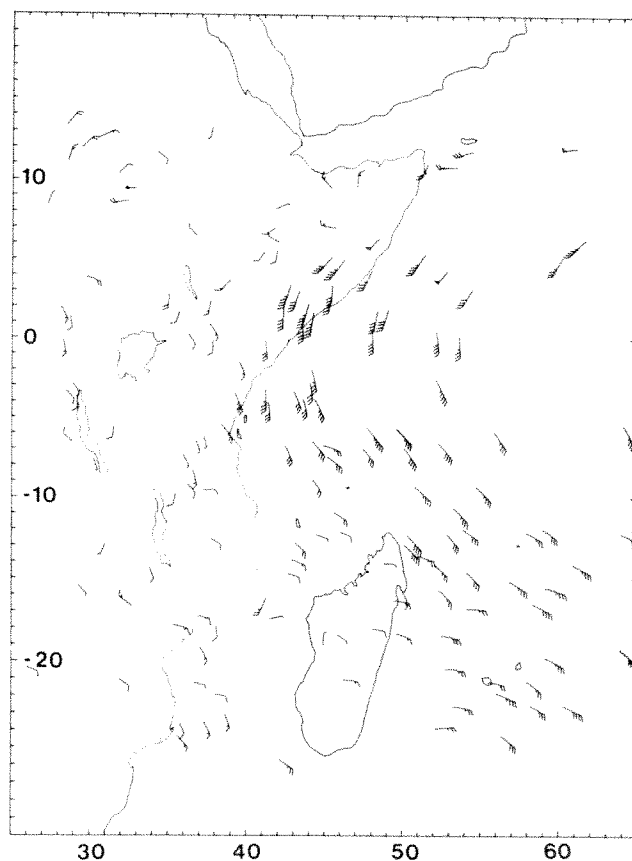


Fig. 2 Wind vectors determined from cloud displacements on 11 July 1978.

The Somali jet can have a regulating effect on monsoon precipitation over the Indian sub-continent, and a correlation has been found between the amount of rainfall over the western coast of India and the intensity of low-level equatorial winds over Kenya². It has also been found that cross-equatorial winds are stronger during a good monsoon year than during a bad one. The complete structure of this jet is largely unknown because it flows mainly over the ocean. For example, fluctuations of its intensity over Kenya, where it can be studied, may reflect a longitudinal shift of the core of the jet rather than a temporal variation of the intensity. The structure of the jet was intensively studied with an instrumented aircraft for a few days during MONEX-77, but no long-term investigation of variations and geographical extension has been made—this would be particularly difficult with conventional observations because of the marine extension of the jet. However, a geostationary meteorological satellite taking images at intervals would allow such a study from the analysis of cloud displacements. METEOSAT can serve this function and has been working since the end of 1977. It gives a view of the western Indian ocean up to about 60°E and is positioned in the neighbourhood of longitude 0° . METEOSAT produces images of the Earth and its cloud cover every half-hour in three spectral bands—one band in the visible spectrum and two in the IR spectrum (thermal IR, $10.5\text{--}12.5 \mu\text{m}$, and water vapour IR, $5.7\text{--}7.1 \mu\text{m}$, bands) with a ground resolution of 2.5 km for the visible channel and 5 km for the IR channels. From successive images cloud elements can be tracked to infer wind vectors.

The displacement of each cloud element can be determined by using the optical correlator built in our laboratory. This apparatus makes it possible to visualise several successive high resolution images on film loops and to move each image in relation to the others. Pictures can be aligned very precisely by using the landmarks; cloud motions are then measured by moving the pictures in such a way that the observed cloud appears motionless during the film loop; the relative displacements of the

pictures and the location of the cloud elements are recorded and the results are processed on a computer that plots them on a map. This method is relatively laborious but is very precise and moreover, the operator himself chooses the cloud element. In this study, three images were used: two successive visible images taken around noon and a thermal IR image taken at the time of the first visible image. The loop is made with the two successive visible images, and the IR one is used to discriminate the level of the clouds.

At low levels, the deviation between cloud vectors and raw wind data is often small—less than 5 knots. Three factors which might contribute to this error are: (1) an error in the tracking of the clouds; (2) a wrong height given to a cloud vector; and (3) a wrong cloud vector due to its displacement by non-advective effects (for example, orographic clouds or clouds related to local thermal effects). The first error is minimal when an optical correlator is used. The second one is the most difficult to exclude: for convective clouds the level of the top can be easily found on the IR picture, but the cloud motion more closely follows the wind near the base of the cloud. When the cloud top is not higher than the 600 m bar level, the wind given is assumed to be representative of the 900 m bar level. This is particularly so over the Indian Ocean where the trade winds are relatively deep. The third kind of error cannot be detected by objective techniques: it is very weak over the ocean but increases over land.

From METEOSAT low-level clouds (cumulus) are clearly visible over the entire marine region except for the northern Somalia coast, where the cold Somali oceanic current inhibits cloud activity (Fig. 1). Intensive convective activity prevails over Madagascar where it is difficult to infer wind vectors due to orographic effects. Over the flat land of Kenya and southern Somalia, wind vectors can be deduced from cloud motions within the low-level jet. Over the African continent and outside the region where the Somali jet is flowing, cloud motions are very weak and it is difficult to infer wind vectors, as the clouds are related to local convective phenomena.

Figures 2 and 3 give examples of raw wind vectors (not interpolated on a grid) as deduced from cloud motions on two days of the 1978 summer monsoon (11 and 16 July). It can be seen that data cover a large part of the western Indian Ocean. The only regions for which there are no data are certain parts of the African continent, the Arabian peninsula and the marine region off the northern Somali coast. The low-level wind field agrees quite well with previous studies based on conventional but scarce data⁴. On 11 July (Fig. 2) the Somali jet is clearly visible. It flows from the east of the northern tip of Madagascar to Africa where it can be seen over the flat lands of eastern Africa before flowing towards India. The results illustrate the importance of the mountainous ranges from Kenya to Ethiopia and of the Madagascar barrier. Strong winds can be seen around the Mascarene Islands showing that the roots of the jet may be found to the east of 60° E. North of the Equator the Coriolis force deflects the air and gives rise to westerlies over the Arabian Sea. An interesting feature of the clockwise gyre of air concerns its geographical extension. Thus on 11 July it was particularly large (larger than deduced from conventional data) and at the Equator strong winds can be found from 40° E to 55° E; the core of the jet is located close to the African coast. Another feature seen in Fig. 2 is a well-defined depression over Sudan corresponding to the early stage of an African easterly wave.

On 16 July (Fig. 3) the wind field was quite different. The Somali jet can also be seen but only to the north of 10° S and it is very weak. An intensification can be noted over the western Arabian sea off the Somali coast. The geographical extension is very weak and the root in the Mozambique channel is stronger than on 11 July. The most intriguing feature is the wind discontinuity found to the east of Madagascar; it seems to be related to the weakening of the low-level jet. This discontinuity corresponds to the normal deflection of the winds by a frontal zone moving westwards in the Southern Hemisphere. As can be seen in Fig. 1, this frontal system is associated with a mid-latitude cyclonic perturbation centered far south of Madagascar.

Thus extra-tropical systems of the Southern Hemisphere may interact with the cross-equatorial flow of the monsoon. This point needs further consideration with a study using wind fields on days around 16 July.

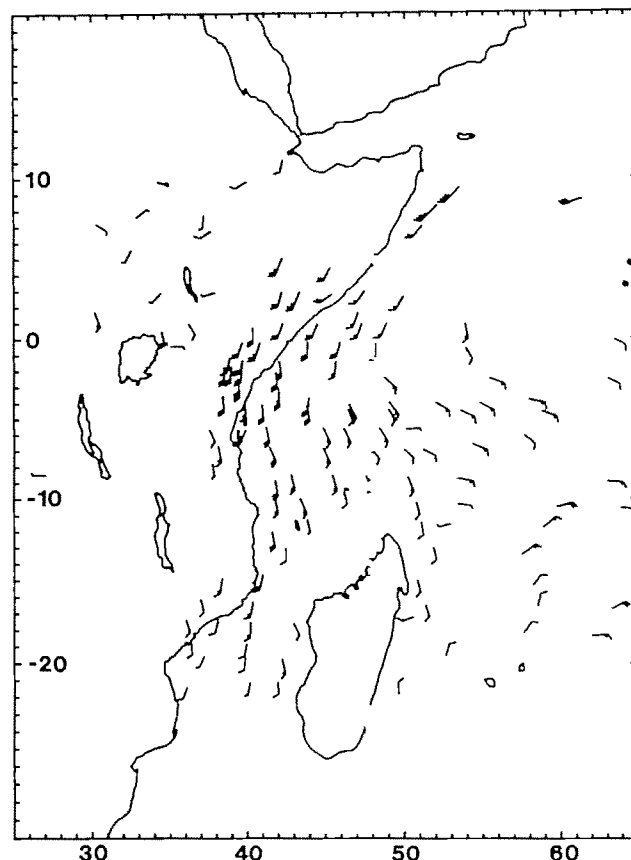


Fig. 3 Wind vectors determined from cloud displacements on 16 July 1978.

Our preliminary study has shown that the large-scale features as well as the temporal fluctuations of the Somali low-level jet can be studied from successive images taken from a geostationary platform. Since a complete view of the western Indian Ocean can be obtained, the causes of the fluctuations may be found. A daily extensive study was performed, covering 15 days in July 1978 as well as the end of May during the onset of the Indian summer monsoon. Our work stresses the value of a geostationary satellite over the western Indian Ocean region. Thus GOES-1, taking images of the Indian Ocean during MONEX-79, will be an important component of the MONEX observational systems.

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Stratospheric aerosol optical thickness measurements at 35°S

IN the stratosphere the attenuation in the radiance of a light beam is predominantly due to scattering by air molecules and aerosols, and absorption by ozone. For the height range 10–30 km, aerosols can account for at least 60% of the attenuation at 700 nm (ref. 1) and are responsible for most of the long and short term variations². During 1969–76 the stratospheric optical thickness due to aerosol scattering was measured at Adelaide 35°S, using a lidar³. The observations reported here show that the aerosol optical thickness varied by more than a factor of three during that time.

The transmittance⁴ of the atmosphere, $T(h_1, h_2)$, between heights h_1 and h_2 is a measure of the fractional attenuation of a vertically directed beam and is given by

$$\begin{aligned} T(h_1, h_2) &= T_M(h_1, h_2) T_A(h_1, h_2) T_O(h_1, h_2) \\ &= \exp \left[- \int_{h_1}^{h_2} \{ \beta_M(h) + \beta_A(h) + \beta_O(h) \} dh \right] \\ &= \exp [-\tau_M(h_1, h_2) - \tau_A(h_1, h_2) - \tau_O(h_1, h_2)] \quad (1) \end{aligned}$$

where β_M and β_A are the molecular and aerosol volume scattering coefficients, β_O the ozone volume absorption coefficient, and τ_M , τ_A and τ_O are the respective optical thicknesses. For lidar studies it is necessary to introduce the volume scattering function $B(\theta, h)$ which is a measure of the radiation scattered per unit solid angle in the direction θ at the height h in the atmosphere. The total radiation scattered out of a beam is given by

$$\beta(h) = \int_{4\pi} B(\theta, h) d\omega$$

and the fraction of the scattered radiation directed in the backwards direction per unit solid angle is given by $F(\pi) = B(\pi, h)/\beta(h)$. Then the transmittance due to aerosols, $T_A(h_1, h_2)$, between the heights h_1 and h_2 can be related to the integrated backscatter function by

$$\begin{aligned} -\ln [T_A(h_1, h_2)] &= \tau_A(h_1, h_2) = \int_{h_1}^{h_2} \beta_A(h) \\ &= F_A^{-1}(\pi) \int_{h_1}^{h_2} B_A(\pi, h) dh \quad (2) \end{aligned}$$

For the stratosphere suggested values of $F_A(\pi)$ range between 0.013 (refs 5, 6) and 0.0199 (ref. 7).

The laser power $p(h)$ returned from a height h and detected by the lidar receiver is given by

$$p(h) = P_0 K T^2(0, h) [B_M(\pi, h) + B_A(\pi, h)] / h^2$$

where P_0 is the power transmitted, K is a system constant which includes the geometry and efficiency of the lidar optics and electronics, and $B_M(\pi, h)$ and $B_A(\pi, h)$ are the volume back-scattering functions for air molecules and aerosols at the height h . Rearranging gives

$$\begin{aligned} B_A(\pi, h) &= p(h) h^2 / \{ P_0 K T_M^2(0, h) \cdot T_O^2(0, h) \cdot T_A^2(0, h) \} \\ &\quad - B_M(\pi, h) \quad (3) \end{aligned}$$

The solution of equations (2) and (3) for B_A and T_A is accomplished by an iterative technique developed by Elterman⁸. At the height or heights where the total scattering is a minimum the aerosol scattering is assumed to be zero, thereby normalising the lidar signal to the known molecular atmosphere at these heights. This procedure is justified by the detection of regions of very low aerosol content by direct sampling techniques. Values of B_A and T_A are then determined for successive height increments by iteration of equations (2) and (3) at each level. The

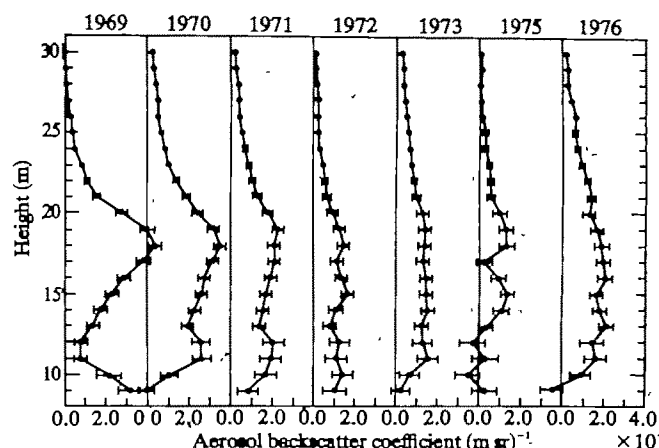


Fig. 1 Yearly means values of aerosol backscatter coefficient B_A . Error bars are ± 1 standard deviation.

constant $F_A(\pi)$ was taken as 0.0199; a choice of 0.013 would have changed the integrated backscatter function for the average of the data for 1969 by <2%. The molecular parameters B_M and T_M are calculated from the atmospheric density profile measured by radiosonde balloons launched from the Adelaide Airport, 7 km from the lidar site, on the night of the lidar measurements or on the following morning. The ozone transmittance was calculated from Eltermans tables¹.

The average yearly profiles of $B_A(\pi, h)$, shown in Fig. 1, show how the values of this function have varied with height and time. Error bars represent one standard deviation and include the statistical error in the signal photoelectron count rate and a 1% error⁹ in the radiosonde-measured density profile. Note that although each nightly profile was normalised to the molecular profile in one or more regions, the average profiles for some years show no region where the aerosol contribution to scattering is consistently zero. Failure of the ruby laser allowed only one observation between December 1973 and February 1976.

By integrating curves similar to Fig. 1 over the height range 10–30 km, the vertically integrated aerosol backscatter function^{6,10} is formed; the average monthly values are plotted in Fig. 2. The aerosol optical thickness is indicated by the scales on the right. Scale A refers to a value of $F_A(\pi)$ of 0.0199 and scale B to value of 0.013.

A maximum in aerosol optical thickness occurred in late 1969 followed by a decline thereafter until 1976. The occurrence of the peak value is related to the eruption of the Fernandina volcano in June 1968 and its timing is possibly a result of the gas-to-particle conversion rate¹¹ and the stratospheric meridional transport rates. There is a suggestion of an annual

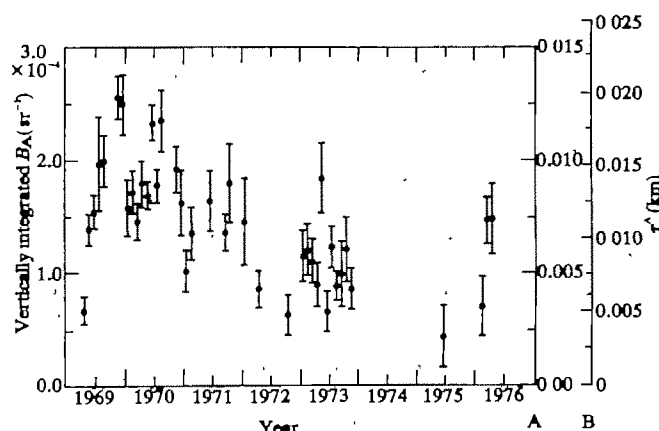


Fig. 2 Monthly mean values of vertically integrated backscatter coefficient (10–30 km), and corresponding values of aerosol optical thickness assuming values of $F_A(\pi) = 0.0199$ (scale A), and 0.013 (scale B).

variation in 1969 and 1970 with lower values early in the year. The abnormally high value in May 1973 and low value in June 1975 are each the result of observations on a single night and may not truly represent the average conditions in these months.

The peak values of aerosol optical thickness range between 0.013 and 0.020 (depending on the assumed value of F_A). These are considerably less than the values for 700 nm determined by Elterman from searchlight measurements in 1964 and 1965¹ (0.027) and are also less than his value for 1970¹². The average Adelaide value for 1973 is between 0.005 and 0.007. This is higher than the lidar-derived value of 0.004 obtained over California¹⁰ for the same period, but much lower than Elterman's¹ value of 0.014 for the 'normal' stratosphere (when converted to 694 nm). The average of values for early 1976, 0.006–0.009 is slightly higher than the 1973 average and may indicate the presence of some residual dust from the eruption in October 1974 of Volcán de Fuego in Guatemala.

To obtain a reliable estimate of the minimum value optical thickness for the period of observation, a weighted average was calculated from those eight monthly values which were not statistically distinguishable from the lowest value. A value of $(7.4 \pm 0.2) \times 10^{-5}$ per steradian was obtained for the integrated backscatter function, which corresponds to an optical thickness of between 0.004 and 0.006. Thus the extreme values of aerosol optical thickness varied by a factor of at least three during the period 1969–76.

To relate the aerosol optical thickness for the lidar wavelength of 694 nm to the optical thickness for the solar spectrum, the extinction due to scattering was calculated for the two spectral distributions using the stratospheric aerosol size distribution given by Bigg¹³. The extinction of solar radiation is 87% of that for monochromatic radiation at 694 nm. Thus during the period 1969–76, the optical thickness of stratospheric aerosols for solar radiation varied from about 0.018 to 0.004.

An estimation of the reduction in the ground level solar flux at Adelaide during 1969–76 due to stratospheric aerosols can be obtained from the analysis of Cadle and Grams¹⁴. The energy lost due to upward scattering from a solar beam incident at a zenith angle of 60° varied between 0.5 and 0.15%.

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Anomalous results for tidal flow through the Pentland Firth

THE flow of water across the vertical component of the Earth's magnetic field induces a potential gradient in the water; measurements of the difference in voltage between the opposite sides of a tidal channel provide an indication of the magnitude of the tidal flow. Longuet-Higgins¹ derived the relationship between the potential gradient and the tidal flow for the case of

uniform flow in a channel of semi-elliptic cross-section. By recording the difference in potential between the ends of submarine telephone cables valuable measurements have been obtained of flow through the Dover Strait^{2,3} and through various channels in the Irish Sea^{4,5}. However, recent cable measurements for tidal flow through the Pentland Firth are shown here to include anomalous results.

The study of the movement and dispersal of certain water masses over periods of years and, consequently, over large spatial zones is of increasing interest in connection with studies of dispersal of radioactive waste or fisheries investigations. Cable measurements are of particular value in such studies as they provide, at minimal cost, a continuous long-term record of the integrated flow across a particular section⁶. However, in recent years many of these cables have either been abandoned or replaced by 'repeated' cables. In the latter, power is fed along the cables to amplify the communications signal at repeated intervals and this tends to distort the measurement of the flow-induced voltage⁷.

Unexpected results found from recording on cables crossing the Pentland Firth (Fig. 1) are particularly interesting. Two cables, running along closely parallel paths, link the Orkneys with the Scottish mainland. Recordings of voltage were made on these cables from February to October 1976, coinciding with the JONSDAP '76 oceanographic exercise in the North Sea. The measurements coming respectively from the two cables were very similar so only the results from cable 2 are presented. Tidal analyses of the recordings were made by dividing the data into six discrete sets each lasting 29 d. Despite the high noise level, the analyses produced consistent results for the principal harmonic constituents (Table 1). However, an examination of the propagation of the M_2 tidal constituent shows that the phase of this constituent calculated from the cable data (that is 18° for flow measured positive towards the east) does not accord with the general pattern of flow as deduced from other data sources (Fig. 1).

Values for the phase of the M_2 constituent of surface currents were calculated from the Admiralty Tidal Stream Atlas. Figure 1 shows values of 271° and 256° obtained for positions in the Firth and a value of 235° at a position slightly east of the Firth. As part of JONSDAP '76 current meter measurements were made at position 53 (58°37' N, 2°25' W). Tidal analyses of these measurements produced an M_2 value of 302° for flow along the major axis of the ellipse aligned 10°W of S. The phase of the vertical tide in this region is indicated by the co-phase lines in Fig. 1 and also by the values from the coastal gauges. Over the region in which the phase of the currents varies from 271° to 302°, the phase of the vertical tide varies from about 250° to

Table 1 Tidal analyses of voltages recorded on the Pentland Firth telephone cable

	Amplitude				Phase			
	1976		1978		1976		1978	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
O1	20	22	29	31	23°	24°	7°	19°
K1	44	47	82	20	172°	22°	193°	28°
μ_2	45	24	50	6	251°	11°	254°	7°
N2	52	10	56	21	325°	7°	339°	6°
M2	231	3	236	3	18°	3°	19°	1°
L2	17	42	18	75	111°	21°	148°	51°
S2	105	11	111	20	61°	8°	66°	5°
M4	10	47	6	22	203°	14°	190°	92°
M6	6	57	12	16	69°	44°	66°	25°
	mV	%*	mV	%†				

* Standard deviation of results from six separate monthly analyses expressed as a percentage of the mean value of the constituent.

† Standard deviation of results from three separate monthly analyses.

320°. Thus maximum flows apparently occur within 1 h of tidal high water throughout this region. Therefore the anticipated M_2 phase for flow across the cable section would be in the range 280°–340° compared with the recorded value of 18°.

An examination of the non-tidal (or residual) component from the cable signal shows a reasonable correlation ($r = 0.71$) between the easterly flows through the Pentland Firth and the southerly flows measured by current meters at the nearby position 53. There was no indication of either a significant wind-driven component or a seasonally varying flow component in the residual signal.

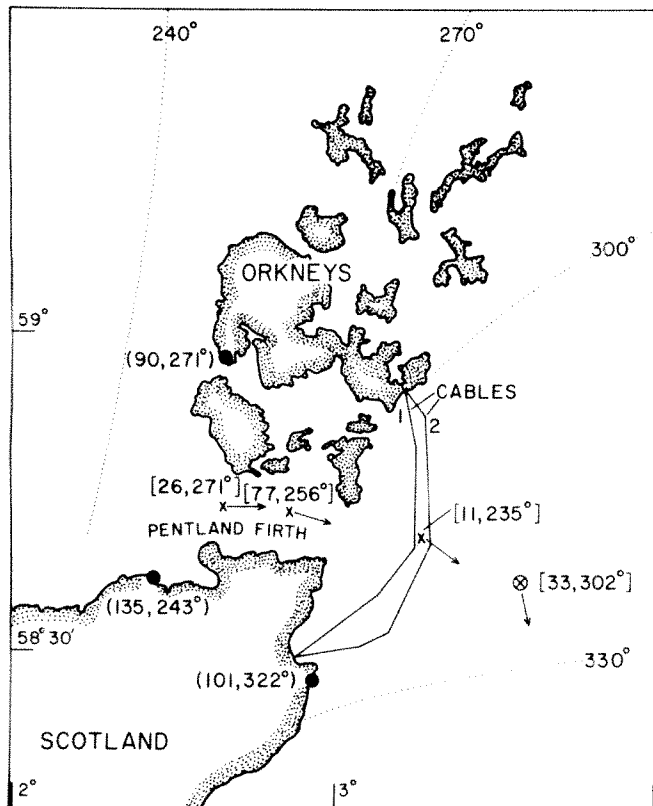


Fig. 1 Propagation of the M_2 tidal constituent in the region of the Pentland Firth. ●, Tidal elevation data in cm; ×, tidal stream data in cm s^{-1} (from Admiralty Tidal Stream Atlas); ⊗, current meter data from JONSDAP '76; co-phase lines shown dotted.

One explanation for the anomalous M_2 phase follows from the work of Robinson⁸ who showed that cables respond in a complex fashion to flow over a wide area surrounding the termination points of the cable. He also showed that non-uniformity in sea-bed conductivity can complicate the general integral. In this region, the variability in both the coastline and the tidal stream patterns tends to invalidate the application of simplified formulae of the kind derived by Longuet-Higgins. In addition, the northerly latitude and part east-west alignment of the cable increase the noise level on the cable⁹. The question also arose as to whether any error had been introduced either in the instrumentation or in the data processing.

To establish the validity of these results the recordings were repeated over a 3-month period, July–September 1978. One of the cables had been severed in the interim period and so measurements could only be made on cable 2. To avoid repeating possible errors from the earlier experiment, the recording equipment was installed by a scientist not involved in the earlier recordings. Also, the programmes used to convert the original data into hourly values for subsequent analyses were rewritten. The data were sub-divided into three discrete sets, each of 29 d

duration, for the purpose of tidal analyses. The results, shown in Table 1, closely agree with the values obtained from the 1976 recordings. Thus the voltage recordings from the Pentland Firth submarine cables seem to provide a useful indication of residual flow through the channel but produce an anomalous M_2 tidal signal.

R. I. R. Palin and A. J. Harrison were responsible for the instrumentation and data collection, their assistance in data translation also contributed significantly to this study. This work was funded by a Consortium consisting of the NERC, the Ministry of Agriculture Fisheries and food, and the Departments of Energy, and Industry.

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A simple approach for identifying and measuring acidification of freshwater

ACIDIFICATION of lakes and rivers accompanied by the loss of fish populations is a problem in Scandinavia^{1–4}, northeastern USA^{5,6}, and southeastern Ontario, Canada^{7,8}. These areas have granitic or other siliceous bedrock types, thin and patchy podsollic soils, and extremely soft and poorly buffered surface waters, and receive precipitation which is decidedly acidic (volume-weighted average pH 4.0–4.6). Sulphate, whose principal source is precipitation, is usually the major anion in these acidified waters. In similar areas such as northern Scandinavia and northwestern Ontario, in which precipitation is not acidic (pH > 5.0) such oligotrophic pristine softwaters generally have pH levels > 5.5, and bicarbonate is the major anion^{9–11}. Acidification of such waters entails a decrease in the bicarbonate buffer (alkalinity) with only minor decreases in pH, and then after exhaustion of the bicarbonate an increase in acidity to pH levels well below 5.0. Because of the severe damage to fish populations, and other biological consequences, the decreases in pH that characterise the later stages of the acidification process are of major concern. We need, therefore, a simple 'early warning' indicator that identifies lakes and rivers which are undergoing the first stage of acidification, the loss of alkalinity, but which have not yet reached the stage of marked pH decreases. Furthermore, we need quantitative estimates of the degree of acidification. We show here that excess sulphate concentrations, or current calcium concentrations and alkalinity can provide such an estimate.

If bicarbonate is still present, simply measuring alkalinity will not reveal whether or not acidification has occurred unless pre-acidification alkalinity data are available for comparison. Unfortunately, few such data exist. However, in unacidified waters bicarbonate alkalinity is present in approximately equivalent concentrations as the sum of calcium and magnesium (less that of sea-spray origin). As the ratio Ca/Mg is also relatively constant, and pH is closely related to alkalinity and acidity, plots of pH and calcium should distinguish between

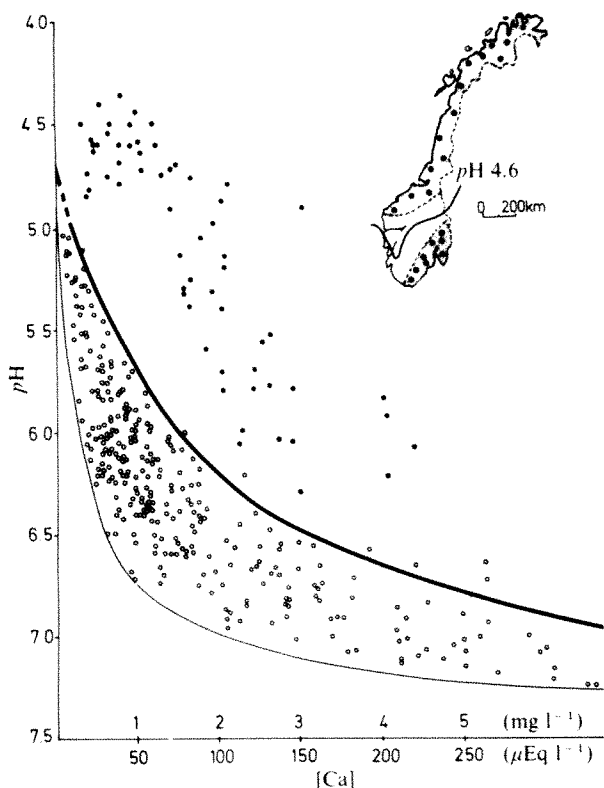


Fig. 1 pH and calcium concentrations in lakes in northern and northwestern Norway sampled as part of the regional survey of 1975, in lakes in northwestern Norway sampled in 1977 (○) and in lakes in southernmost and southeastern Norway sampled in 1974 (●). Southern Norway receives highly acid precipitation (pH 4.2–4.5) and a large number of lakes have lost their fish populations due to high acidity^{2,4}. Inset shows areas in which these lakes are located. Areas south of isoline receive precipitation more acid than pH 4.6.

acidified and unacidified waters. This is the case for small Norwegian lakes sampled in 1975 and 1977: most of the lakes in northern and northwestern Norway fall below an empirically drawn curve, whereas lakes in southernmost and southeastern Norway lie above this curve (Fig. 1).

The curve also separates lakes on the west coast of Sweden where pH in precipitation is 4.2–4.4 from lakes in west-central Sweden (W. Dickson, personal communication) where pH in precipitation is less acid (Fig. 2). Furthermore, it also works for North America. Lakes in the Experimental Lakes Area (ELA)¹¹, northwestern Ontario¹¹, and in the Sierra Nevada, California¹² (Fig. 3), areas not receiving highly acidic precipitation, fall below the curve, whereas waters in acidified areas such as the Adirondack Mountains, New York⁶, Hubbard Brook, New Hampshire¹³, and Sudbury, Ontario⁷, fall above the curve.

Figures 1–3 clearly show that the empirical curve distinguishes between lakes situated in non-acidified and acidified areas in Scandinavia and North America. This acidification indicator is particularly useful in that it identifies lakes that have lost some alkalinity but in which pH levels are still high enough for fish populations and other components of the biota to be apparently intact and normal; these waters may be threatened by future inputs of acid components. Thus an 'early warning' is given.

This indicator depends on the observation that in oligotrophic pristine freshwaters calcium is accompanied by a proportional amount of bicarbonate. In such areas, one finds waters in which this is not the case, for example waters containing high concentrations of natural organic acids (highly coloured waters), or waters in areas where the geological environment is such that calcium and bicarbonate are not the major cation and anion, respectively. When applied to data from such waters, the indicator implies an apparent acidification where none has occurred.

The acidification of a lake is the loss in alkalinity that has taken place in the lake water. To quantify acidification we thus need to know the pre-acidification alkalinity and the present-day alkalinity (or acidity in case of acid lakes):

$$\text{Acidification} = \text{pre-acidification alkalinity} \\ - \text{present day alkalinity.}$$

As few historical alkalinity values are available for lakes that are acidified today, an estimate of the pre-acidification alkalinity is therefore needed. Present-day calcium concentrations can be used to estimate alkalinity because in regions not receiving acid precipitation softwater oligotrophic waters contain proportional concentrations of calcium and alkalinity. For example, calcium and alkalinity data from 59 lakes in northern and northwestern Norway gave a least-squares linear regression alkalinity ($\mu\text{Eq l}^{-1}$) = $-29 + 1.32 \text{ Ca}$ ($\mu\text{Eq l}^{-1}$), $r = 0.92$. Similarly, data from 98 lakes in the ELA¹¹ gave alkalinity ($\mu\text{Eq l}^{-1}$) = $-32 + 1.42 \text{ Ca}$ ($\mu\text{Eq l}^{-1}$), $r = 0.87$. (If alkalinity is determined by the standard acidimetric titration to pH 4.5, actual bicarbonate concentrations are overestimated by $32 \mu\text{Eq l}^{-1}$, the amount of free H^+ -ion in solution at pH 4.5. For these regressions the alkalinity data obtained by titration were corrected for this $32 \mu\text{Eq l}^{-1}$.)

These data suggest that the relationship between calcium and alkalinity in pristine softwater lakes is of a general nature. Thus pre-acidification alkalinities can be estimated from present calcium concentrations.

One factor must, however, be considered when estimating pre-acidification alkalinities from present-day calcium concentrations. There is evidence for an increase in the calcium concentrations in acidified areas due to increased chemical weathering and washout from the soil^{10,14,15}. Thus present-day calcium concentrations might overestimate the pre-acidification alkalinity. On the other hand, an increased washout of calcium from a lake catchment probably also results in an equivalent amount of alkalinity which is consumed during the acidification process.

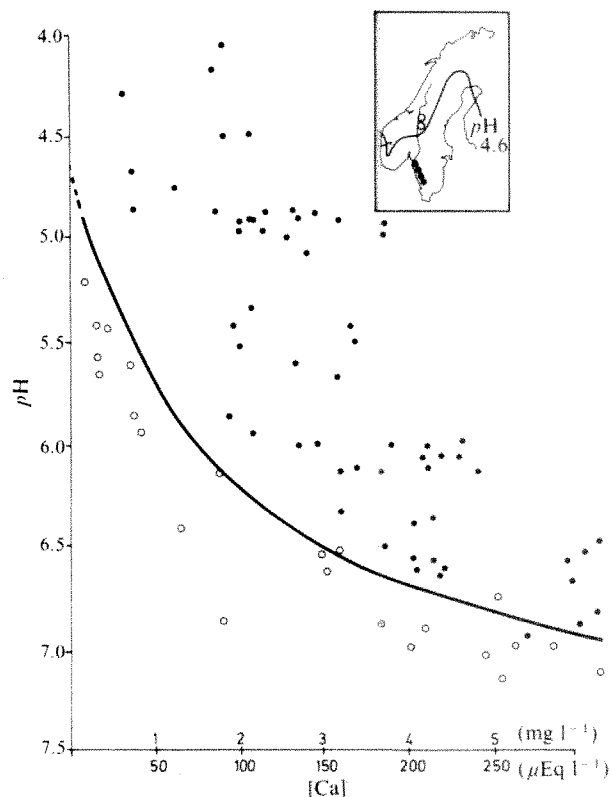


Fig. 2 pH and calcium concentration in lakes in southern (●) and west-central Sweden (○) (data from W. Dickson). Inset shows locations of lakes sampled. Areas south of isoline receive precipitation more acid than pH 4.6.

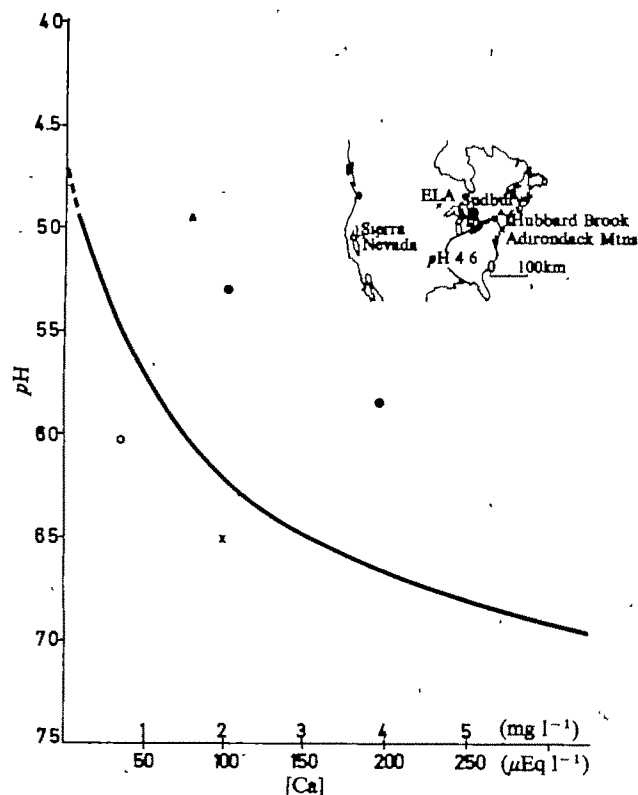


Fig. 3 Average pH and calcium concentrations in 170 lakes in the Sierra Nevada, California¹² (○), 109 lakes in the Experimental Lakes Area, northwestern Ontario¹¹ (×), 178 lakes in the vicinity of Sudbury, Ontario⁷ (⊙), 216 lakes in the Adirondack Mountains, New York (●) (Schofield, personal communication), and average for 11 yr at Hubbard Brook, New Hampshire¹³ (Δ). Waters in areas receiving acid precipitation lie well above the solid curve, whereas those in other areas lie below. Inset shows locations of these areas. Areas east of the isoline receive precipitation more acid than pH 4.6.

Regional surveys of lakes in southern Norway have been conducted annually since 1974 (ref. 16). Strong acid concentrations using the Gran technique¹⁷ were measured in the 1978 samples, and when combined with pH, calcium and sulphate data, estimation of the degree of acidification can be made for 46 lakes. The acidification estimated this way is closely related to the weighted average concentration of H^+ and sulphate in precipitation at each lake (Fig. 4a, b). These data indicate that significant acidification has occurred in areas receiving precipitation with volume-weighted average concentrations of H^+ above about $20\text{--}25\ \mu\text{Eq l}^{-1}$ (pH 4.7–4.6) and SO_4 concentrations above $1\ \text{mg l}^{-1}$ ($20\ \mu\text{Eq l}^{-1}$). This 'threshold' pH level of 4.6–4.7 was also determined by Wright¹⁸ in an analysis of historical pH data from 217 lakes in southern Norway and Sweden. Lakes in which pH had declined significantly during the past 20–25 yr are located exclusively in areas receiving precipitation more acid than about pH 4.6.

The estimated acidification of lakes in southern Norway is also strongly related to the concentrations of excess sulphate in the lake water, that is, sulphate in excess of that of marine origin as measured by chloride concentrations and the $SO_4:Cl$ ratio in seawater (Fig. 4c). This suggests that the bicarbonate lost in acidified lakes has been replaced by an equivalent amount of sulphate. The excess sulphate concentration in lakes less a 'general Northern Hemisphere' concentration then provides another measure of the acidification. Figure 4a, b indicates that about $10\text{--}20\ \mu\text{Eq l}^{-1}$ ($0.5\text{--}1.0\ \text{mg SO}_4\ \text{l}^{-1}$) is a reasonable estimate of the natural background concentration for lakes in southern Norway. Almer *et al.*¹⁰ suggest $20\text{--}60\ \mu\text{Eq l}^{-1}$ as a general background for Sweden.

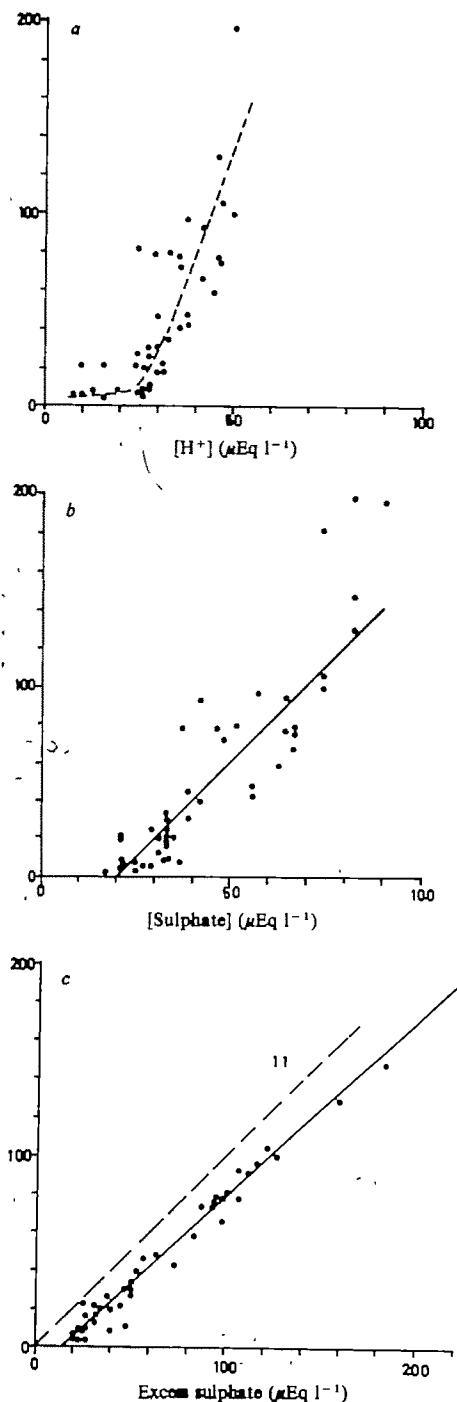


Fig. 4 Estimated acidification in 46 lakes in south Norway versus H^+ -concentration (a) and excess sulphate (b) in precipitation at each lake. Precipitation data were interpolated from data given by Dovland *et al.*¹⁹. c, Estimated acidification versus excess sulphate concentrations in 46 lakes. Least squares regression line: $y = -12 + 0.89x$ ($r = 0.99$).

Thus, in areas in which very little sulphur is supplied by weathering and where the ratio of non-marine calcium to magnesium is fairly constant, acidification of freshwaters can be estimated from either present-day excess sulphate concentrations or by present-day calcium concentrations and alkalinity.

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Palaeogene komatiites from Gorgona Island

UNMETAMORPHOSED mafic and ultramafic rocks overlain by Neogene oceanic sediments are exposed on Gorgona Island which is a remnant of the Coastal Cordillera along the Pacific margin of Colombia¹. Gorgona is one of the rare places in the world where young pyroxenitic komatiites exhibiting typical quenched spinifex textures (Fig. 1) occur. The spinifex rocks form lenticular bodies (? lava flows) within cumulate ultramafics and troctolitic gabbros and are closely associated with diabases and tholeiitic pillow basalts¹. We show here that in textures, rock and mineral chemistry, the Gorgona spinifex rocks are almost identical with the Archean peridotitic to pyroxenitic komatiites from Munro Township (North-east Ontario)². For this reason the term komatiites is used for the Palaeogene rocks, although one could call them high Mg-tholeiites.

Mineral composition: quenched coarse olivine blades up to 5 cm long and 1 mm thick dominate the spinifex texture. The blades are Fo_{90–91} with narrow edges of Fo_{85–86}. Clinopyroxenes and plagioclases are restricted to the interstitial groundmass (Fig. 1). High-aluminium calcic pyroxenes exhibit coarse skeletal crystals; whereas low-aluminium calcic pyroxenes, unzoned plagioclase laths (An_{75–81}) and devitrified and chloritised glass form fine-grained, arborescent crystal aggregates. Chromite occurs mostly in idiomorphic grains.



Fig. 1 Photomicrograph of the spinifex-textured komatiite from Gorgona Island. Weakly serpentinised skeletal plates of olivine are randomly scattered around a groundmass consisting of quenched clinopyroxene aggregates, few plagioclases and devitrified glass.

Rock composition: the major and trace elements have been mainly determined by X-ray fluorescence methods³, the rare earth elements by instrumental neutron activation analysis (INAA in Karlsruhe) (see Table 1).

Outstanding chemical features of these rocks are the high MgO, chromium and nickel contents relative to ocean floor basalts. The olivine compositions seem to be compatible with crystallisation from a liquid with magnesium-values corresponding to the bulk rock composition, giving K_D values (ol/rock)⁴ of about 0.3. We think that the unusual chemical

Table 1 Rock composition of komatiites from Gorgona Island

	Average of four determinations wt%	Calculated anhydrous wt%	Trace elements (p.p.m.)			
SiO ₂	44.2	46.2	Nb	<1	La	1.1
TiO ₂	0.66	0.69	Zr	30	Ce	~1±1
Al ₂ O ₃	12.0	12.6	Y	14	Nd	~1±1
Fe ₂ O ₃	3.2	1.3	Sr	58	Sm	1.7
FeO	8.2	10.3	Rb	<1	Eu	0.75
MnO	0.18	0.19	U	<1	Gd	3.4
MgO	15.9	16.6	Th	<4	Tb	0.49
CaO	10.1	10.5	Pb	<6	Ho	0.89
Na ₂ O	1.13	1.18	Ga	15	Tm	0.27
K ₂ O	0.02	0.02	Zn	57	Yb	1.5
P ₂ O ₅	0.06	0.06	Cu	105	Lu	0.17
Cr ₂ O ₃	0.18	0.19	Ni	718	Ba	<1
NiO	0.09	0.09	Co	93	Sc	31
H ₂ O	4.0	—	Cr	1250	S	109
Total	100.0	100.0	V	256		

$$\begin{aligned} \text{Mg-value:} & \quad \text{FeO:Fe}_2\text{O}_3 = 9:1 \\ \frac{100 \text{ Mg}}{\text{Mg} + \text{Fe}^{++}} &= 77.6 \\ &= 74.2 \end{aligned} \quad K_D = \frac{X_{\text{FeO}}^{\text{ol}} X_{\text{MgO}}^{\text{rock}}}{X_{\text{FeO}}^{\text{rock}} X_{\text{MgO}}^{\text{ol}}}$$

composition of these rapidly chilled extrusive rocks within the close neighbourhood of oceanic crust throws new light on the petrological models of generation of ocean floor basalts; by far the most common rock type on Earth. There are several possibilities for the origin of ocean floor basalts: do they represent relatively unmodified primary magmas produced by partial melting from a lherzolite type source rock⁴, did they originate from other ultramafic mantle material followed by crystal fractionation⁵ or could magma mixing of mantle-derived primary magmas together with highly evolved differentiation products along with crystal fractionation have played an important part^{6,7}?

We believe that the Gorgona komatiites represent the least modified liquid generated by partial melting of the upper mantle. They have certain chemical characteristics similar to the melt inclusions in olivine and plagioclase from Mid-Atlantic ridge basalts⁷ (DSDP, Legs 45 and 46, Sites 395 and 396). These include low TiO₂ and Na₂O contents, and high magnesium-values. However, the Gorgona komatiites have higher MgO and nickel contents and are closer to the primary magma, proposed on the basis of experimental evidence⁶, than are the most 'primitive' ocean-floor basalts described to date. Green *et al.*'s primary magma which could have segregated from residual harzburgite at ~20 kbar, 1,430 °C, was experimentally derived from basalt glass (south Mid-Atlantic Ridge, DSDP Leg 3, Site 18, sample 3-18-7-1)⁸ which contains microphenocrysts of olivine (Fo_{89.5}) and plagioclase (An₇₉). This glass also shows very similar rare earth patterns to the Gorgona komatiites. Both are strongly depleted in light rare-earth elements and have low concentrations of large-ion lithophile elements. These aspects will be discussed in more detail elsewhere⁹. Geochemical investigations of the surrounding mafic rocks¹⁰ show that Gorgona Island consists mainly of normal fractionated olivine tholeiites, diabases, troctolites and gabbros, which could be explained by the existence of a shallow magma chamber^{6,7}. The question still remains of whether Gorgona Island represents an uplifted part of a large Pacific oceanic crust or a smaller marginal basin.

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Gametogenesis and calcification of planktonic Foraminifera

THE processes of gametogenesis and development in benthic Foraminifera are well known^{1,2}; however, only recently has there been substantial success in the culture of planktonic species³. As part of an investigation of planktonic life cycles, we have maintained in laboratory culture mature foraminifers of *Hastigerina pelagica* and *Orbulina universa* for up to 68 d with more than 80% of the individuals producing gametes. Juvenile stages were subsequently cultured up to initial calcification and regular chamber formation. Although other workers^{4–6} have cultured adults and induced gametogenesis, this is the first known success in the maintenance of gametes and the culture of second generation Foraminifera.

Live, adult, planktonic foraminifers were obtained from pelagic waters off Bermuda by hand capture according to a technique of Bé *et al.*³. Although this method limits specimens to those visible to a diver in 3–20 m of water, it yields healthy individuals undamaged by net tows or conventional samplers. Each organism was isolated in 500–700 ml of filtered natural seawater and aerated lightly. Normal ambient temperatures (19–23 °C) and lighting (12 h, 40–50 foot candles, fluorescent source) were used. To maintain axenic cultures, the seawater was filtered through successive 0.45-µm and 0.1-µm Millipore filters before the introduction of the foraminifers and repeated periodically to remove accumulated by-products. Adult specimens were removed to separate Petri dishes for biweekly feedings of *Thalassiosira fluviatilis* and nauplii of *Artemia* sp. grown in sterile conditions. *H. pelagica* readily accepted both, suggesting that it is an indiscriminate omnivore in nature. *O. universa* did not accept crustaceans, although carnivorous diets have been reported for it as well as for other spinose species^{1,3,5,7}. In any case, our results indicate that planktonic Foraminifera can be maintained readily on an exclusively carnivorous diet although healthiest individuals result from a combined diet of phytoplankton and live Crustacea.

Based on observations of 29 *H. pelagica* and two *O. universa*, gametogenesis can be said to be a predictable development

preceded by several characteristic morphological changes. Between 24 and 36 h before gamete release, the normally expanded calymma decreases until no bubbles or only a single bubble layer is visible around the chambers. Simultaneously, all pseudopodial filaments are retracted into the calcareous test and the main body of cytoplasm constricts into the inner chambers until the last and typically largest chamber is evacuated. The cytoplasm progressively darkens from pale orange or tan to deep orange or golden brown. Previous workers have attributed intense orange-red hues to redistributed lipid storage products⁶; however, in our specimens, orange pigmentation is a dietary phenomenon. While cytoplasmic coloration intensified in all specimens, only those fed high proportions of *Artemia* were deep orange. Others fed exclusively *Thalassiosira* tended to brown and, in one case of gametogenesis within 5 d of capture, to dark grey or black. The latter coloration may be due to local pelagic species of Crustacea in Bermuda, including the copepods *Candacia ethiopica*, *Euchaeta marina* and *Harpacticus* sp., which have black pigments and are sufficiently large to be a feasible food source for these foraminifers.

In *O. universa*, cytoplasmic regression and loss of the calymma coincided with a loss of all major spines. In contrast, in

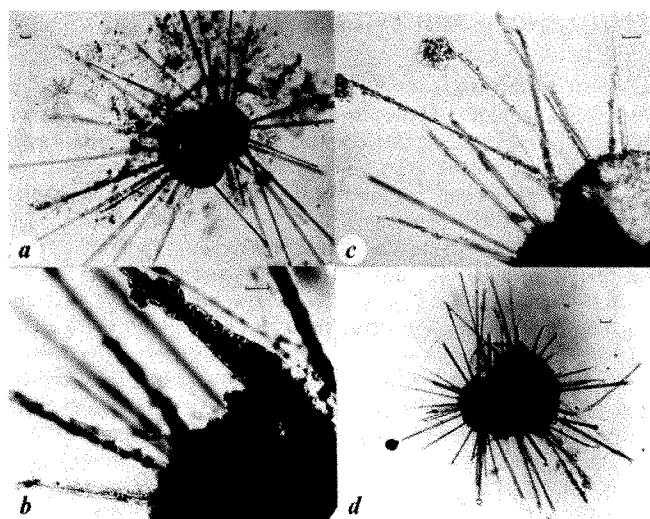


Fig. 1 Light micrographs of major gametogenic stages of *H. pelagica*. Scale bars, 25 µm. *a*, Onset of explosive release. Gametes and cellular residue are emitted only from major apertures and are densest near remains of terminal chamber. *b*, Masses of gametes flowing along spines in cytoplasmic strands during early to middle stages of gradual release. A few remnants of the parent bubble capsule are evident. *c*, Gamete clumps encased in hyaline material on spines near aperture. Unoccupied spines are dissolving inside a sheath formed by the parent organism. *d*, Terminal stages 10–12 h after release began. Few gametes remain in vicinity of parent test; most spines are resorbed leaving only membrane sheath or base.

no *H. pelagica* were all spines shed and the degree of spine loss or disintegration varied between individuals; remaining spines occasionally served to transport gametes to the periphery (Figs 1*b*, 3*b*). Spines in *H. pelagica* may be lost by resorption of their external bases⁵, by release of the spine from its base, or by progressive disintegration of the spine from its peripheral end (Fig. 1*c*). In five *H. pelagica*, spines began disintegration after onset of gamete release and were effectively dissolved in 4–6 h. The process is assumed to be autolytic because surface seawater is supersaturated in CaCO₃ and would not induce rapid dissolution. Most spines were encased in a residual, membraneous sheath which, as the spine disintegrated, thinned, curving at the peripheral end. More fibril spines were often held in place by secondary mucoid strands. Experiments now in progress

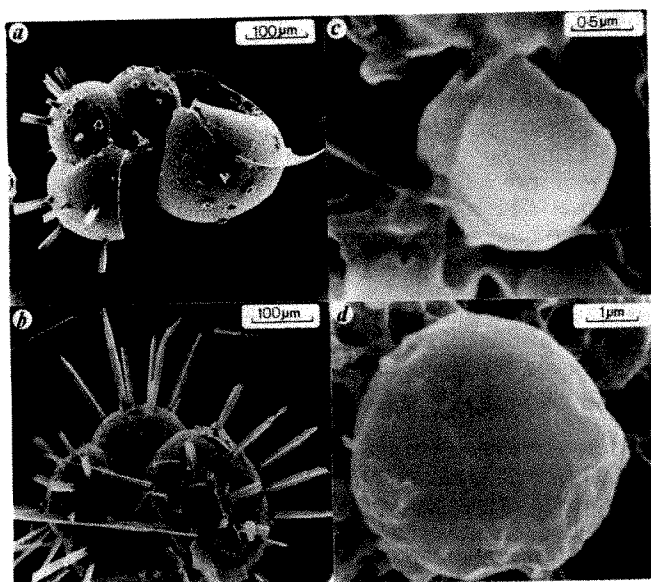


Fig. 2 *a*, Glutaraldehyde-fixed adult at 8-h stage of gamete release. Remains of organelles and developing gametes are found near lip of major aperture. *b*, Post-gametogenic test left 3 weeks in culture. Spines show dissolution pattern. *c*, Biflagellated gamete of *H. pelagica*, fixed 12–15 h after release. The flagella measure 7–10 μm and 12–15 μm respectively. *d*, Initial chamber (proloculus) formed 28 h after gamete release.

indicate that patterns of disintegration within the sheath resemble those of empty foraminiferan tests; however, the triradial spines of vacated tests require up to 6 weeks to produce the same degree of etching as can be produced in 4 h in a gametogenic individual (Fig. 2*b*). The fragility of *Hastigerina* tests was noted by Berger⁸ and a similar spicule dissolution phenomenon shown for other pelagic protozoans⁹. These factors along with limited latitudinal distribution, may explain the relative scarcity of *Hastigerina* in marine sediments.

The actual release of gametes may be explosive or gradual and occurs 2–3 h after the test surface becomes rugose and the cytoplasm dense and granular. In dehiscence, cytoplasm fills the final chambers and gametes are discharged with protoplasm.

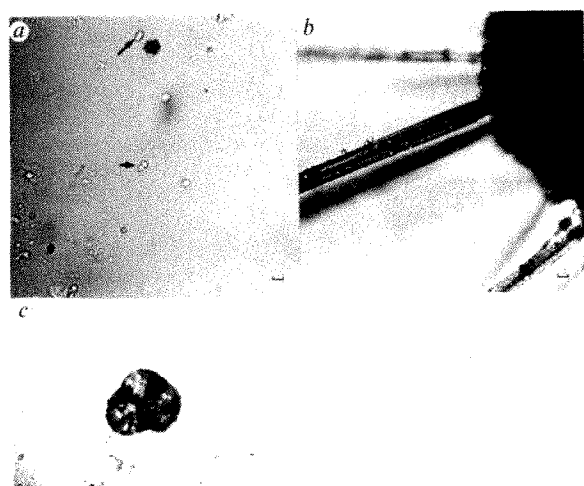


Fig. 3 *a*, Syngamy found in culture water 36 h after release, and *b*, on spines of parent test. A pair in which the process is nearly complete can be seen at top centre of *a*. Scale bar, 5 μm . *c*, Three-chambered *H. pelagica* produced in culture within 15 d of gametogenesis. Chambers shown range 27–40 μm in diameter.

The final chamber, formed 4–10 d before the onset of gametogenesis in 20% of our cultures, is lost entirely or part of its outer wall destroyed by forcible expulsion of its contents (Fig. 2*a*). Cytoplasmic residue is later released in a characteristic, streaming pattern (Fig. 1*a*), as reported for *Globigerinella aequilateralis*⁵, and closely resembling phases described for *Elphidium crispum*¹. In gradual release, intensification of the cytoplasm is followed by gametes being extruded along the spines. In spite of rapid flagellar movement, the gametes are normally held in a line by a mucoid coat similar to that covering dissolving spines (Fig. 1*b*). Substantial clumps form at the spine tips within 2 h of the onset of release (Fig. 1*c*) and the flow may continue for 8–10 h. Most gametes, however, reach the periphery and separate from the clumps within 3 h. At this stage, the gametes are dark, ovoid bodies, 2–5 μm long, and have two distinct, unequal flagella with a common base (Fig. 2*c*). The gametes can move just after release although rapid motion does not begin until they are 50–100 μm from the surface of the parent test. Movement is typically a stationary oscillation with rapid flagellar beating or rotation as though due to spinning flagella. Flagellar movement was too rapid to be clearly discerned during swimming. Mobile flagellated gametes survived in culture up to 12 d although most of them calcified or died within 5 d.

Syngamy was rare, observed among gametes of only four *H. pelagica*. No single parameter was common to the cultures in which copulation took place, nor were there apparent morphological differences from other gametes. An estimated maximum of 10% of the population formed syngamous pairs, and frequency of copulation was not increased by combining gametes from two specimens reproducing within the same 36 h. The pattern of copulation was invariable, one gamete remaining relatively still while a second moved rapidly around its periphery, periodically withdrew 10–15 μm , and then forcibly pushed against it. Contact generally occurred between the antilflagellar ends of the gametes with adherence evident at the point of impact (Fig. 3). While thus attached, the two pairs of flagella straightened and beat rapidly, and the gamete pair whirled in the water. The more mobile of the two then broke off, withdrew, and repeated the battering process. Separation became progressively more difficult due to coalescence. This activity continued up to 30 min, ending in a single perceptible organism, about 5 μm in diameter, with two pairs of inactive flagella (Fig. 3*a*). Nuclear material could not be resolved in the living material, but a transfer or fusion is assumed.

Calcification to a single chamber, presumed a proloculus, took place as soon as 9 h after gamete release although most cultures did not show calcification for several days. Single-chambered organisms thus formed were smooth spheres, 8–20 μm across, with few or no apertures perceptible (Fig. 2*d*). The degree of calcification varied, with some individuals forming overlapping layers; however, that may have been a culture abnormality. In only one of our cultures have we found chamber formation beyond prolocula. Two polythalamous individuals were produced (Fig. 3*c*), each forming four chambers within 15 d of gametogenesis, the last chamber appearing within 2 d of the third. The organisms measured 65–80 μm in diameter with the largest chambers 35 and 40 μm in diameter. Both were planispiral with irregularly arranged pores about 1 μm wide and a single 10- μm aperture in the final chamber. No spines have been produced on either individual. None of our second generation specimens has matured beyond this stage and the main difficulties foreseen in further culturing are the provision of appropriate food sources and the formation of culture aberrants with a small sample size.

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Carcinogenicity testing of herbicide 2,4,5-trichlorophenoxyethanol containing dioxin and of pure dioxin in Swiss mice

SERIOUS environmental contamination is a hazard in the use of derivatives of 2,4,5-trichlorophenol (TCP) as weedkillers¹ or defoliants² and in accidents during the manufacture of TCP³ (as in Seveso, Italy in 1976⁴⁻⁶). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin) is an inevitable toxic⁷ teratogenic⁸ and weakly mutagenic^{9,10} by-product which is always present as a contaminant. When TCP derivatives, including 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), were tested for carcinogenicity in (C57BL/6 × C3H/An)F₁ and (C57BL/6 × AKR)F₁ mice (experiments lasting 18 months) none increased tumour incidence¹¹, while in another study there was a significant increase in the incidence of tumours in C3Hf mice treated with 2,4,5-T¹². During a chronic toxicity study with TCDD (a potent inducer of microsomal enzymes¹³) in Sprague-Dawley rats some tumours were observed¹⁴. In another 2-yr study TCDD ingested at 0.1 µg per kg body weight per day caused an increased incidence of hepatocellular and squamous carcinomas in

Sprague-Dawley rats, but reduced the incidence of some other tumours. Smaller doses of dioxin did not affect tumour incidence¹⁵. We report here that both 2,4,5-trichlorophenoxyethanol (TCPE), a derivative of TCP, and dioxin enhance liver tumours in male mice in a dose-dependent manner. Amyloidosis is a long term complication often associated with severe skin lesions induced by TCDD.

Because TCPE is almost insoluble in water it was suspended in a salt solution containing 0.5% carboxymethylcellulose. Dioxin was dissolved in sunflower oil and determined by gas chromatography. Various doses of a combination of TCPE and dioxin, of pure dioxin and of vehicles alone were administered (Table 1). In group 1, TCPE contained 0.3% of contaminants. Ten-week-old, outbred Swiss/H/Riop mice (25-30 g) were used in groups of 100 males plus 100 females, except for groups administered pure dioxin and sunflower oil alone, each of which consisted of 45 males only. Animals were kept in plastic cages at 25 ± 2 °C. Standard diet and water were provided *ad lib*. TCPE contaminated with dioxin, pure dioxin or vehicle alone were administered by gastric tube once a week for 1 yr.

Animals were followed up for the rest of their lives. Autopsies were performed after spontaneous death or when the mice were moribund, and all organs were examined histologically. For light microscopy, sections were stained with haematoxylin and eosin. Amyloid deposits were identified with Congo red stain under polarised light¹⁶ and by electron microscopy. Pathological findings were assessed and analysed statistically. The effective number of animals was the number of survivors at the time the first tumour was observed. Significance was calculated by the χ^2 test. A difference in tumour frequency between groups was considered significant only when *P* was less than 1%. The carcinogenesis study was carried out in three phases (groups 1-3, 4-8 and 9-12) and comparisons were made with the respective control groups.

The LD₅₀ of an acute, peroral dose of TCPE containing 0.1 p.p.m. dioxin is 1,320 mg kg⁻¹. The largest dose of TCPE that induced no noticeable tissue lesions in the organs of the mice during 6 months of administration was 70 mg per kg. That was therefore taken as the maximum tolerated dose.

Table 1 Cumulative data on tumour incidence

Groups	Treatment			Sex	Effective no. of mice	No. of tumour bearing mice	Animals with tumours of				Average life span (d)
	TCPE (mg per kg)	Dioxin (µg per kg)	Vehicle* (mg per kg)				liver no (%)	lung no.	lymphoma no.	other organs no.	
1	67.0	0.112 (1.6 ppm)	50	M	88	69	42† (48)	50	7	16	595
				F	83	61	7 (8)	52	15	25	652
2	70.0	0.007 (0.1 ppm)	50	M	98	78	57‡ (58)	48	11	16	571
				F	96	59	9 (9)	39	15	23	582
3	Control			M	93	63	2 (26)	44	8	17	577
				F	84	57	4 (5)	41	23	18	639
4	7.0	0.07 (10 ppm)	50	M	93	79	25 (27)	54	18	22	641
				F	96	60	10 (10)	38	19	19	589
5	7.0	0.0007 (0.1 ppm)	50	M	94	77	23 (24)	50	28	17	660
				F	93	71	8 (9)	42	36	21	590
6	0.7	0.00007 (0.1 ppm)	5.0	M	97	78	24 (25)	51	20	17	643
				F	94	64	5 (5)	38	22	21	566
7	—	—	50	M	96	74	32 (33)	44	14	22	615
				F	84	55	4 (5)	38	18	17	565
8	Control			M	96	78	32 (33)	58	22	15	651
				F	91	57	4 (4)	31	24	19	549
9	—	7.0	10	M	43	27	13 (30)	11	6	7	424
10	—	0.7	10	M	44	36	21† (48)	18	12	4	633
11	—	0.007	10	M	44	39	13 (29)	27	10	6	649
12	—	—	10	M	38	27	7 (18)	15	6	7	588

* Carboxymethylcellulose in groups 1-8, sunflower oil in groups 9-12

† *P* < 1%.

‡ *P* < 0.1%.

Table 2 Occurrence of amyloidosis with skin lesions induced in mice by dioxin

Groups	Treatment		Effective no. of mice	Skin lesion no.	Skin lesion + amyloidosis no.
	Dioxin ($\mu\text{g per kg}$)	Vehicle (mg per kg)			
9	7.0	10.0	43	25	17
10	0.7	10.0	44	13	10
11	0.007	10.0	44	5	5
12	—	10.0	38	0	0

The cumulative data in Table 1 show that only the incidence of liver tumours of the males significantly differed between groups. Subsequent comparisons therefore relate to the males. The incidence of liver tumours in groups 1, 2 and 10 was twice that in the respective controls (groups 3 and 12). The smaller doses of TCPE and dioxin given to groups 5 and 6 (1/10 and 1/100 of maximum tolerated dose) caused less frequent tumours; there were no significant differences from groups 7 and 8. There were no significant differences in the frequency of liver tumours between groups 1 and 2 (given dioxin at 0.112 and 0.007 $\mu\text{g per kg}$) or groups 4 and 5. The latter two groups received equal amounts of TCPE but group 4 received 100 times more dioxin than did group 5. Thus, dioxin in this dose range did not influence tumour frequency.

Groups 11 and 2 received equal amounts of dioxin (0.007 $\mu\text{g per kg}$) but there was no significant difference in liver tumour incidence between group 11 and its control, group 12. Thus dioxin has no liver tumour enhancing effect at this dose. The increased incidence of liver tumours in group 2 was caused by TCPE and not by dioxin. In group 10, which received 100 times more dioxin than did group 11 (0.7 $\mu\text{g per kg}$), liver tumour incidence was twice that in the control, group 12. Thus dioxin has a liver tumour enhancing effect at a higher dose. Dioxin contamination in group 4 was less than 0.7 $\mu\text{g per kg}$ —the threshold dose of TCDD in our experiment—and its hepatocarcinogenic effect could not be observed.

Spontaneous and induced liver tumours were not histologically different. The ratio of benign hepatomas to hepatocellular carcinomas in the control groups was not affected by treatment; only the absolute number of liver tumours increased. No cirrhosis was seen concurrently with tumours.

Average life span decreased considerably in group 9 (Table 1) which received the largest dose of TCDD. TCDD caused severe chronic, ulcerous skin lesions (probably similar to chloracne in humans) followed by generalised lethal amyloidosis (Table 2), which can be regarded as a process secondary to chronic skin lesion¹⁷. This group was not evaluated in the carcinogenic bioassay for early death.

Until more is known about the people who have been exposed to them, the carcinogenicity of 2,4,5-T and structurally related chemicals in humans cannot be evaluated¹⁸⁻²¹. But it is clear that not only dioxin contamination, but also exposure to TCPE during its production or use should be controlled and reduced. Amyloidosis should be considered a possible later complication of the most severe forms of human chloracne induced by TCDD.

A preliminary report of this study was presented at the International Conference on Ecological Perspectives on Carcinogens and Cancer Control in Cremona in 1977²². This work was initiated by the late Professor B. Kellner. We thank Mrs Rose Gaál Szabo for technical assistance.

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Initiation of development of diapausing embryo by mammary denervation during lactation in a marsupial

MOST macropodid marsupials conceive at postpartum oestrus and the 100-cell embryo enters into and remains in embryonic diapause while there is a suckling young in the pouch¹. Removal of the pouch young results in reactivation of the quiescent corpus luteum and of the diapausing blastocyst. Prolactin inhibits corpus luteum development tonically during lactational quiescence in the tamar wallaby, *Macropus eugenii*². However, it is not known whether the hormonal milieu associated with lactation, or the neural stimulus of suckling itself is the primary signal to the hypothalamus which results in the maintenance of embryonic diapause in *M. eugenii*. I therefore examined the role of the neural pathway from the mammary gland in tamar wallabies carrying diapausing embryos. I report here that after denervation of the suckled mammary gland, the quiescent corpus luteum and the diapausing blastocyst resumed development, whereas none of a sham-operated group initiated blastocyst development. The pouch young grew at a normal rate and continued to obtain milk from denervated glands, despite the presence of a developing embryo in the uterus.

Only one of the four available teats is suckled by a particular pouch young. Each teat has a separate mammary gland, but the anterior and posterior teats on each side are less than 1 cm apart³. In experimental animals, the suckled gland and its ipsilateral partner where chosen for denervation, whereas, because of the diffuse nature of the innervation, in control animals the contralateral pair were denervated.

Denervation was effected by cutting around the chosen pair of nipples, and clearing the gland from the connective tissue until the ilio marsupialis muscle, through which the mammary nerves pass, was clearly visible. The minor blood vessels and ducts leading to the mammary lymph glands were ligated, the muscle layer cut through, and the remaining fascia and connective tissue cleared from the entire gland until it was connected only by the intact major artery and vein and there was no sign of any remaining nerves. After repair of the wound the pouch young, aged either 35 or 60 d, was replaced on the nipple.

Pouches were checked daily for the first 7-10 d. Subsequently, the females were replaced in their outdoor enclosures until laparotomy on either day 22 or 23 post-denervation.

After laparotomy, all control and pregnant experimental animals were killed. The corpora lutea were weighed directly, and embryos measured and aged, as previously described^{4,5}.

Table 1 Influence of denervation of the mammary gland on embryonic diapause; status of embryos 22 d after denervation of the suckled (experimental) or non-suckled (control) mammary gland

No. of animals	Pouch young status	Blastocyst or vesicle recovered	Day 22/23 embryos	Developed§ CL or follicle but no embryo	No blastocyst,§ unfertilised egg, CL or follicle	Total activated after operation
Experimental						
9	5 remained on teat	0	3*	1	1	7/9
	4 lost 5-14 d	0	1*	2	1	
Control						
4	3 remained on teat	2†	0	0	1	0/4
	1 lost 9 d	1‡	0	0	0	

Anaesthesia was induced by 2.0 ml sodium pentobarbitone (60 mg ml⁻¹) in 0.9% NaCl, maintained by halothane in oxygen. CL, corpus luteum.

* Embryo sizes (crown-rump length), 10 mm, 14.8 mm, 13.3 mm, 11.1 mm. † Blastocyst sizes (diameter), 0.25 mm, 0.26 mm. ‡ Vesicle size (diameter), 0.6 mm.

§ Three of the five denervated animals which were not pregnant underwent an infertile cycle, and had a developing follicle and enlarged corpus luteum at laparotomy. The remaining two denervated animals and one of the four controls had no apparent postpartum ovulation, as there was no corpus luteum in the ovary, and no egg or blastocyst in the uterus.

Mammary glands were fixed in buffered formalin for histological examination. Non-pregnant experimental animals were examined by laparotomy for post-luteal follicular development or corpora lutea and allowed to recover.

After denervation of the suckled mammary gland, the quiescent corpus luteum resumed development, as indicated by increased size and weight, and post-luteal follicular growth was observed in seven of nine experimental animals (Table 1). In four of nine animals embryos developed despite the presence of a suckling pouch young. Twenty-one days after denervation the embryos of these four fitted within the size range of normal 22-d embryos⁵. The five pouch young which remained on the denervated glands grew normally, and when measured and aged on a growth curve⁶ all had maintained a normal growth rate. Pouch young were lost in four cases. Histological examination of the glands confirmed that the nerve supply remained severed. In the

control group, none of the four animals initiated development, although one which lost its pouch young between 9 and 13 d after the operation carried a 10-d vesicle at laparotomy on day 22 (Table 1). Thus, surgery itself did not cause resumption of development.

These results show that the neural stimulus derived from suckling is essential to maintain the quiescent state of the diapausing blastocyst, but that an intact mammary neural connection is not essential for maintenance of this stage of lactation in this species. Milk secretion after denervation continued apparently unimpaired in five of the nine experimental animals. Histologically, the glands seemed normal when compared with glands obtained from animals carrying pouch young of the same age (60-84 d). However, it is not possible to say whether the remaining four were lost (at days 5, 11, 12 and 14 post-denervation) due to a failure of lactation (such as the absence of oxytocin and the milk ejection reflex), or to post-operative behavioural abnormalities. Milk was observed slowly leaking from the denervated teat before replacement of the pouch young, and in animals which lost their pouch young the gland became swollen. In other species, the myo-epithelium can be induced to contract by rapid mechanical stretching⁷; pouch young could certainly pull the denervated teat during their movements in the pouch and this may counteract the lack of oxytocin release. Oxytocin in *M. eugenii* thus may be a facultative but not obligatory mechanism for effective milk discharge, as Cross⁹ suggests for the goat.

The ability of denervation to allow the corpus luteum to resume growth, and lactation to continue, contrasts with the results of hypophysectomy in this species, in which the corpus luteum also resumes development but lactation and follicular growth fail¹⁰. However, lactation continues after removal of the corpus luteum although follicular development begins and animals return to oestrus 11 d later². It must be concluded that suckling, mediated through the neural pathway and prolactin, inhibits the corpus luteum, and that it is corpus luteum secretions (such as progesterone or oestradiol-17 β) which inhibit subsequent follicular development in the intact animal.

These findings provide evidence for the role of the neural pathway from the mammary gland as a primary signal to the hypothalamus in maintaining embryonic diapause during lactation in a marsupial species. Figure 1 shows how this finding is consistent with present evidence for the control of reproductive activity in this species during lactational quiescence.

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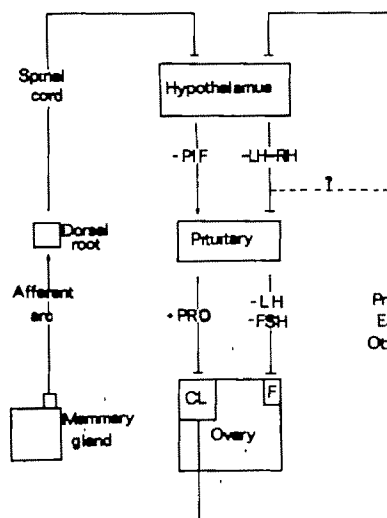


Fig. 1 An hypothetical scheme showing pathways consistent with present evidence for the control of lactational quiescence in the tamar wallaby. Removal of the pouch young and thereby the suckling stimulus leads to resumption of corpus luteum growth and of embryonic development, with oestrus and ovulation occurring at the end of the 27-d pregnancy¹. Removal of the pituitary leads to resumption of corpus luteum and blastocyst growth¹⁰ but ovulation and follicular development fail¹⁰. Hypophysectomy followed by prolactin injection prevents resumption of corpus luteum development², and bromocriptine administration in intact animals results in reactivation of the corpus luteum¹¹. Removal of the corpus luteum leads to oestrus and ovulation 12 d later², presumably due to the removal of small amounts of steroids derived from the quiescent corpus luteum that may inhibit pituitary gonadotrophin secretion. It is not clear, however, whether such corpus luteum inhibition acts directly on the pituitary, or through the hypothalamus, as suggested elsewhere^{12,13}. CL, corpus luteum; E β , oestradiol-17 β ; F, follicle; FSH, follicle-stimulating hormone; LH, luteinizing hormone; LH-RH, luteinizing hormone releasing hormone; PIF, prolactin inhibiting factor; PRO, prolactin; Prog, progesterone; †, stimulation; ‡, inhibition; +, increased levels; -, decreased levels.

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Abelson virus-transformed haematopoietic cell lines with pre-B-cell characteristics

THE Abelson murine leukaemia virus (A-MuLV) complex, which consists of a replication-defective focus-forming virus and its helper Moloney leukaemia virus, causes a thymus-independent lymphosarcoma in BALB/c mice¹. Infection *in vivo* leads to the production of tumours which are usually classified as 'null' lymphoid cells², although immunoglobulin (Ig)-synthesising B-cell lines have been reported³⁻⁵ and macrophage lines may occasionally be produced⁶. In some cases, particularly with pretreatment of the mice with the mineral oil pristane, there is a high incidence of Ig-producing plasmacytomas⁷. Additional data on Ly phenotypes have suggested that most A-MuLV tumours, including those with no demonstrable Ig synthesis, may be B-cell lineage derivatives⁸. Here, we provide evidence for A-MuLV transformation of B-cell precursors resulting in two cell lines which retain the capacity for inducible IgM synthesis. Line ABC-1 was derived from BALB/c mouse bone marrow cells transformed *in vitro* by the Abelson virus⁹ and line ATV-3 from a tumour in a BALB/c mouse inoculated with A-MuLV.

The ABC-1 cells are large, vacuolated, undifferentiated blast cells. Immunofluorescence and cytotoxicity tests indicated that no cells had cell surface immunoglobulin (SmIg) or expressed detectable levels of Thy-1.2, suggesting that there were no mature B or T lymphocytes present. Additional characterisation of this cell line (Table 1) revealed that no cells expressed Ia or TL 1,2,3,4 antigens or peanut agglutinin binding sites (Gal(β1-3)-GalNac), the latter being a marker for thymocytes and T-cell precursors¹⁰. All cells contained cell surface exposed G_{M1} gangliosides as indicated by binding of ¹²⁵I-cholera toxin or fluoresceinated cholera toxin. The cells did not react with a rabbit antiserum specific for antigens on a gp100 molecule associated with a human non-T, non-B or 'null' lymphoblastic leukaemia^{11,12}. Preliminary experiments suggest that the ABC-1 cells, in common with other A-MuLV-transformed lines⁸, have low but detectable levels of terminal deoxynucleotidyl transferase (M.B., unpublished observations).

The use of affinity-purified antibodies to mouse IgM conjugated with fluorescein indicated an absence of cells with detectable cell surface fluorescence, although 1-4% showed cytoplasmic staining. No cytoplasmic fluorescence was seen in controls of AKR leukaemic T lymphocytes and normal thymocytes. In normal spleen, plasma cells stained intensely.

The presence of cytoplasmic IgM in a minor proportion of cells and the absence of staining for surface membrane Ig indicate the presence of cells with the characteristics of the earliest detectable cells in the B-cell lineage, that is, 'pre-B' cells^{13,14}.

We next attempted to induce lymphoid differentiation in ABC-1 cells which had been in continuous culture for only 8 months. Dimethyl sulphoxide (DMSO), 1%, failed to induce any increase in the percentage of cells expressing cytoplasmic

Table 1 Surface membrane phenotype of an *in vitro* Abelson virus-transformed line ABC-1

Marker	% Positive cells		
	A-MuLV line: ABC-1	Controls (BALB/c) Spleen	Thymus
SmIg	<0.01	40	2
Thy-1.2	<1	19	83-95
Ia	<1	30-45	3
TL 1,2,3	<5	<5	11
TL 4	<5	<5	46
Peanut agglutinin	1	3	>95
Cholera toxin receptors	100	100	>95
Fc receptors	25	34	5
Human gp100 cALL antigen	<0.1	ND	<0.1

Surface membrane immunoglobulin (SmIg) was detected by direct immunofluorescence using several anti-Ig antisera, including rabbit anti-mouse Ig (non-class specific) and a rabbit antiserum specific for IgM. Thy-1.2 was determined by indirect immunofluorescence using an AKR anti-CBA thymocyte antiserum (Searle) followed by a fluorescein-labelled rabbit anti-mouse Ig and a two-stage ⁵¹Cr-release cytotoxicity test using the same anti-Thy-1.2 antiserum plus rabbit complement (1:15). Values given refer to results by ⁵¹Cr release and represent the percentage specific lysis. Ia expression was tested by indirect immunofluorescence techniques and a two-stage cytotoxicity test (using trypan blue exclusion). Anti-Ia was a (B10.A × A) anti-B10.D2 antiserum (anti-Ia^d). Values for controls are derived from indirect immunofluorescence experiments in which it was necessary first to remove surface membrane Ig of spleen cells by 'capping'. TL antigens were detected by complement-mediated cytotoxicity. Anti-TL antisera were produced by immunisation of appropriate congenic strains of mice. Anti-TL-4 was raised by immunising (A × C57BL/6-Tla^a) mice with a C57BL thymic lymphoma line (ERLD). Anti-TL 1,2,3 was raised by immunising (A-Tla^a × C57BL/6) with a thymic lymphoma line (ASL1) derived from an A strain mouse. cALL, common acute lymphoblastic leukaemia antigen. ND, not determined.

Table 2 Stimulation of cytoplasmic IgM production in an *in vitro* transformed line ABC-1

Treatment	% cells staining for cytoplasmic IgM on			
	Day 0	Day 1	Day 4	Day 7
None	1-4	1-4	1-4	1-4
<i>Escherichia coli</i> LPS				
5 μg ml ⁻¹	—	20	23	25
20 μg ml ⁻¹	—	25	39	25
Dimethyl sulphoxide, 1%	—	ND	23	4
Butyric acid, 1 mM	—	1	18	4
Pokeweed mitogen conditioned medium	—	7	34	ND

Concentrations of each inducer shown are those which, out of the range of concentrations used, were found to induce maximal stimulation of cytoplasmic IgM-positive cells. Pokeweed mitogen conditioned medium refers to supernatant from syngeneic spleen cell cultures (5 × 10⁶ fresh cells ml⁻¹) incubated for 72 h with pokeweed lectin(s) (15 μg ml⁻¹). The cells were removed by centrifugation (1,300 g) and the conditioned medium used at one part conditioned medium to two parts fresh medium. Abelson virus-transformed cell cultures were set up at 10⁶ cells per ml on day 0 and assayed on days 1, 4 and 7 for the presence of surface membrane Ig and Thy-1.2 by fluorescence techniques (see legend Table 1). Thy-1.2 was also assayed on five occasions by a ⁵¹Cr-release cytotoxicity test. Smears were also made and stained with anti-IgM antibodies (see legend to Fig. 1). Rabbit anti-mouse IgM (μ-chain specific) antiserum was raised by immunisation with purified MOPC 104E myeloma protein (Ig). The rabbit antibodies were isolated on a MOPC 104E-4B Sepharose immunoabsorbent column, passed over mouse myeloma IgG-4B Sepharose to remove anti-γ chain cross-reactivity and conjugated to fluorescein or rhodamine isothiocyanate.

IgM or *de novo* expression of cell surface Ig. Lipopolysaccharide (LPS), at doses of $5\text{--}20\ \mu\text{g ml}^{-1}$, rapidly induced an increased expression of cytoplasmic IgM, maximally 9–10% 2 d after addition (data not shown).

Cells which had been in continuous culture for longer (16 months) showed a more pronounced stimulation of cytoplasmic IgM-positive cells (20–40%). Several compounds were tested for their ability to elicit differentiation; these were added over a range of doses to fresh cultures (Table 2). Cells were assayed on days 1, 4 and 7 for the presence of Thy-1.2, SmIg and cytoplasmic IgM. DMSO, LPS, butyric acid and conditioned medium from BALB/c spleen cell cultures stimulated with pokeweed lectin(s) (PWM), all increased the percentage of cytoplasmic IgM-positive cells. No cell surface Ig-positive cells were detectable after these treatments. Of the compounds tested, only LPS induced an increase in cells with cytoplasmic IgM production within 1 d, but there was no significant difference in proliferation over this period ($8.7 \times 10^5\ \text{ml}^{-1}$ and $9.3 \pm 0.3 \times 10^5\ \text{ml}^{-1}$ for control and LPS-stimulated cells, respectively). Stimulation by butyric acid and PWM conditioned medium was not evident at day 1 but was seen at day 4.

In contrast to the responses elicited by the above compounds, dextran sulphate ($2\text{--}50\ \mu\text{g ml}^{-1}$), concanavalin A ($5\text{--}10\ \mu\text{g ml}^{-1}$), PWM ($5\ \mu\text{g ml}^{-1}$), dibutyl cyclic AMP

($1.25\text{--}5 \times 10^{-4}\ \text{M}$), cholera toxin ($0.01\text{--}1.0\ \mu\text{g ml}^{-1}$), phytohaemagglutinin ($2.5\text{--}20\ \mu\text{g ml}^{-1}$) and purified protein derivative of tuberculin ($5\text{--}50\ \mu\text{g ml}^{-1}$) all failed to stimulate an increase in cells with cytoplasmic IgM, Thy-1.2 or SmIg.

Several cell lines were also isolated from tumours in BALB/c mice which had been injected intraperitoneally at 5–7 d of age with Abelson virus. All these lines were negative for Thy-1.2 and SmIg. We attempted to induce lymphoid differentiation in two lines in which there were no cells showing detectable cytoplasmic fluorescence with anti-IgM; DMSO, dextran sulphate, LPS, thymopoietin, iododeoxyuridine, and interferon ($475\ \text{units ml}^{-1}$) with or without 1% DMSO or dextran sulphate, did not induce expression of Thy-1.2, cell surface Ig or cytoplasmic IgM.

A third line (ATV-3) had 5–22% (mean 15%) cells with cytoplasmic IgM, none of which had detectable cell surface immunoglobulin. After 48–72 h culture with $10\ \mu\text{g ml}^{-1}$ LPS the proportion of cells with cytoplasmic IgM had increased to 35% and 70% in two experiments, with no concomitant appearance of cell surface Ig.

Synthesis of IgM by both ABC-1 and ATV-3 lines was confirmed by SDS-polyacrylamide gel electrophoresis and fluorography (Fig. 1). Both lines apparently synthesised μ heavy chain and light chain.

The synthesis of IgM by these lines and the induction of increased numbers of detectable IgM-containing cells imply that A-MuLV had transformed immature B-lymphoid lineage committed precursors. A minority of cells in both lines seems to have a maturation arrest at the level of 'pre-B' cells whereas a substantial proportion are presumably less mature but can be induced to express pre-B features. No *bona fide* 'mature' B cells (cell surface Ig positive) have been inducible in these lines. It is interesting that LPS and PWM-activated cell supernatants induce pre-B-cell characteristics in ABC-1 cells, whereas concanavalin A, phytohaemagglutinin or pokeweed lectin itself do not, as this parallels the differential mitogenic effects of these same compounds on purified murine T and B cells *in vitro*¹⁵. Previous work has indicated that polyclonal B-cell activators may selectively depress DNA synthesis of A-MuLV-transformed cells¹⁶. LPS has also been reported to stimulate a chemically induced pre-B-cell line to express cell surface Ig¹⁷. These data and analyses of Ly antigen phenotypes⁸ support the view that A-MuLV-transformed cells are predominantly B-lineage committed cells.

Baltimore *et al.* have recently provided further evidence for B-cell differentiation in several A-MuLV-transformed lines^{18,19}, some of which are similar to the two reported here in synthesising μ chains with no cell surface representation¹⁸. These lines apparently synthesised light chains only following stimulation with LPS^{18,19}.

Abelson virus therefore seems to transform mainly B-lineage lymphoid cells. In the two lines reported here in detail, transformation *in vitro* (ABC-1 line) or *in vivo* (ATV-3 line) resulted in the production of lines with features indicative of early maturation compartments in the B-lymphocyte lineage. The availability of such cells with at least a partial competence for reversal of maturation arrest should be useful for studying early molecular events in the control of immunoglobulin gene diversification and expression.

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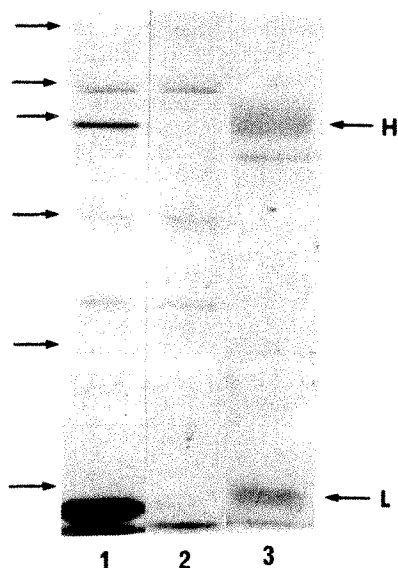


Fig. 1 Fluorography of SDS-polyacrylamide gel electrophoresis showing μ heavy and light chain synthesis by Abelson virus-transformed cells (ATV-3). Slot 1, immune precipitate with rabbit anti-mouse μ chain antibodies; slot 2, immune precipitate with normal rabbit serum; slot 3, Coomassie blue-stained tract containing MOPC-104E IgM. Positions of heavy (H) and light (L) chains from MOPC-104E indicated. Molecular weight markers (arrowed) are (from top to bottom): β -galactosidase (135,000); phosphorylase α (94,000); lactoperoxidase (80,000); catalase (60,000); alcohol dehydrogenase subunit (yeast, 37,500) and carbonic anhydrase (29,000). 1×10^6 cells were incubated at 37°C for 16 h with $250\ \mu\text{Ci } ^{35}\text{S}$ -methionine in methionine-free medium. The labelled cells were washed three times in methionine-free medium and extracted in 3 ml 0.01 M Tris, pH 8, 150 mM NaCl and 0.5% NP40 for 15 min at 4°C . The cell extract was centrifuged at $100,000g$ for 1 h at 4°C ; $250\ \mu\text{l}$ were incubated overnight with $20\ \mu\text{l}$ of rabbit anti-mouse μ chain or normal rabbit serum and the immune complexes isolated by *Staphylococcus aureus* precipitation. The bacteria were pelleted, washed three times in the above buffer with 0.25% gelatin, and bound complexes were eluted by boiling in $70\ \mu\text{l}$ of 10% glycerol, 2% SDS, 0.05 M Tris, pH 6.8, plus $10\ \mu\text{l}$ 1 M dithiothreitol in 0.1% bromophenol blue indicator. The eluted complexes as well as the reduced MOPC-104E and marker proteins were subjected to SDS-PAGE.

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Retinoic acid inhibition of Epstein-Barr virus induction

TUMOUR promoters of the diterpene type efficiently induce Epstein-Barr virus (EBV) and additional persisting herpes viruses in genome-harboring lymphatic cells^{1,2}. The tumour-promoting activity of these substances to some extent parallels their efficiency in virus induction²: the most active tumour promoters induce EBV even at concentrations of 10^{-10} – 10^{-9} M, whereas 100–500-fold higher concentrations are required for induction by weak promoters. Treatment of cells with tumour promoters results in a fast and reproducible increase in the activity of ornithine decarboxylase (ODC)^{3,4} up to 250-fold levels of normal activity. Recent reports^{5,6} described the inhibition of ODC induction by pretreating the cells with retinoic acid. Moreover, retinoic acid has been reported to act as an inhibitor in experimental 2-stage carcinogenesis models⁷. We have therefore investigated the possible interaction of retinoic acid with virus induction by tumour promoters. The effect of retinoic acid was also studied on the induction of early antigens (EA) of EBV by the pyrimidine analogue, iododeoxyuridine (IUdR)^{8,9}, by treatment of IgM-producing cells with anti-IgM antibodies¹⁰ and by superinfection with EBV from P3HR-1 cells^{11,12}. The data indicate that retinoic acid specifically interferes with induction by non-viral reagents without affecting EBV antigen synthesis after superinfection.

cis-Retinoic acid (Nordmark) was dissolved in ethanol diluted in tissue culture medium and applied to Raji cells in concentrations ranging between 10^{-8} and 5×10^{-5} M. No cellular growth inhibition was noted up to 10^{-5} M; at 5×10^{-5} M there was a slight decrease in growth over a period of 5 d.

Concomitant treatment of these cells with 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) and retinoic acid resulted in effective inhibition of EA induction by TPA (Fig. 1). At 10^{-5} M, more than 90% of EA induction by TPA was suppressed and some inhibition was still obvious at 10^{-6} M. Kenner *et al.*⁶ reported that pretreatment of the cells with retinoic acid 1 h before TPA addition was most effective in inhibiting ODC induction. We therefore carried out analogous experiments, but found no difference in the inhibition of the EA response, regardless of whether the cells were pretreated or simultaneously treated with retinoic acid. Pretreatment of the cells with retinoic acid (10^{-6} M) for 12 h followed by subsequent washing and addition of TPA still resulted in more than 90% inhibition of EA induction. Additional experiments with retinoic acid, using a different tumour promoter (Pimelea factor P2), yielded analogous results to TPA treatment.

We then investigated the specificity of the inhibitory effect of retinoic acid on EBV antigen induction by tumour promoters. Raji cells induced by IUdR or anti-IgM-antiserum to EA synthesis were treated simultaneously with varying concentrations of retinoic acid (Figs 2, 3). Although IUdR induction of EA seems to be less strongly inhibited than TPA induction, some inhibition is obvious over a relatively wide range of retinoic acid concentration. EA induction by anti-IgM is clearly inhibited by retinoic acid.

Further experiments were designed to test the influence of retinoic acid on EA induction by superinfecting Raji cells with EBV derived from P3HR-1 cells^{11,12}. Retinoic acid did not affect the percentage of EA-positive cells, even when applied at 10^{-5} M. Similarly, the spontaneous induction rate of P3HR-1 and B95-8 cells, which is approximately 6 and 4%, respectively, was not significantly altered by applying retinoic acid at 10^{-7} – 10^{-5} M for 3 and 5 d.

These data show that retinoic acid inhibits virus induction by chemical inducers and anti-IgM antiserum without affecting antigen synthesis after superinfection or spontaneous induction. This suggests that the different inducing reagents activate the persisting genomes indirectly by a common mediator which seems to be inhibited by retinoic acid. EA induction by superinfection or spontaneous induction of viral antigens in tissue culture cells apparently follow different pathways. Such observations are consistent with the recent demonstration of a correlation between inducibility by promoters, IUdR and anti-IgM antiserum and EBV genome equivalents per cell (K.B. *et al.* in preparation), whereas the response to superinfection seems to be independent of genome numbers in EBV genome-carrying cells.

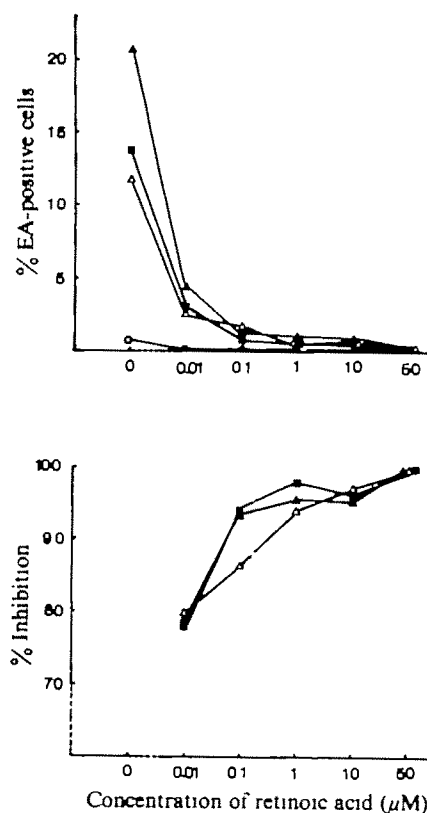


Fig. 1 Effect of retinoic acid on induction by TPA in Raji cells (clone no. 7, K.B. *et al.*, in preparation). The cells (5×10^5 per ml) were incubated in 10 ml medium (RPMI 1640 containing 10% fetal calf serum) in the presence of 20 ng ml^{-1} TPA and various concentrations of retinoic acid. The % of EA-positive cells was determined at indicated times by indirect immunofluorescence¹¹. a, The % of EA-positive cells; b, the % of inhibition in the presence of retinoic acid compared with the control cultures (without retinoic acid). O, Day 1; ■, day 3; ▲, day 5; △, day 7.

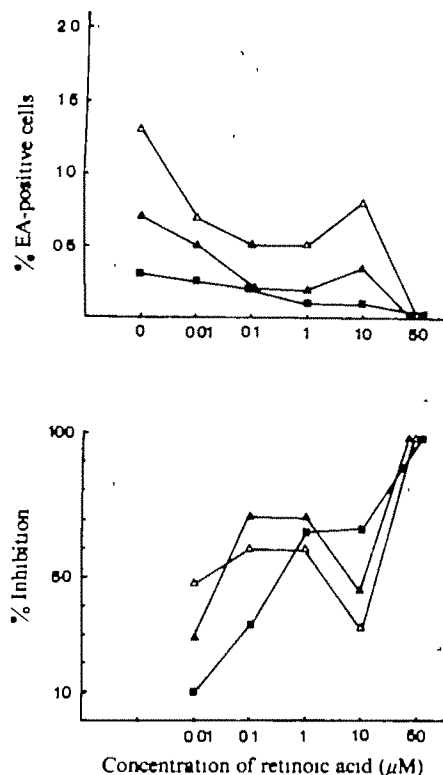


Fig. 2 Effect of retinoic acid on EA induction by IUdR in Raji cells (clone no. 7): 10 ml of the cells (5×10^5 per ml) were incubated in the presence of $100 \mu\text{g ml}^{-1}$ IUdR and various concentrations of retinoic acid. ■, Day 3; ▲, day 5, △, day 7.

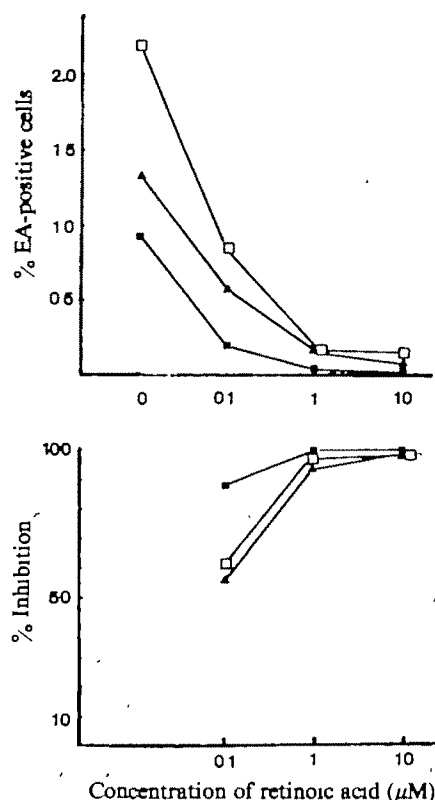


Fig. 3 Effect of retinoic acid on EA induction by anti-IgM antiserum. Raji cells were incubated with 1% of rabbit anti-human IgM antiserum (Behring-Werke) and retinoic acid in conditions outlined in Figs 1 and 2. ■, Day 1; ▲, day 2; □, day 4.

It is uncertain whether the inhibition of ODC activity by retinoic acid has any direct relationship to inhibition of virus induction by retinoic acid. Preliminary data indicate that neither putrescine, which should interact with ODC by feedback inhibition^{13,14}, nor colchicine, which is also reported to inhibit TPA-induced ODC activity¹⁵, reduce the number of TPA-induced Raji cells (H.z.H., unpublished data). This seems to dissociate virus induction from ODC stimulation and stresses the specificity of the virus induction process by diterpene esters.

If herpes virus persistence in general is affected by the application of retinoic acid, this could have important implications for the prevention of recurrent herpetic infections.

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Teratocarcinomas rarely develop from embryos transplanted into athymic mice

SEVEN-DAY-OLD mouse embryos transplanted to extra-uterine sites give rise to tumours that have been classified as either benign teratomas or malignant teratocarcinomas¹. The ratio of teratomas to teratocarcinomas varies from one mouse strain to another, suggesting the existence of teratocarcinoma-permissive and nonpermissive strains². Control of teratocarcinogenesis in this tumour system resides in the adult recipient of the embryonic graft and might involve the immune response. We report here that we have tested the hypothesis that the absence of cell-mediated immunity enhances the growth of transplanted embryos and facilitates the development of teratocarcinoma. Contrary to our expectations, our data indicate that embryos grafted into recipients with partial or total deficiency of cell-mediated immunity grow poorly and develop almost exclusively into benign teratomas.

We compared the outgrowth of 7-d-old embryos derived from teratocarcinoma-permissive (C3H/HeJ) and teratocarcinoma-nonpermissive (C57BL/6J) mouse strains. These embryos were transplanted under the kidney capsule of normal syngeneic mice, thymectomised, irradiated and bone marrow-reconstituted (TIR) mice, and athymic (*nu/nu*) mice. Results are summarised in Table 1.

Tumours obtained in the syngeneic recipients were as previously described². In C3H/HeJ mice, two out of three of the

Table 1 Weight of tumours and the ratio of teratomas to teratocarcinomas obtained from 7-d-old embryos transplanted under the kidney capsule of adult recipients

Embryo	Recipient	No of grafts	Weight of tumours (mg±s.e.m.)	Teratoma/teratocarcinoma
C3H	C3H, untreated	94	3,569±330 (a)	33/61 (e)
	C3H, TIR	21	557±117 (b)	17/4 (f)
	Nude, <i>nu/nu</i>	14	814±240 (b)	10/4 (g)
C57BL	C57BL, untreated	71	2,178±279 (c)	64/5 (h)
	Nude, <i>nu/nu</i>	21	538±97 (d)	20/1 (j)

An embryonic portion of 7-d-old embryos was isolated and transplanted under the kidney capsule of adult recipients which were: untreated, 2-month-old C3H/HeJ and C57BL/6J; athymic; thymectomised, irradiated and bone marrow-reconstituted (TIR) mice. Athymic (*nu/nu*) mice were on BALB/c background. TIR animals were thymectomised as weanlings, irradiated 2 weeks after thymectomy with 850 rad and reconstituted with 2×10^7 syngeneic bone marrow cells. Animals that survived and recovered completely were used as recipients 4 weeks after reconstitution. Recipient animals were killed 2 months after grafting. Diagnosis of teratoma and teratocarcinoma was made histologically. *P* value, as calculated by Student's *t* test, was <0.005 for *a* relative to *b*, *c* to *d*, and *a* to *c*. *P* values, as calculated by χ^2 test, were as follows: <0.005 (*e* relative to *f*), <0.025 (*e* to *g*), >0.75 (*f* to *g*), <0.005 (*e* to *h*), >0.1 (*g* to *j*), >0.9 (*h* to *j*).

tumours were teratocarcinomas, whereas in C57BL/6J mice, less than 10% of tumours were classified as teratocarcinomas. The proportion of teratocarcinomas in C3H/HeJ mice was significantly higher than in nude and TIR recipients. In addition, all embryo-derived tumours in nude and TIR recipients were considerably smaller than those in control mice. Tumours as well as the adjacent kidney parenchyma of both nude and TIR animals often contained aggregates of small lymphocytes.

Our results show that the removal or absence of the T-cell immune system in recipients of embryonic grafts not only does not stimulate the development of teratocarcinomas but apparently inhibits teratocarcinogenesis. Two hypotheses could explain these observations. First, in agreement with the immunostimulation theory of Prehn³, one could postulate that an interaction between the transplanted embryo and the T-cell immune system is necessary for teratocarcinogenesis. TIR mice are not completely devoid of T-cell functions⁴, but the number of T cells they have might be insufficient for immunostimulation. The alternate hypothesis is that the development of teratocarcinomas was inhibited by natural killer cells and/or macrophages. Nude mice have high levels of natural killer-cell activity⁵ and, as such cells are derived from bone marrow⁶, their activity is probably also restored in TIR mice. Depletion of T lymphocytes increases resistance to syngeneic tumours, and activated macrophages have been suggested as cells mediating such resistance⁷. The presence of lymphocytic infiltrates in the embryo-derived tumours obtained in nude and TIR mice supports the latter hypothesis. Because of the unique nature of these embryo-derived tumours we have been able to show that the T-cell status of the recipient mice affects the nature of the tumour (benign or malignant) in a situation where the incidence of tumours is unaffected.

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Antigen-specific suppressive factor produced by a transplantable I-J bearing T-cell hybridoma

THE demonstration of a definite antigen-binding specificity possessed by suppressor T cells and suppressor factors¹⁻⁶ has provided an important strategy to determine the T-cell receptor for antigen. Although the gene coding for the determinant on the suppressor molecule has been unambiguously mapped in the I-J subregion of the mouse major histocompatibility complex (MHC)^{3,7}, the chemistry and the mode of action of the active molecule have been only poorly analysed—probably because of the difficulty in collecting sufficient materials from suppressor T cells for each studies. The establishment of T-cell hybridomas with specific suppressor function would provide an ideal tool to analyse the suppressor molecule and the mechanism of its action. We have been successful in obtaining a number of hybrid cell lines continuously secreting molecules having both antigen-specific suppressor function and I-J determinants. High frequency of fused cells with I-J determinants was reproducibly made by the combination of two procedures; the method to

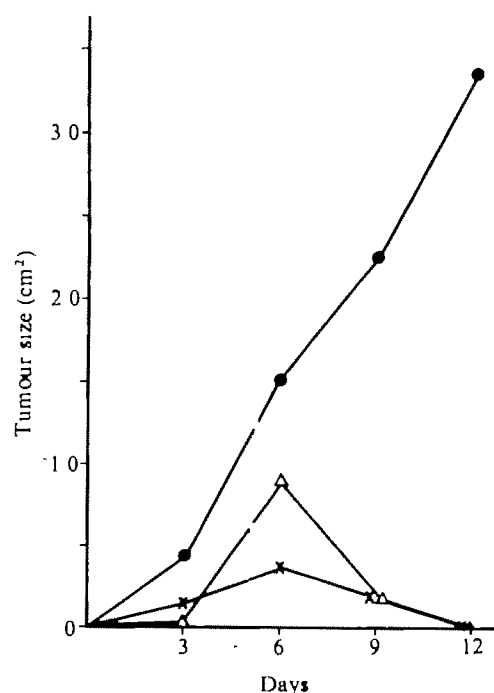


Fig. 1 Growth of I-J^b positive hybridoma 9F181a in F₁ mice but not in mice with parental haplotypes. 2×10^6 9F181a cells in 0.1 ml were inoculated into C3H (H-2^b) (X), C57BL/6 (H-2^b) (Δ) or (C3H x C57BL/6)F₁ (H-2^b x b) mice (●). Tumour size was measured on the day indicated and expressed as the mean tumour area, being represented by the product of two perpendicular diameters. Each group consisted of four animals.

Table 1 Antigen-specific suppressive effect of secreted or extracted materials from the I-J^{b+} hybridoma 9F181a on the *in vitro* secondary anti-DNP IgG PFC responses

	Suppressor factor	Dose	Anti-DNP IgG PFC response to	
			DNP-EA	DNP-KLH
Expt A	Not added	—	1,733 ± 125	6,295 ± 1194
	Extract of BW5147	3 × 10 ⁵ cells	1,762 ± 300	5,371 ± 1194
	Extract of 9F181a	3 × 10 ⁵ cells	1,697 ± 691	417 ± 107
Expt B	Not added	—	1,520 ± 274	1,492 ± 72
	Ascites of 9F181a-bearing mice	50 µl	1,521 ± 290	213 ± 73
		10 µl	1,307 ± 299	149 ± 88
		1 µl	1,379 ± 89	625 ± 368
		0.1 µl	1,648 ± 494	1,762 ± 137

4 × 10⁶ spleen cells from C57BL/6 mice primed with 100 µg of DNP-KLH or DNP-EA and 1 × 10⁹ pertussis vaccine 4 weeks before culture in Falcon 3008 plastic plate in 1 ml of RPMI-1640 (GIBCO) enriched with 10% fetal calf serum in the presence of 0.1 µg of antigen and 2 × 10⁻⁵ M 2-mercaptoethanol. The secreted or extracted materials from the hybridoma 9F181a were added to the culture at the start of cultivation. The culture was maintained in humidified atmosphere of 5% CO₂ in air at 37 °C. Anti-DNP IgM and IgG PFC were assayed on day 5 of culture by Cunningham and Szenberg as described previously². Expt A: 100 µl of the extract corresponding to 3 × 10⁵ BW5147 cells or hybridoma 9F181a cells was added to the appropriate group at the beginning of culture. For the preparation of the extract, cells were collected, washed three times with serum-free medium and resuspended with 0.01 M sodium phosphate-buffered saline pH 7.2 at a concentration of 1 × 10⁸ cells per ml. They were frozen in dry ice in acetone and thawed with warm water at 37 °C 10 times each. The cell-free extract was obtained by ultracentrifugation of the freezing and thawing materials at 20,000g for 1 h. Several other experiments gave similar results. Expt B: Various doses of ascites from (C57BL/6 × C3H)F₁ mice intraperitoneally implanted with 2 × 10⁷ hybridoma 9F181a cells 14 days previously were added at the beginning of the culture. Five other experiments gave similar results. Results are expressed as mean numbers of anti-DNP IgG PFC of four or five cultures ± s.d.

enrich antigen-specific Ig⁻ T cells from primed spleen cells by adsorption to and elution from antigen-coated Petri dishes, and the selection of I-J⁺ hybrids by sorting out with fluorescence activated cell sorter (FACS) after staining the fused cells with appropriate fluorescence reagents⁸. Here we report the derivation of an I-J bearing hybrid cell line, which is transplantable to H-2^{b/k} F₁ hybrid mice, secreting the antigen-specific suppressor molecule. Some physicochemical and immunochemical properties are also described.

The hybrids were made by fusion of AKR thymoma cell line BW5147, which lacks the enzyme hypoxanthine—guanine phosphoribosyl transferase (HGPRT⁻), with the suppressor T cell of C57BL/6 mice specific for keyhole limpet haemocyanin (KLH). The KLH-binding suppressor T cells were enriched by

allowing Ig⁻ spleen cells from KLH-primed mice to bind to KLH-coated Petri dishes⁹. Cells thus obtained constituted about 0.1–0.5% of the original spleen cells, but were enriched for the suppressor activity about 100-fold. The method for cell fusion used was as described elsewhere⁸. In brief, a mixture of 1 to 5 × 10⁶ enriched suppressor T cells and 1 to 50 × 10⁶ of BW5147 thymoma cells was suspended in Dulbecco's modified Eagle's medium (DMEM). The mixture was centrifuged at 400g and the pellet resuspended in 2 ml of polyethylene glycol (PEG; MW 2,000)—dimethyl sulphoxide (DMSO) solution (1 weight of PEG plus 1 volume of 15% DMSO-DMEM). Within 1 min, a further 2 ml of 50% (w/v) PEG solution without DMSO was poured into the test tube, and the reaction mixture gently stirred with a Pasteur pipette. The suspension was then gradually diluted with 16 ml of serum free DMEM and further diluted with 180 ml of DMEM containing 13% of fetal calf serum (FCS). They were then incubated at 37 °C in 5% CO₂ for 3–4 h. After incubation cells were washed four times with DMEM and cultured for 1 week in HAT medium (RPMI-1640 supplemented with 10% FCS and hypoxanthine, aminopterin, thymidine (HAT)) on Falcon plates (Falcon 3008), each well containing about 2 to 10 × 10⁴ cells in 1 ml. Cells grown in HAT medium were collected and stained with an anti-I-J^b antiserum (B10.A(5R) anti-B10.A(3R)) and fluorescein-conjugated rabbit anti-mouse Fab, both of which had been extensively preabsorbed with BW5147 cells to avoid nonspecific staining. The stained cells were then sorted by fluorescence activated cell sorter, FACS II (Becton-Dickinson), and fluorescence positive cells were cloned in multi-well microplates (Falcon 3040) by limiting dilution method or single cell manipulation.

Several hybridisation experiments have been carried out, and so far seven antigen-specific suppressor T-cell lines established. The detailed properties of these cell lines will be described elsewhere. The cell line 9F181a described here displayed certain characteristics not possessed by other functional T-cell hybridomas: this line was transplantable to H-2^{b/k} F₁ mice and secreted the antigen-specific suppressor molecule having I-J determinants, thus providing an opportunity to compare the suppressor molecules secreted and extracted from the suppressor T-cell hybridoma.

The transplantability of 9F181a was studied by inoculating 2 × 10⁶ cells in 0.1 ml intracutaneously into the shaved back of C57BL/6 (H-2^b), C3H (H-2^k) and (C57BL/6 × C3H)F₁ (H-2^{b/k}) mice respectively. The active growth of the tumour as a

Table 2 Immunochemical characterisation of the suppressive molecule in the secreted and extracted materials from the 9F181a hybridoma

Suppressor factor absorbed with:	Anti-DNP IgG PFC per culture	
	Ascites	Extract
Not added	1,023 ± 401	1,169 ± 114
Unabsorbed	86 ± 49	12 ± 23
KLH	1,492 ± 115	1,251 ± 233
Ascites	32 ± 64	35 ± 45
Anti-mouse Igs	96 ± 65	47 ± 56
Anti-mouse Fab	149 ± 175	59 ± 70
Anti-H-2 ^b	1,204 ± 141	1,180 ± 276
Anti-I-J ^b	1,364 ± 92	947 ± 159
Anti-I-J ^k	149 ± 74	24 ± 47

20 µl of ascites or extracted materials from the 5 × 10⁵ hybridoma cells were absorbed by passing through the following immunoabsorbents: KLH (5 mg KLH per ml beads), *Ascaris suum* extract (5 mg protein per ml beads), anti-H-2^b (C3H.Q × HTH anti-C3H.B10; 1 ml γ-globulin fraction of antiserum per ml beads), anti-I-J^b (B10.A(5R) anti-B10.A(3R); 1 ml γ-globulin fraction of antiserum per ml beads), anti-I-J^k (B10.A(3R) anti-B10.A(5R); 1 ml γ-globulin fraction of antiserum per ml beads), polyvalent rabbit anti-mouse Igs (5 mg purified anti-mouse Ig antibodies per ml beads), and rabbit anti-mouse Fab (5 mg purified anti-mouse Fab antibodies per ml beads). The beads used were Sepharose 4B. The method for preparation of immunoabsorbents was as described previously³. The absorbed materials were added to the culture of spleen cells of C57BL/6 mice primed with DNP-KLH. The assay of anti-DNP IgG PFC is the same as in Table 1.

Table 3 Molecular weight of the suppressor factor in ascites of mice bearing 9F181a

Fraction added	Anti-DNP PFC per culture		% Suppression of IgG PFC
	IgM	IgG	
No factor	688 ± 58	2,544 ± 59	—
Fraction I	756 ± 58	2,956 ± 211	0
Fraction II	756 ± 365	2,590 ± 89	0
Fraction III	596 ± 89	2,865 ± 431	0
Fraction IV	779 ± 380	413 ± 325	84
Fraction V	668 ± 175	2,062 ± 325	19

The materials fractionated on Sephadex G-200 as indicated in Fig. 2 were concentrated by vacuum dialysis at 4 °C and tested for suppressive activity. The fractionated material at a dose corresponding to 30 µl of original ascites was added to the culture of spleen cells of C57BL/6 primed with DNP-KLH. Results are expressed as mean numbers of anti-DNP IgM and IgG PFC ± s.d. The degree of suppression was calculated as follows:

$$\% \text{ Suppression} = (1 - \text{experimental PFC/control PFC}) \times 100$$

solid form was observed in (C57BL/6 × C3H)_{F1} mice, but not in parental C3H or C57BL/6 mice (Fig. 1). If the cells were transplanted intraperitoneally into F₁ mice, a large quantity of ascites was produced. This ascites contained a strong antigen-specific suppressor activity (see below). The transplantability of 9F181a is partially explained by its surface phenotypic expressions: Even 13 months after hybridisation the cell line expressed H-2 antigens of both parental cells, H-2^b, and H-2^k, together with I-J^b determinants introduced from C57BL/6 suppressor T cells.

Previous studies have firmly established that the extract from sonicated splenic T cells of KLH-primed mice can specifically suppress the *in vivo* and *in vitro* secondary IgG antibody responses against DNP-KLH^{2,10}. The active molecule involved in this suppression was shown to carry determinants coded for by genes mapped in I-J subregion of MHC³. It was therefore of interest to establish whether the ascites as well as the extract obtained from this hybridoma contains a similar molecule having I-J determinants and antigen-specific suppressor activity. Various doses of ascites obtained from (C57BL/6 × C3H)_{F1} mice bearing 9F181a were added to the culture of 4 × 10⁶ DNP-KLH- or DNP-EA (hen's egg albumin: hapten coupled to irrelevant carrier)-primed C57BL/6 spleen cells which were concomitantly stimulated with the corresponding antigen (0.1 µg ml⁻¹) in the Mischell-Dutton culture system. A separate experiment was carried out to study the suppressive activity of the extract of 9F181a. The dose of extracted material corresponding to 3 × 10⁵ viable cells was added to the culture. The number of plaque forming cells producing anti-DNP IgG antibody was measured on day 5 by the method of Cunningham and Szenberg as described previously².

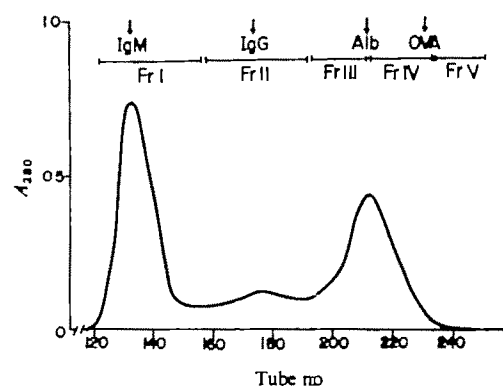


Fig. 2 Elution profile of ascites from mice bearing 9F181a after fractionation on Sephadex G-200. Two ml ascites was dialysed in 0.005 M borate buffered saline pH 8.0 for 16 h. The dialysed material was then fractionated on Sephadex G-200 column (2.5 × 90 cm × 2). Fractions (3 ml) were monitored by a spectrophotometer at 280 nm. Five fractions were separated as indicated, and were tested for suppressive activity (see Table 3). Arrows indicate the position of marker proteins (mouse IgM, mouse IgG, mouse albumin and hen's egg albumin: OVA) eluted at the identical condition. the MW of Fr. I is >200,000; Fr. II, 100,000–200,000; Fr. III, 70,000–98,000; Fr. IV 42,000–68,000; Fr. V, 30,000–40,000

The results in Table 1 show that addition of 1–50 µl of ascites to the cultured spleen cells suppressed anti-DNP IgG antibody response to DNP-KLH but not to DNP-EA. Even 10 µl of ascites showed a maximal suppressive effect, and 1 µl was enough to show a significant suppression. The extracted material from 9F181a also showed the same carrier specific suppressor activity, and the same dose of the extract from parental BW5147 had no effect on the response against both antigens. IgM antibody-forming cells to DNP-KLH which comprised 20 to 30% of total antibody secreting cells were not suppressed by both extracted and secreted materials (data not shown).

Immunochemical properties of the hybridoma-derived suppressor factors were studied by absorption with immunoadsorbents composed of specific antigen (KLH), irrelevant antigen (*Ascaris* extract), anti-H-2, anti-I-J, polyvalent anti-mouse IgG and anti-mouse Fab antibodies. As shown in Table 2, the suppressive activity of both ascites and extract was completely removed by KLH, anti-H-2^b and anti-I-J^b, but not by *Ascaris* extract, anti-I-J^k, polyvalent antimouse IgG and anti-mouse Fab. These results indicate that the suppressor molecules both secreted and extracted from 9F181a have an antigen-binding site and I-J coded determinants.

The molecular size of the hybridoma-derived suppressor molecule was investigated. Figure 2 shows the elution profile of

Table 4 Suppressive effect of extracted and secreted materials from 9F181a on the response of C57BL/6 and (C57BL/6 × C3H)_{F1} but not of C3H mice

Suppressor factor	Dose	Anti-DNP IgG PFC per culture		
		C57BL/6	(C57BL/6 × C3H) _{F1}	C3H
Not added	—	1,854 ± 288	3,424 ± 345	6,160 ± 725
Extract of BW5147	3 × 10 ⁵ cells	2,054 ± 245	3,194 ± 435	5,025 ± 1,109
Extract of 9F181a	3 × 10 ⁵ cells	134 ± 114	586 ± 122	5,382 ± 530
Ascites of 9F181a-bearing mice	20 µl	682 ± 272	1,008 ± 568	6,543 ± 1,264

Extract equivalent to 3 × 10⁵ BW5147 or hybridoma 9F181a cells and 20 µl of ascites was added to the culture of spleen cells of C57BL/6 (H-2^b), C3H(H-2^k) and (C57BL/6 × C3H)_{F1} (H-2^{b/k}) mice immunised with DNP-KLH and pertussis vaccine to test the requirement of H-2 histocompatibility for effective suppression. The numbers of anti-DNP IgG PFC after 5 days of culture with and without the factor were compared. Assay of anti-DNP IgG PFC as in Table 1.

ascites on Sephadex G-200. The fractionated materials as indicated in Fig. 2 were tested for their suppressive activity. This was present in fraction IV (Table 3). The MW of fraction IV was between 42,000 and 68,000—similar to that of previously reported suppressor factor from splenic T cells².

We have previously demonstrated that the suppressor T-cell factor from primed mice was able to suppress only the responses of H-2 histocompatible strains¹¹. Thus, we attempted to examine whether or not such a genetic restriction exists in the effect of the suppressor factor derived from the hybridoma 9F181a. As the cell line was made by fusion of BW5147 (H-2^b) and C57BL/6 (H-2^b) suppressor T cells, the secreted and extracted materials from 9F181a were tested for their activity to suppress the response mounted by spleen cells of parental haplotypes, H-2^k and H-2^b and of their F₁ hybrid (H-2^{b/k}). The results in Table 4 show that the responses of C57BL/6 and (C57BL/6 × C3H)F₁ were suppressed by both secreted and extracted materials, whereas that of C3H (H-2^k) was not. This result is consistent with the fact that I-J^b but not I-J^k is phenotypically expressed on 9F181a. The finding that the putatively homogeneous suppressor factor derived from the hybridoma had this strict H-2 restriction supports our previous postulate that the I-J bearing factor can interact with other types of lymphoid cells only if they share an identical I-J subregion¹¹.

A T-cell hybridoma with antigen-specific suppressor activity has recently been reported by Kontiainen *et al.*¹². The suppressive factor from their hybridoma was shown to suppress mainly the primary IgM antibody response, while the secondary IgG antibody response was less affected. The antigen-specific suppressor factor from our hybridoma preferentially suppressed secondary IgG response without measurable suppression of the IgM antibody response. The same was true for the hybridoma-derived factors reported by Taniguchi and Miller⁶ in the *in vivo* suppression of the secondary antibody response to other protein antigen, in which antigen-specific hybridoma derived factors could suppress only the IgG antibody response. Whether such a difference would be inherent to the structure of the factors is not yet known.

The establishment of T-cell hybridoma cell lines continuously producing homogeneous materials with antigen-specific activity would make possible a more exact analysis of the molecule and its mode of action. The transplantable hybridomas secreting a large quantity of the active molecules in ascites or in the culture supernatant would be of particular value in the study of the chemistry of the molecule.

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Interferon enhances the excitability of cultured neurones

INTERFERON has varied biological effects on cells^{1,2} and induces modifications of the cell surface both *in vitro*^{3–6} and *in vivo*⁷. The particular capacity of the neuronal membrane to generate propagated action potentials (well retained in culture⁸) makes neurones excellent material for the study of substances that affect the cell surface. We have therefore determined the effect of interferon on the electrical activity of nerve cells. Most of the experiments reported here were carried out on the cat cerebral and cerebellar cortex, because the electrical patterns of these structures have been well defined *in vitro*^{9,10}. Furthermore, as human leukocyte interferon exhibits a high degree of antiviral activity on cat cells¹⁰, we were able to study the effect of highly purified interferon preparations on the spontaneous and evoked electrical activity of these neurones. In other experiments, we studied the effect of rat interferon on rat neurones in culture. Our results, which show that human and rat interferon specifically enhance the excitability of cat and rat nerve cells, respectively, may have implications for the therapeutic use of interferon as well as for the understanding of some of the manifestations of viral diseases.

Explants of cerebral and cerebellar cortex from newborn kittens or rats were cultivated in Maximow assemblies at 35.5 °C in a drop of nutrient medium containing 40% fetal calf serum⁸. After 3 weeks, the culture was transferred into a moist chamber and fixed on the stage of an inverted phase contrast microscope.

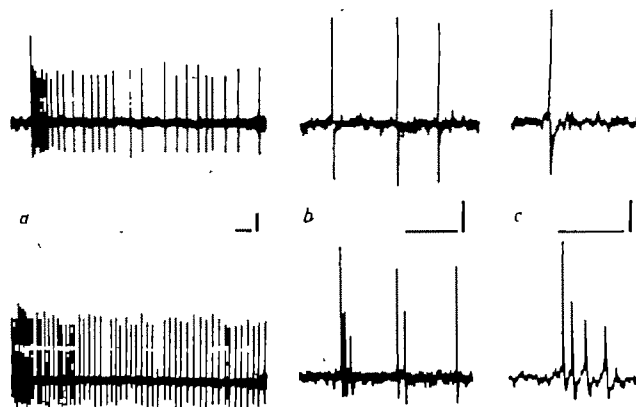


Fig. 1 Effect of human leukocyte interferon on the spontaneous activity of a Purkinje cell (kitten cerebellar cortex, 43 d in culture). The upper (before interferon) and lower tracings (4 h after interferon) are recorded from the same cell for different sweep rates (a, b, c). Note that interferon increases the spike frequency and the duration of the burst (a). b and c (faster sweeps) show the tendency of the interferon-treated neurone to fire repeatedly (2–4 successive action potentials) compared with the single spike obtained from the same neurone before interferon. Horizontal bars, 100 ms; vertical bars, 1 mV, negativity downwards.

Under visual control, the two recording electrodes (glass micropipettes filled with 3 M KCl with a resistance of about 30 MΩ) were placed against the neuronal perikarya to record extracellularly the electrical activity of two neurones (or groups of neurones) for several hours. Focal electrical stimuli (15 V, 0.2–1 ms) were applied through a pair of electrodes (filled with isotonic saline, with the same resistance, 0.5–1 MΩ) which could be placed successively in various sites of the explant. The responses were amplified by a differential field effect transistor preamplifier (a.c. recording-time constant 250 ms), and stored on tape.

Table 1 Statistical analysis of the latency of 12 different cortical responses before and at least 1 h after addition of human leukocyte interferon

Stimulation site	No. of responses before and after interferon	Mean latency (ms $\pm$ s.d.)
1	Before 32	40 $\pm$ 9.4
	After 15	17 $\pm$ 4.7*
2	Before 30	36 $\pm$ 6.5
	After 16	29 $\pm$ 5.5†
3	Before 17	38 $\pm$ 5.5
	After 15	23 $\pm$ 7.4*
4	Before 31	16 $\pm$ 3.1
	After 21	10 $\pm$ 1.7*
5	Before 15	34 $\pm$ 5.9
	After 15	14 $\pm$ 7.1*
6	Before 15	67 $\pm$ 9.8
	After 15	30 $\pm$ 4.8*
7	Before 18	42 $\pm$ 7.1
	After 30	24 $\pm$ 4.7*
8	Before 15	109 $\pm$ 17.2
	After 16	75 $\pm$ 10.3*
9	Before 29	57 $\pm$ 15.5
	After 29	15 $\pm$ 8.5*
10	Before 16	68 $\pm$ 17.0
	After 15	12 $\pm$ 2.5*
11	Before 16	33 $\pm$ 5.6
	After 16	22 $\pm$ 5.2*
12	Before 25	33 $\pm$ 10.1
	After 25	13 $\pm$ 5.4*

Results were from three different kitten cerebral explants. Analysis by *t* test.* Highly significant, $P < 10^{-5}$.† $P < 0.0001$.

Crude human leukocyte interferon prepared as previously described¹¹ was purified over 3,000-fold and had a specific activity of 10^7 units per mg protein. It had a titre of 5.12×10^{-5} as assayed (using vesicular stomatitis virus) on both human diploid fibroblasts and cultures of kidney from the same kitten whose brain cortical cells were used in the experiment. A control human leukocyte preparation of approximately the same protein content (4 mg ml^{-1}) was prepared as previously described¹¹. Rat cell culture interferon prepared according to the techniques used in the production of mouse interferon¹² had a titre of 8×10^{-5} on rat fibroblasts and a specific activity of 8×10^5 units per mg protein. Because of the inherent complexity of the system it was only feasible to use a single interferon concentration for each species. We chose 10^4 human leukocyte interferon units and 8×10^3 rat cell culture interferon units.

When human leukocyte interferon was added to the culture medium of an explant from kitten cerebellar cortex there was an increase in the frequency of the spontaneous unit discharges and a tendency for individual neurones to fire repeatedly (Fig. 1). These changes were first observed after approximately 30 min and became more marked after several hours. In explants of kitten cerebral cortex the spike and slow wave pattern, which was usually observed at a frequency of about 1 per min, occurred every 30–40 s after the addition of interferon, and often showed a tendency to repeated firing. Rat interferon induced comparable effects on the neurones from newborn rat cerebellar and cerebral cortex. It is important to emphasise that although interferon induced quantitative modifications, it did not modify the basic spontaneous electrical pattern characteristic of these neurones⁸ (the cerebellar bursts¹³ and the cerebral spike and wave^{8,14}).

As interferon enhanced the spontaneous electrical activity of cat and rat neurones, we studied the effect of human interferon on the evoked electrical activity of cat neurones. In the experiments, the responses were plotted for different stimulating sites before and at various intervals after the addition of interferon. The general pattern of the evoked responses was similar to that characteristic of spontaneous activity and was reproducible for several hours. As in the experiments on spontaneous electrical

activity, an effect of interferon was first observed at approximately 30 min and persisted for the whole 4–5-h recording session.

The most important effect of interferon was a marked shortening of the latency between stimulation and the initial response (Fig. 2a, b). This decrease in latency was observed for all the stimulating sites at both recording electrodes, and the statistical analysis of these results is presented in Table 1. Interferon also lowered the threshold to stimulation. Thus, for a given intensity, a single shock (0.7–1.0 ms) evoked a single wave before interferon (Fig. 2c, upper tracings), whereas a similar but repetitive pattern was observed after the addition of interferon (Fig. 2c, lower tracings). Furthermore, the duration of the shock necessary to elicit a single response could be decreased from 0.7 ms to 0.3–0.2 ms after the addition of interferon. Lastly, when treated neurones were subjected to a repetitive stimulation, they were able to respond at a higher rate (6–8 potentials

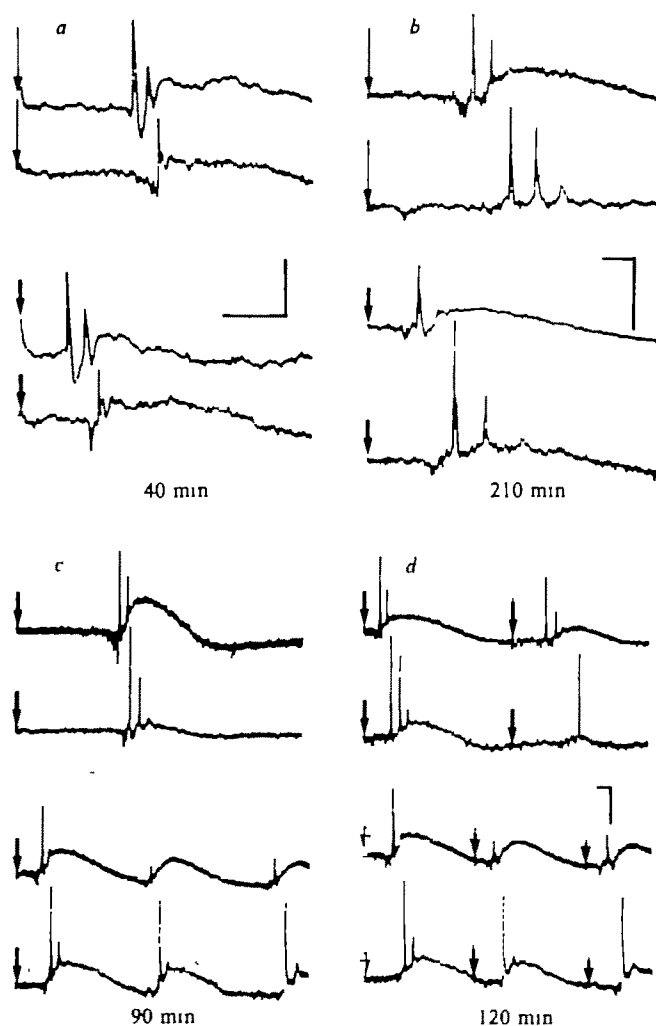


Fig. 2 Patterns of evoked electrical activity in cultures of kitten motor cortex (a, c = 46 d) and hippocampus (b, d = 58 d) before (two upper tracings in each panel) and after (two lower tracings in each panel) treatment with 10^4 units of human leukocyte interferon. a and b illustrate the shortening of the latency after addition of interferon (see time in minutes in lower tracings) for the same stimulating site and for the same neurones. In c, a single shock of 0.7 ms evoked a single spike and wave from both neurones before interferon, and three successive spikes and waves 90 min after the addition of interferon. Note that the latency of the repetitive firing in the lower two tracings is considerably shorter than that of the single response shown in the two upper tracings. In d, two shocks at intervals of 250 ms evoked only two responses before interferon, whereas 2 h after the addition of interferon, three shocks at intervals of 175 ms still evoked three successive responses with a constant shortened latency. Note in all panels that the polarity and amplitude of the waves and the order of firing of both neurones remain unchanged after interferon. Horizontal bars, 20 ms; vertical bars 1.5 mV; negativity downwards. The length of the arrows indicating stimulation is directly proportional to the intensity of the current.

per s instead of 4–5 potentials per s) (Fig. 2d). It is interesting that interferon did not, however, increase the amplitude or modify the polarity of the electrical potentials.

We must stress that neither human nor rat interferon preparations was toxic for neurones in culture. The behaviour of interferon-treated cells did not change during the several hours of observation, and after staining (with Bodian silver impregnation) the neurofibrillar nets had the same normal morphological appearance as those in control cultures. Furthermore, when comparable amounts of human and rat interferon were added to fresh cat and rat explants, these cultures could be maintained for 1 month with interferon without any detectable damage.

The evidence that interferon was responsible for these effects may be summarised as follows. Only human leukocyte interferon, which exerts antiviral activity on cat cells, enhanced the spontaneous activity and evoked responses of cat cerebellar and cerebral cortex: a control leukocyte preparation and rat cell culture interferon did not exert these effects. Likewise, rat cell culture interferon, but not control preparations, enhanced the electrical activity of rat neurones.

An enhanced excitability of cerebral and cerebellar neurones in culture has also been observed using bicuculline¹⁵, picrotoxin¹⁵ and strychnine^{14,16}. Some of the effects of these substances (shortened latency, lowered threshold, tendency to repetitivity) are comparable to those we have described for interferon. Some striking differences do exist, however, between the effects of interferon on neurones and those reported for other pharmacological agents¹⁷. (1) Interferon treatment did not increase the amplitude or reverse the polarity of the cortical waves (as do most of the other excitatory substances). (2) The effect of interferon was seen after 30 min and lasted several hours. (Most other substances induce an immediate effect which lasts only a few minutes.)

Our data do not permit us to determine whether the enhanced excitability results from a direct effect on the neurone (modification of transmembrane ionic exchanges) or at the level of the synapse (facilitation of excitatory or blocking of inhibitory synapses). We believe this to be the first report of an effect of interferon on the function of nerve cells in culture. The nature of this effect is consistent with other known actions of interferon on cells, that is, enhancement of a particular cell function in the absence of cell toxicity¹². Although most of the effects of interferon require its incubation with cells for periods over 12 h, some early effects of interferon suggestive of modification of the cell surface have been observed within several hours; these include changes in thymidine transport¹⁸, release of labelled uridine¹⁹, and enhanced cytotoxicity of sensitised T lymphocytes²⁰ or natural killer cells²¹. However, the enhancement by interferon of the excitability of neurones within 30 min seems to be one of the earliest effects yet described.

Our observations may, however, be of more general interest. If the interferon-induced enhancement of neurone excitability in culture also occurs *in vivo*, our results may be relevant to some of the untoward manifestations of viral diseases.

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Stimulant effects of enkephalin microinjection into the dopaminergic A10 area

THE overt behaviour of various species is affected in different ways by opiate drugs. Mice and cats show behavioural stimulation following a systemic morphine injection whereas rats and dogs generally exhibit decreased behavioural activity^{1–4}, although in certain conditions, stimulant effects can also be seen in the latter species^{4,5}. Tatum *et al.* suggested that there are several distinct sites of action of opiate drugs in brain and that the relative dominance of one system over another determines whether the stimulant or depressant effects predominate⁴. Indeed, direct microinjections of morphine into the ventral tegmental area (VTA) have been shown to result in facilitation of self-stimulation behaviour whereas similar injections into the periaqueductal gray matter cause only an attenuation of this behaviour⁶. The discovery of endogenous ligands for opiate receptors in the brain⁷ has focused attention on the role of these peptides in various aspects of behaviour. Their analgesic properties following intraventricular and intracerebral injection are well documented⁸. Recently, it has been reported that infusion of α -endorphin into the substantia nigra induced stereotyped behaviour⁹, whereas local injections into the nucleus accumbens of the long-acting synthetic enkephalin analogue, [D-Ala²]-Met⁵-enkephalinamide (AME), produced an increase in locomotor activity¹⁰. We now report that infusion of AME (0.03–2.0 μ g) bilaterally into the dopaminergic (DA) A10 area of the VTA induces a short-latency behavioural stimulant effect reminiscent of effects produced by stimulation of the mesolimbic DA pathway¹¹. This effect is antagonised by pretreatment with the opiate receptor blocker naloxone.

Twenty-seven male Wistar rats, weighing 300–320 g, were anaesthetised with Nembutal (50 mg per kg), and two stainless-steel guide cannulae (23 gauge) were implanted stereotactically into an area 0.5 mm dorsal to the ventral tegmentum. Cannulae placements were confirmed histologically; the correct loci, quantified according to the coordinate system of König and Klippel, averaged $A = 2.1 \pm 0.3$, $L = 0.9 \pm 0.2$, and $DV = -2.6 \text{ mm} \pm 0.5$. Behavioural testing began 14 d after surgery.

Cannulae were sealed and kept patent by insertion of a 30-gauge inner stainless-steel stylet which protruded 0.5 mm beyond the outer guide. Microinjections were made by replacing each inner stylet with a 30-gauge needle attached to a micro-syringe by polyethylene tubing. A volume of 0.5 μ l was injected into each cannula over a 30-s period. The rats received injections of 2.0, 1.0, 0.12 or 0.03 μ g of AME or the vehicle (0.9% NaCl) on each side of the brain. Subjects were adapted to circular photocell cages for 2 h before injection and activity was measured for an additional 120 min after infusions into the

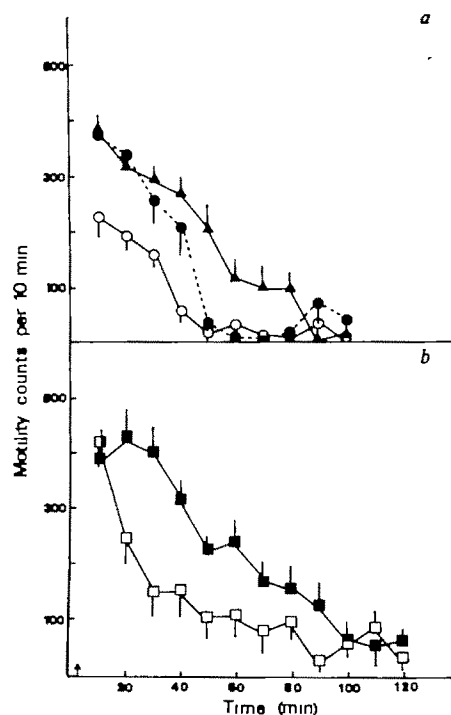


Fig. 1 Motility scores after microinjection of D-Ala²-Met⁵-enkephalinamide (AME, Calbiochem) or NaCl (0.9%) into the VTA. Ordinate: time in minutes; abscissa: number of times light beam interrupted per 10-min period (data presented are $\bar{x} \pm$ s.e.m.). a, Δ , $2 \times 0.12 \mu\text{g}$ AME ($n = 8$); $\bullet$, $2 \times 0.03 \mu\text{g}$ AME ($n = 8$). Total scores of both AME groups are significantly different from mean total scores of subjects injected with NaCl, O. ($P < 0.001$; 2-tailed t -tests.) b, $\blacksquare$, $2 \times 1 \mu\text{g}$ AME + NaCl (1 ml per kg, i.p.); $\square$, $2 \times 1 \mu\text{g}$ AME + naloxone (1.2 mg per kg in 1 ml per kg NaCl, i.p.). Mean total motility scores for the two groups are significantly different ($P < 0.02$, 1-tailed t -test). Arrow indicates injection. Whole-body locomotor activity was measured in circular photocell cages (60 cm diameter, BRS/LVE). In those animals receiving two drug injections, the injection sequence was balanced within the group and spaced at 48-h intervals. One day before the second injection $1 \mu\text{l}$ of 0.9% NaCl was infused through the cannulae to reduce possible misleading effects of residual drug deposits. Measurements were made during the light phase of the rat's diurnal cycle.

VTA. On one test, the subjects were pretreated with naloxone HCl (1.2 mg per kg intraperitoneally (i.p.)) immediately before bilateral infusions of $1 \mu\text{g}$ AME.

Figure 1 shows that bilateral injections of AME, in doses of $2 \times 1 \mu\text{g}$ ($n = 11$), $2 \times 0.12 \mu\text{g}$ ($n = 8$) or $2 \times 0.03 \mu\text{g}$ ($n = 8$), produced a clearcut increase in locomotor activity in all animals. This effect was evident in the first 10 min post-infusion and lasted up to 90 min. The mean total number of photocell counts for the $2 \times 1 \mu\text{g}$ dose was $2,656 \pm 367$ (s.e.m.) interruptions in 120 min. Seven additional animals were tested at the high dose of $2 \times 2 \mu\text{g}$ AME, which also led to a significant increase in the motility score ($2,781 \pm 300$ over 120 min). However, this score was not significantly different from that obtained with the $2 \times 1 \mu\text{g}$ dose and therefore the data were not included in Fig. 1. Significant increases in motility were observed in the groups receiving $0.12 \mu\text{g}$ and $0.03 \mu\text{g}$, with mean total counts of $1,930 \pm 163$ and $1,513 \pm 117$ per 120 min, respectively. Pretreatment with naloxone effectively antagonized the effects produced by $2 \times 1 \mu\text{g}$ AME (Fig. 1b). The activity score after naloxone was $1,616 \pm 234$ over 120 min, which was significantly less than the score obtained with a control dose of $2 \times 1 \mu\text{g}$ ($P < 0.02$ 1-tailed independent t test) but still above the score obtained after control infusions of NaCl ($\bar{x} = 828 \pm 68$ in 120 min $n = 16$).

The behavioural activation was defined more precisely by time sampling the behaviour of 11 rats according to a standard ethogram. The behavioural data indicated that the categories of

walking, rearing and sniffing were increased significantly only in the AME-treated group, at the expense of sleeping, sitting or lying quietly on the floor (Table 1). There was no indication of stereotyped behaviour such as repetitive head movements or prolonged sniffing on the same spot.

These results clearly indicate a behavioural stimulant effect after intracerebral infusions of AME into the VTA. No initial depressant effect was observed in any animal at any of the doses used. Although a significant stimulant effect was obtained with the lowest dose, there was evidence of a dose-response relationship, as the level of activity produced by this dose was significantly lower than that seen after the dose of $2 \times 1 \mu\text{g}$ ($P < 0.005$, 1-tailed t test).

Increased behavioural activity has been reported after microinjection of D-Ala into the nucleus accumbens¹⁰ and thalamus¹². However, the effects observed in the present study were obtained at much lower doses (0.03 – $2.0 \mu\text{g}$ compared with $10 \mu\text{g}$) and at significantly shorter latencies. This short-latency effect agrees with earlier observations of facilitation of self-stimulation behaviour following microinjection of morphine ($0.5 \mu\text{g}$) into the same area of the brain⁶. The antagonism of the behavioural stimulant effect of AME by naloxone is particularly important as it strongly suggests that the effect is mediated by opiate receptors.

Recent immunohistofluorescence data have identified enkephalin-positive perikarya in the interpeduncular nucleus, and enkephalin-containing nerve fibres in the VTA¹³. Thus, the present findings and the results of a previous localisation study with morphine⁶ suggest that the stimulant effects of AME are probably mediated by its action within the VTA or interpeduncular nucleus. The fact that infusions dorsal to this area were less effective (unpublished observations) is also consistent with this interpretation. It is important to recognise the difficulties in the precise localisation of intracerebral infusions. Thus, as the region of stimulation is inferred from the cannulae placements, the possible contribution of the adjacent substantia nigra cannot be ruled out. However, the behavioural data point to the involvement of the VTA in the effects reported here, as the increased locomotor activity and

Table 1 Effect on behaviour of $2 \times 2 \text{ g}$ D-Ala²-Met⁵-enkephalinamide (AME)

Behaviour	AME	Control	Statistic Z
Walking	2.5 (1–9)	0 (0–3)	
Rearing	6.5 (3–12)	2 (2–3)	2.09*
Sniffing	34.5 (32–39)	24 (20–33)	1.82
Explorative combined	46 (39–54)	30 (22–39)	2.18*
Teeth chattering + 'freezing'	0 (0–1)	0 (0–1)	
Sitting	1 (0–4)	6 (3–21)	
Lying	0 (0–4)	6 (1–15)	
Sleeping	0 (0–1)	2 (0–13)	
Passive combined	2 (0–8)	22 (9–31)	2.18*
Burying	1 (0–6)	0 (0–1)	
Picking up bedding material	0 (0–2)	0 (0)	
Gnawing	2 (0–18)	0 (0–15)	
'Wet dog' shivering	0 (0)	0 (0–1)	
Grooming	3.5 (0–10)	6 (0–23)	

For observations, the rats were placed in circular glass chambers (30 cm diameter) with bedding material distributed evenly over the floor, and given 1 h to adapt to this environment. The figures represent the number of occasions on which the specified behaviour was observed in the period 10–52 min after injection (median values and ranges are given for AME ($n = 6$) and control ($n = 5$) groups, respectively). Observations were made every 10 s during the first 2 min of each 10-min period (total: 60 samples per rat in 52-min test period). Statistical estimates were obtained with a Wilcoxon test (2-tailed). Walking + sniffing scored as walking; rearing + sniffing scored as rearing, sniffing + sitting or lying scored as sniffing.

* $P < 0.05$.

exploration elicited by AME are reminiscent of the intense exploratory activity seen after microinjections of dopamine into the nucleus accumbens¹¹.

The nucleus accumbens is one of the important projection areas of the DA cell bodies in the VTA¹⁴ and there is strong evidence for the involvement of this mesolimbic DA pathway in the stimulant effects of amphetamine and other related compounds¹⁵. Although the role of dopamine in the locomotor effects produced by AME injections into the nucleus accumbens is uncertain¹⁰, there is evidence that opiates may produce behavioural excitation by activating DA neurones¹². Recent electrophysiological data also support this contention¹⁶. Thus, it is tempting to speculate that the mesocorticolimbic DA system may mediate some of the locomotor stimulant effects of enkephalin in particular and opiates in general. The mesolimbic DA pathway has also been implicated in positive reinforcement¹⁷ and motivation¹⁸ and it remains to be determined whether this pathway might mediate the reinforcing properties of enkephalin¹⁹. If such a relationship were confirmed, it would have important implications for understanding the addictive properties of opiates.

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Brain ACTH and endorphin reduced in rats with monosodium glutamate-induced arcuate nuclear lesions

IMMUNOASSAY and immunocytochemical techniques, in some instances with additional characterisation by bioassay and gel filtration, have demonstrated the presence of ACTH-like¹⁻¹⁰, β -lipotropin (LPH)-like^{6,9,11-13} and β -endorphin-like^{6,14,15} activity within the central nervous system. However, the source of these peptides in the central nervous system is uncertain³. Hypophysectomy is not associated with any change in hypothalamic content of ACTH^{1,5,10} or of β -endorphin-like material¹⁴, suggesting a non-pituitary, presumably central nervous system, origin. Immunoreactive perikarya containing

ACTH-like^{8-10,16,17}, β -LPH-like^{9,11,13,16,17} and β -endorphin-like¹⁵⁻¹⁷ material have thus far only been demonstrated in the arcuate nucleus and adjacent lateral areas of the mediobasal hypothalamus. We considered that if the arcuate nucleus were the source of ACTH-like activity within other areas of the central nervous system, its destruction would result in a decrease of such activity. Neonatal administration of sodium glutamate produces central nervous system lesions, restricted largely to neurones of the arcuate nucleus and to the retina¹⁸, while sparing fibres of passage¹⁹. We report here that, using neonatal sodium glutamate administration to produce such arcuate nucleus destruction in rats, the content of ACTH and β -endorphin-like material in the hypothalamus and other brain regions is markedly reduced in the absence of any change in whole pituitary, anterior lobe or plasma ACTH concentrations.

Timed pregnant Holzman rats were housed in air-conditioned quarters on a lighting schedule of 14 h light, 10 h darkness with *ad libitum* food and water. Female offspring received 2.0 mg per g monosodium glutamate (MSG) intraperitoneally on the 2nd and 4th days of life, and 4.0 mg per g on the 6th, 8th and 10th days of life²⁰. Animals were killed at 5 months of age. Another group of animals received five doses of 10% NaCl as an isotonic control.

Animals treated with MSG demonstrated the endocrinological, metabolic and behavioural abnormalities and the hypothalamic neurotoxicity (arcuate nucleus) characteristic of the MSG syndrome, in confirmation of our previous studies^{20,21}. Animals were decapitated, brains rapidly removed and mediobasal hypothalamus, medial preoptic area, amygdala and sections of cortex removed as previously described²². Five sections of a given area were homogenised in 2 ml 0.1 M HCl, a total of four pools of a given area being obtained from 20 animals. Anterior and neurointermediate pituitary lobes were separately removed, individually placed in 100 μ l HCl and frozen for subsequent assay. At that time 0.9 ml 0.1 M HCl was added to anterior and 0.4 ml to neurointermediate lobe and these were homogenised in a siliconised tube with a Teflon pestle. Trunk blood was obtained at the time of decapitation for immunoassay of plasma ACTH. Determination of immunoreactive ACTH¹ and endorphin²³ content of brain areas and of pituitary lobes was carried out as previously described; an aliquot of the homogenate was removed for measurement of protein concentration²⁴. Immunoassay of plasma ACTH was carried out by methods used in our laboratory²⁵. The ACTH antibody used exhibits approximately 8-30% cross-reactivity with the 31,000 molecular weight (31K) molecule, reacts on an almost equimolar basis with ACTH₁₋₂₄ and ACTH₁₋₃₉, and exhibits no cross-reactivity with α -MSH, β -MSH, ACTH₁₋₁₀, ACTH₂₅₋₃₉, β -LPH or α -, β -, or γ -endorphin. The β -endorphin antibody reacts with β -LPH, 31K ACTH and β -endorphin on a near-equimolar basis, but does not cross-react with γ -LPH, β -MSH or the enkephalins.

Glutamate-treated animals exhibited a highly significant reduction in immunoreactive concentrations of ACTH-like and β -endorphin-like material in the mediobasal hypothalamus, and also in the medial preoptic nucleus and amygdala. There were no significant changes in whole pituitary, anterior lobe or plasma ACTH content (Table 1). No significant difference in any of these parameters was noted among animals in different oestrous cycle stages.

These findings demonstrate: (1) that destruction of cell bodies of the arcuate nucleus is associated with a four- to fivefold decrease in detectable ACTH-like and endorphin-like immunoreactive material within the hypothalamus, and (2) that this occurs in the absence of any changes in whole pituitary, anterior lobe or plasma ACTH concentrations. These findings indicate that the arcuate nucleus is a major source of the cell bodies giving rise to fibre tracts containing ACTH and endorphin detected in hypothalamic and other sites. The lesser decreases in amygdalar concentrations might indicate that at least some of the immunoreactive material present in this area could arise locally, as suggested by Pacold *et al.*⁵, or that

Table 1 Effect of monosodium glutamate on CNS content of ACTH and β -endorphin

Region	(n)	ACTH (fmol per 100 μ g protein)			β -Endorphin (fmol per 100 μ g protein)		
		Control	MSG	P	Control	MSG	P
Medial basal							
Hypothalamus	4*	52.7 $\pm$ 5.7	9.6 $\pm$ 2.8	<0.001	99.6 $\pm$ 6.6	22.7 $\pm$ 9.8	<0.001
Medial preoptic area	4*	32.7 $\pm$ 6.5	7.9 $\pm$ 3.2	<0.05	117.0 $\pm$ 32.6	35.6 $\pm$ 11.2	<0.05
Amygdala	4*	30.2 $\pm$ 4.8	14.1 $\pm$ 5.4	<0.1	25.1 $\pm$ 2.4	12.4 $\pm$ 1.7	<0.05
Cortex	4*	0.7 $\pm$ 0.2	1.1 $\pm$ 0.1	NS	<0.7	<0.7	
Pituitary (pmol)	20	168.0 $\pm$ 7.3	159.0 $\pm$ 11.5	NS			
Anterior	20	125.5 $\pm$ 7.7	133.1 $\pm$ 11.7	NS			
pituitary (pmol)							
Plasma (fmol ml ⁻¹)	20	36.0 $\pm$ 5.5	30.9 $\pm$ 6.4	NS			

*Four pools, five animals each (mean $\pm$ s.e.m.).
NS, not significant.

surviving cell bodies (possibly in the mediobasal hypothalamic area adjacent to the arcuate nucleus) preferentially innervate the amygdala. Watson, Richard and Barchas¹⁰ have noted that when the β -LPH-positive cells (arcuate nucleus) were destroyed by hypothalamic lesions on one side of rat brain, there was also a reduction of staining for ACTH and β -LPH fibres.

Additional studies in enucleated animals, animals with stereotactically produced arcuate nuclear lesions, and deafferentation should show whether other areas affected by MSG administration are involved in the decrease in brain ACTH concentration seen in such treated animals. It is also unlikely that any of the endocrine effects associated with MSG administration²¹ (decreased growth, hypothyroidism and hypogonadism) are responsible, because, as noted, hypophysectomy is not associated with any change in brain ACTH concentration.

The data also demonstrate that brain concentrations of a pituitary-like peptide can vary independently of pituitary or plasma concentrations of that peptide. Similarly, marked alterations in pituitary and plasma ACTH concentrations following short or long term adrenalectomy are not associated with changes in median eminence or mediobasal hypothalamic ACTH concentrations²⁶. This, in addition to the data derived from hypophysectomised animals, suggests a non-pituitary source of these peptides, in keeping with our recent demonstration of synthesis of immunoreactive 31K ACTH/endorphin-like material²⁷ on incubation of dispersed bovine arcuate nucleus area with labelled precursor amino acid. (If ACTH/endorphin-like activity detected in arcuate nucleus perikarya represented a specific uptake and concentrating system of pituitary-derived material, with subsequent axonal transport and processing, this might explain the present findings, but not those of the labelled amino acid incubation studies.)

The MSG-lesioned animal might serve as a model to explore some of the behavioural effects of ACTH-related peptides, although the multiplicity of effects²¹ on other endocrine systems and neurotransmitters would limit the interpretations and specificity of such observations.

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Nicotinamide is a brain constituent with benzodiazepine-like actions

BENZODIAZEPINES are a group of drugs with wide therapeutic application as anxiolytics, hypnotics, anticonvulsants and muscle relaxants. To exert their therapeutic and pharmacological effects, they apparently interact with a brain-specific benzodiazepine receptor characterised by a high-affinity binding site for this group of compounds^{1,2}. In analogy to the identification of morphine receptors in the brain and the subsequent isolation of opioid peptides, the discovery of the benzodiazepine receptor prompted the search for an endogenous brain constituent, which physiologically may display benzodiazepine-like actions³. We describe here the isolation and benzodiazepine-like actions of nicotinamide, a compound which might exert these actions in the brain physiologically.

From acetone or HClO₄ extracts of bovine or rat brain, three compounds were isolated by conventional column chromatography and identified by mass spectrometry, which showed some, although rather low, potency in inhibiting specific binding of ³H-diazepam to the benzodiazepine high-affinity binding site, tested *in vitro*¹: nicotinamide (IC₅₀ = 3.9 mM), hypoxanthine

($IC_{50} = 0.7 \text{ mM}$) and inosine ($IC_{50} = 0.9 \text{ mM}$). Of these, only nicotinamide showed the main neuropharmacological central effects characteristic of a benzodiazepine, described below, although, because only 0.3% enters the brain after injection into the internal carotid artery⁴, rather high doses of the compound are needed to elicit central effects after systemic application. However, nicotinamide showed the following effects on cat spinal cord activities, recorded as described previously^{5,6}, after local application by pressure ejection from a cannula ($0.5 \mu\text{l}$, $1 \times 10^{-4} \text{ M}$) placed close to the stimulating electrode in the dorsal horn. (1) Dorsal root reflexes (DRRs) induced by dorsal root stimulation were increased by 30–50%. (2) The excitability of primary afferents, as measured by antidromic potentials (APs) in dorsal rootlets, was unaffected, indicating a lack of direct interaction of nicotinamide with γ -aminobutyric acid (GABA) receptors. (3) Unconditioned monosynaptic ventral root reflexes (VRRs) were not affected. In contrast, presynaptic inhibition of monosynaptic VRRs, induced by conditioning and testing stimuli given to appropriate antagonistic muscle nerve afferents in the hind limb, was increased by 15–20% (Fig. 1). The GABA receptor blocker, (+)bicuculline (0.5 mg per kg , intravenously (i.v.)), inhibited these effects of nicotinamide.

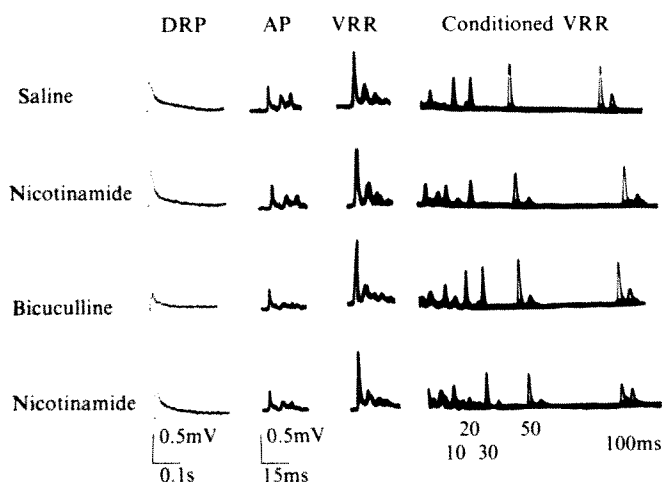


Fig. 1 Enhancement of presynaptic inhibition in cat lumbo-sacral spinal cord by nicotinamide and its reversal by (+)bicuculline. Spinal cord activities of unanaesthetised spinal cats were recorded as described previously^{5,6}. Records are oscilloscope sweeps from one experiment, representative for all five cats tested, showing in four vertical columns from left to right: (1) Averaged dorsal root potentials (DRPs) including the superimposed dorsal root reflexes (eight consecutive responses) evoked in a dorsal rootlet (DR) L_7 by single supramaximal stimuli applied to a DR S_1 ; (2) antidromic potentials (APs) induced in a DR L_7 by constant submaximal volleys given by a semimicroelectrode to the dorsal horn S_1 ; (3) mono- and polysynaptic ventral root reflexes (VRRs) induced in a ventral rootlet S_1 by supramaximal stimuli applied to the nerve afferents of the gastrocnemius muscle; (4) same response as in (3) but now elicited in intervals of approximately 10, 20, 30, 50 and 100 ms following conditioning shocks to the nerve of the biceps femoris muscle. Saline and drugs were administered in the following sequence. Saline: recordings taken 3 min after pressure ejection of saline ($0.5 \mu\text{l}$) through a cannula (outer diameter $100 \mu\text{m}$) in close proximity to the stimulating electrode in the dorsal horn S_1 . Nicotinamide: recordings taken 3 min after the local application of nicotinamide ($0.5 \mu\text{l}$, $1 \times 10^{-4} \text{ M}$); dorsal root reflexes and presynaptic inhibition of conditioned monosynaptic VRRs were augmented. Bicuculline: (+)bicuculline (0.5 mg per kg , i.v.) injected when maximal effects of nicotinamide had been reached; reversed all effects of nicotinamide. Nicotinamide: the action of (+)bicuculline was overcome by the subsequent local injection of nicotinamide ($0.5 \mu\text{l}$, $1 \times 10^{-4} \text{ M}$). Interestingly, in this experiment the presynaptic inhibition by nicotinamide was more pronounced at the 100-ms interval than at shorter intervals, an effect observed only in two out of five cats.

However, the antagonistic action of (+)bicuculline was overcome by the subsequent administration of nicotinamide either locally ($1 \times 10^{-3} \text{ M}$, $0.5 \mu\text{l}$) (Fig. 1) or i.v. (300 mg per kg).

Electrophysiological effects, very similar in size and latency to those observed after local application of nicotinamide, were induced by local administration of the benzodiazepine⁷ Ro 11-7800 ($0.5 \mu\text{l}$, $1 \times 10^{-4} \text{ M}$) or an i.v. injection of diazepam (0.1 mg per kg). The action of both drugs was also reversed by (+)bicuculline. Nicotinic acid ($1 \times 10^{-3} \text{ M}$, $0.5 \mu\text{l}$ applied locally) had no effect on the cat spinal cord activities tested, indicating that the effect of nicotinamide was not due to a possible conversion to nicotinic acid. Inosine and hypoxanthine applied in the same way and at similar concentrations to nicotinamide were without effect on spinal cord activity.

In a conflict test, nicotinamide restored punishment-suppressed behaviour of rats, an action characteristic of clinically effective anti-anxiety agents. In a 1-h test session, in which level pressing for a food pellet reward was combined with an electric foot shock, pretreatment with nicotinamide increased moderately (500 mg per kg , intraperitoneally (i.p.)) and strongly ($1,000 \text{ mg per kg}$, i.p.) punished responding in 5 out of 10 animals.

The anticonvulsant action of nicotinamide was established in an assay highly sensitive for the anticonvulsant action of benzodiazepines. 3-Mercaptopropionic acid (MPA), an inhibitor of the GABA synthesising enzyme glutamic acid decarboxylase, was administered in a dose (50 mg per kg i.p.) precipitating seizures in 100% of the mice (20 animals) within 2–3 min. Nicotinamide (500 mg per kg , i.p.), injected 30 min before MPA, protected 25% of the animals from seizures. Hypoxanthine (500 and $1,000 \text{ mg per kg}$) was without protective effect and inosine was protective (25%) only at $1,000 \text{ mg per kg}$. Nicotinamide has also been shown to protect against audiogenic^{8,9} and isoniazid-induced¹⁰ seizures.

An anti-aggressive action of nicotinamide was demonstrated by the inhibition of aggressive rage reactions in unrestrained cats elicited by hypothalamic stimulation through chronically implanted electrodes¹¹. The current threshold needed to evoke a hissing response and an attack response was elevated by 15% ($P < 0.01$) after i.p. administration of nicotinamide (500 mg per kg , 4 cats) or diazepam (0.5 mg per kg , 4 cats) but not after inosine (500 mg per kg , 4 cats).

The muscle relaxant action of nicotinamide (300 mg per kg , i.p.) was observed in cats and rats. The sedative effect of the compound was apparent in cats (500 mg per kg , i.p.), confirming previous findings in rats^{12,13}, mice and gerbils^{13,14}.

Most remarkably, nicotinamide shows hypnotic action, previously analysed in mice and in man (250 mg per kg subcutaneously and 3 g d^{-1} , orally respectively). It manifests itself as a prolongation of total sleep time and an increase of rapid eye movement sleep^{15–17}, a response also seen after low doses of benzodiazepines^{18,19}. Finally, the finding that nicotinamide treatment increases the 5-hydroxytryptamine level in rat brain¹² is in line with the suggestion that the anxiety-reducing action of benzodiazepines is mediated by the reduction of 5-hydroxytryptamine turnover^{20,21}.

Thus, nicotinamide has properties in common with benzodiazepines (and barbiturates²²) in its action on spinal cord activity, its anticonflict, anticonvulsant, anti-aggressive, muscle relaxant and hypnotic action. It also seems to influence GABAergic mechanisms without binding to the GABA receptor itself, as shown by the electrophysiological findings and the lack of inhibition by nicotinamide of ^3H -GABA receptor binding tested according to Enna *et al.*²³.

If nicotinamide physiologically exerts benzodiazepine-like effects in the brain, its pharmacological potency should be high in view of its low concentration in the brain ($0.1 \mu\text{mol per g}$ rat whole brain)²⁴. This is supported by the electrophysiological experiments using *in situ* drug application, in which the potency of nicotinamide was found to be equivalent to that of a highly potent benzodiazepine. The low apparent affinity of nicotinamide to the benzodiazepine binding site *in vitro* may either

be due to shortcomings of the binding assay in reflecting the *in vivo* affinity of the endogenous ligand to that site, or the benzodiazepine receptor may be part of a receptor complex²⁵, which may contain more than one binding site through which benzodiazepine-like actions can be elicited in the brain. The hypothesis that nicotinamide may exert benzodiazepine-like actions in the brain physiologically, may shed new light on the mental disorders accompanying nicotinamide deficiency states.

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Immunocytochemical localisation of L-glutamic acid decarboxylase in the goldfish retina

A DIRECT way to examine whether certain neurones may use γ -aminobutyric acid (GABA) as a neurotransmitter is to determine by immunocytochemistry if L-glutamic acid decarboxylase (GAD), the enzyme responsible for GABA synthesis, is localised in these cells. We have been studying GABA-ergic pathways in the goldfish retina by autoradiographic and biochemical methods^{1–3}. It would therefore be of interest to localise the GAD-containing cells in this system immunocytochemically. However, although GAD has been purified from mouse brain⁴ and antibodies against the enzyme have been obtained, these antibodies do not cross-react with the GAD found in neural tissues of teleosts⁵. Thus, before immunocytochemical studies of GAD-containing neurones in the goldfish retina were possible, we had to purify GAD from teleost brain and obtain a specific antibody against this enzyme. Such an antibody has now been obtained⁶. We show here that in the goldfish retina, GAD is localised in specific interneurons: type H1 horizontal cells and probably type Ab pyriform amacrine cells. These cell types

have been shown to be both pre- and postsynaptic to red-sensitive cones and red-sensitive centre-depolarising bipolar cells, respectively^{3,7–9}.

Because we were able to obtain the channel catfish (*Ictalurus punctatus*) in large numbers, catfish brains were used for the purification of GAD⁶. The enzyme was extracted by homogenising 600 brains (about 300 g) in 3 l of water containing 0.1 mM pyridoxal phosphate, 1 mM EDTA, 1 mM 2-aminoethylisothionium bromide hydrobromide and 1 mM reduced glutathione, pH 7.2. About 70% of the GAD activity as measured by formation of both ¹⁴C-CO₂ and ¹⁴C-GABA (refs 4, 10) which was recovered in the supernatant after centrifugation of the homogenate at 100,000g for 45 min. The enzyme was purified to electrophoretic homogeneity by a combination of ammonium sulphate fractionation, gel filtration, calcium phosphate gel and preparative gel electrophoresis. Doses of 20 μ g of this enzyme were injected biweekly into two rabbits for 2 months. The ear veins were bled and serum was prepared by centrifugation at 2,000g for 20 min and used for immunocytochemical studies. Anti-catfish GAD immunoglobulins (IgG) from rabbit were prepared by passing the serum through a DEAE Sephacel ion-exchange column and eluting with 50 mM Tris buffer, pH 8.0. The IgG were used for immunodiffusion studies⁶.

GAD-containing neurones in the goldfish retina were localised immunocytochemically using the method of Dubois-Dalcq *et al.*^{11,12}. Eyes from light-adapted goldfish (*Carassius auratus*) were enucleated and isolated eyecups were fixed with 4% paraformaldehyde in isotonic phosphate-buffered saline (PBS; 100 mM NaCl and 25 mM sodium phosphate, pH 7.2) for 1 h at room temperature and overnight at 4°C. Each eyecup was then washed with PBS, cut radially into strips several mm wide, embedded in agar and sectioned at 50 μ m with a vibratome. Sections were incubated at room temperature for 2 h with a 1:250 dilution of rabbit-anti-catfish GAD serum (1:50 to 1:1,000 dilutions of the antiserum give similar staining patterns) or non-immunised serum (control), then washed with PBS and

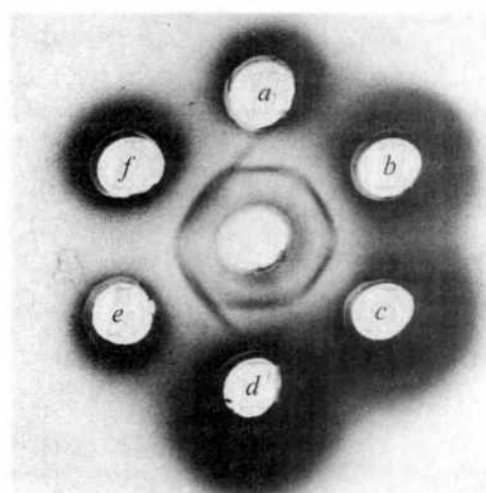


Fig. 1 Ouchterlony double diffusion test with crude GAD from goldfish brain, goldfish retina and catfish brain. Each well contained 15 μ l of solution. Anti-catfish GAD IgG (70 μ g) was in the central well. Wells a to f were homogenates from goldfish brain (a, b), goldfish retina (c, d) and catfish brain (e, f), respectively. The diffusion plate was kept at 4°C for 48 h, washed with PBS at room temperature for 48 h, stained with 1% Coomassie blue for 20 min and destained with 7% acetic acid for 2 h. The spurs between f and a, and d and e suggest that catfish and goldfish GAD are not identical enzymes. However, the formation of a single, sharp precipitin band in b and c indicates that the antibody against catfish GAD cross-reacts strongly and specifically with goldfish GAD.

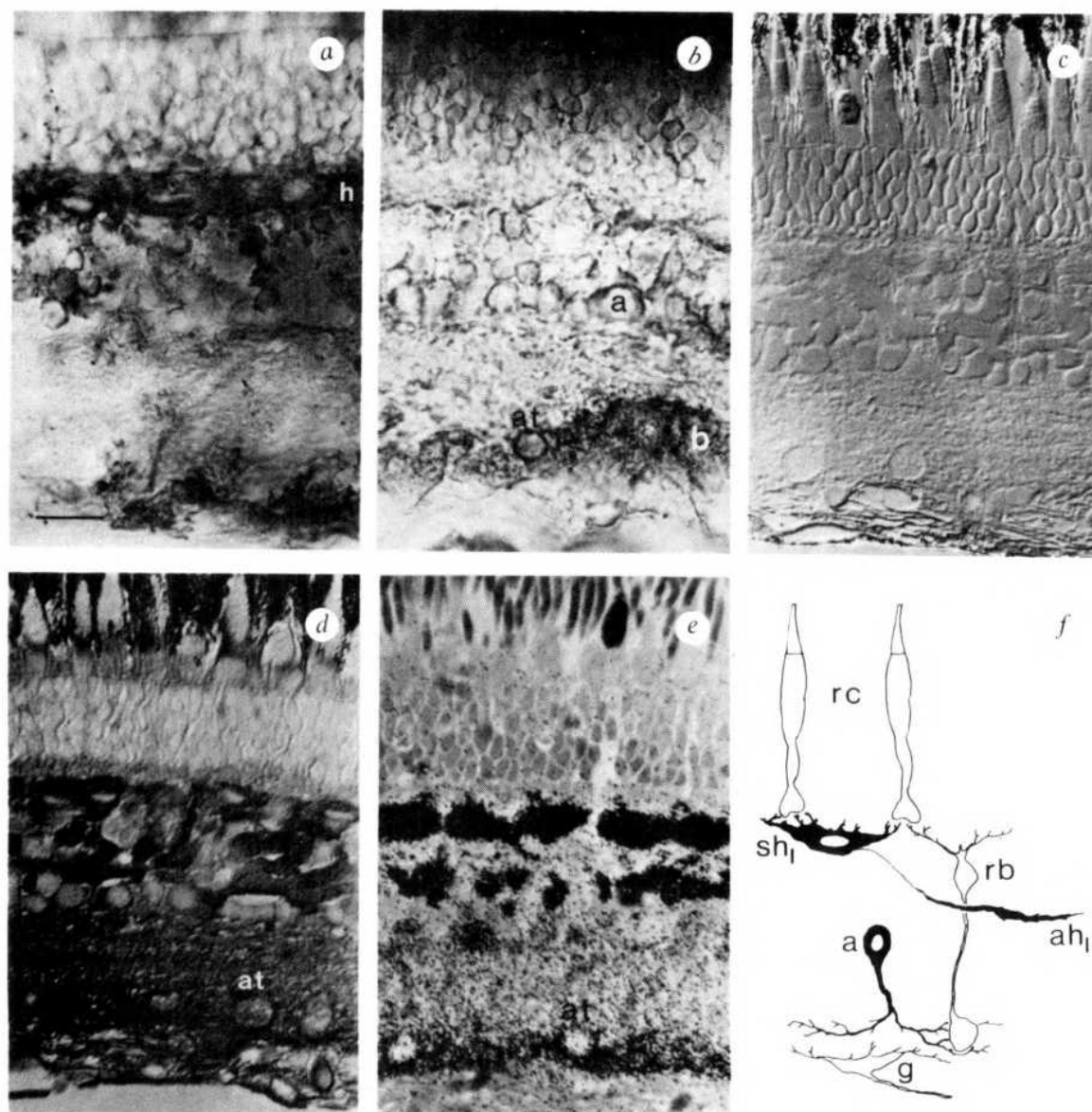


Fig. 2 *a, b*, Immunocytochemical localisation of GAD in 50-μm sections of goldfish retina. Focusing on the outer retina (*a*), HRP-staining is seen over the somata of some cone horizontal cells (*h*). Note that the nuclei of these cells are not stained and therefore probably do not contain GAD. Scale bar 20 μm. Focusing on the inner retina (*b*), HRP-staining is seen over some amacrine cells (*a*, the nucleus is not stained) and in sublamina b of the inner plexiform layer, indicating that GABAergic terminals are probably concentrated in this sublamina (*b*). Note dense HRP-staining around the axon terminal (*at*) of a type b bipolar cell^{3,8,9}. *c*, Control experiment, where a section treated with non-immunised serum shows very little HRP staining. The dark structures between photoreceptors are melanin granules in pigment epithelial cells. *d*, HRP-staining of GAD in a 5-μm section shows that some somata and axons of cone horizontal cells, probably H1 cells are stained. Sublamina b of the inner plexiform layer is also stained, especially around the axon terminal of some type b bipolar cells. *e*, Autoradiographic section of a goldfish retina incubated with 10^{-6} M ^{3} H-GABA. As shown earlier^{1,3}, high-affinity GABA uptake is localised in the somata and axons of H1 horizontal cells, and in some Ab pyriform amacrine cells which make synaptic connections in sublamina b of the inner plexiform layer. *f*, Schematic diagram of probable GABA-ergic neurons (H1 horizontal cells and some Ab amacrine cells) and their functional connections in the goldfish retina as revealed by our immunocytochemical and autoradiographic studies. rc: red-sensitive cones; sh₁ and ah₁: soma and axon of a H1 cell; rb: red-sensitive centre-depolarising bipolar cell (type b); a: pyriform amacrine cell which makes contact with rb; g: retinal ganglion cell.

treated for 2 h with a conjugate of horseradish peroxidase (HRP) and protein A¹¹. After washing, the sections were incubated with diaminobenzidine and hydrogen peroxide¹³. The sections were then fixed in 3% glutaraldehyde in 100 mM phosphate buffer (pH 7.4) for 1 h, dehydrated in ethanol, rinsed with xylene, and immersed in cedarwood oil. The HRP reaction products in these sections were visualised by Nomarski differential interference microscopy. The sections were then embedded in a thin layer of epoxy and sectioned tangentially at 2–5 μm for light microscopy.

Several lines of evidence indicate that the GAD purified from catfish brain and used as the antigen is homogeneous, and that

the antibody against catfish GAD is monospecific⁶. First, in several different polyacrylamide gel electrophoresis systems, the purified enzyme migrated as a single band which contained all the GAD activity. Second, immunoprecipitation studies showed that the antibody inhibited 67% of the GAD activity from catfish brain. Third, the Ouchterlony double immunodiffusion test showed only one sharp precipitin band between the antibody and catfish brain extract (Fig. 1). Finally, this precipitin band contained 86% of the GAD activity on the immunodiffusion gel⁶.

The formation of only one sharp precipitin band between the anti-GAD serum and goldfish brain and retina shows that

goldfish GAD cross-reacts strongly with this antibody. Using the antibody, our immunocytochemical study shows that GAD is localised in some horizontal cells, a few amacrine cells and sublamina b of the inner plexiform layer (Fig. 2). In the goldfish retina, different types of horizontal cells can be classified physiologically and morphologically according to their functional contacts with photoreceptors⁷. Thus, rod horizontal cells receive input exclusively from rods, and cone horizontal cells can be subdivided into H1, H2 and H3 cells, which receive input predominantly from red, green and blue-sensitive cones, respectively. The positions of the HRP-labelled horizontal cells in the inner nuclear layer indicate that these are the perikarya of H1 horizontal cells. To enhance HRP staining in cell bodies and axons of GAD-containing cells, 50- μ m sections were incubated with 0.1% Triton X-100 and the anti-GAD serum (a 1:4,000 dilution was used for most Triton X-100 treated tissue) overnight before the normal immunocytochemical procedure¹⁴. With this treatment, both the perikarya and axons of some horizontal cells are heavily stained (Fig. 2). Again, the positions of the HRP-stained cell bodies indicate that these are H1 horizontal cells. It follows that the HRP-stained axons are most probably axons of H1 cells. H2, H3 and rod horizontal cells, photoreceptors, bipolar cells and Müller (glial) cells do not show detectable HRP staining for this enzyme.

The identities of the HRP-stained amacrine cells cannot be determined without serial reconstruction and electron microscopic studies. The presence of GAD in sublamina b of the inner plexiform layer, especially around the axon terminals of some centre-depolarising bipolar cells (type b)^{8,9}, suggests that GAD-containing amacrine cells make synaptic contacts with these bipolar cells. This result and those of autoradiographic studies of ³H-GABA uptake in the inner plexiform layer, indicate that at least some GABA-ergic amacrine cells are Ab pyriform cells which make contact with red-sensitive centre-depolarising bipolar cells in the goldfish retina^{3,8,9}.

Comparison between the immunocytochemical studies of GAD-containing neurones and autoradiographic studies of GABA uptake reveals a marked similarity in the labelling patterns (Figs. 2, 3). Our findings therefore demonstrate that in goldfish retina, the neurones which possess a high-affinity system for GABA uptake also contain significant levels of GAD. In addition, this study confirms earlier biochemical results showing that at least some isolated axons of cone horizontal cells in the goldfish retina synthesise GABA and contain high specific activities of GAD². These findings, and

those of autoradiographic^{1,2} and electrophysiological¹⁵ studies of the GABA system in the teleost retina, point to GABA as being the transmitter used by H1 horizontal cells of the goldfish retina. The absence of GABA uptake and GAD in all other horizontal cell types (H2, H3 and rod) indicates that they are not GABA-ergic. This raises the possibility that horizontal cells which are in contact with different types of photoreceptors may use different neurotransmitters.

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Fig. 3 A 3- μ m section of a goldfish retina treated with 0.1% Triton X-100 and anti-GAD serum (1:4,000 dilution) overnight and processed for immunocytochemistry. Because this treatment enhanced the penetration of the antiserum and conjugate into the tissue, HRP staining is much more intense than that shown in Fig. 2. In particular, both the somata and axons of the H1 horizontal cells are clearly stained. Scale bar 20 μ m.

Identification of β -adrenergic-sensitive adenylate cyclase in intracranial blood vessels

THE regulation of cerebral blood flow and vascular permeability is important in such clinical conditions as stroke, arterial spasm, vascular headache (for example, migraine) and metabolic effects of seizures. Unfortunately, relatively little is known about the nervous control of cerebral circulation, particularly with regard to the nature of the hormone and neurotransmitter receptors present in cerebral vessels. An influence of the adrenergic nervous system has been suggested by histological studies showing that the blood vessels and branches of the circle of Willis receive innervation from the sympathetic chain¹, and that the smaller, penetrating cerebral vessels (called microvessels) are surrounded by noradrenaline-containing fibres which seem to arise from brainstem noradrenergic nuclei^{2,3}. Although attempts to demonstrate β -adrenergic receptors in cerebral vascular tissue by cytochemical methods have been unsuccessful⁴, indirect evidence for the presence of such receptors is

suggested by reports that adrenergic stimulation can alter cerebral blood flow and vascular permeability^{3,5}, and that catecholamine agonists (such as isoprenaline and adrenaline) can dilate whole-artery preparations of cerebral vessels⁶. We now report the presence of a β -adrenergic-stimulated adenylate cyclase in both superficial and deep cerebral blood vessels. In addition to supplying direct biochemical evidence for the existence of β -adrenergic receptors in the cerebral vasculature, identification of this enzyme should facilitate the evaluation of the actions of pharmacological agents on cerebral blood flow and vascular permeability.

Previous studies have demonstrated that the receptors for several neurotransmitters and circulating hormones are intimately associated with the membrane-bound enzyme, adenylate cyclase, and that activation of this enzyme, resulting in the intracellular formation of cyclic AMP, mediates the effects of the hormone or neurotransmitter⁷. To demonstrate the possible existence of a β -adrenergic-stimulated adenylate cyclase in cerebral vascular tissue, the effects of various catecholamines on the enzymatic synthesis of cyclic AMP from ATP were studied. Broken cell preparations of three types of mammalian blood vessels were used: superficial arteries and proximal pial branches of the circle of Willis; penetrating (intraparenchymal) microvessels; and extracranial carotid arteries. The microvessels were prepared by a modification of published techniques^{8,9}, involving mechanical fragmentation, discontinuous sucrose gradient centrifugation, and sieve filtration (see Fig. 1 legend). Feline blood vessels were used for most experiments, as previous studies have demonstrated a rich

noradrenergic innervation in the cerebral vasculature of this species^{10,11}, and physiological studies have documented β -adrenergic-mediated vasomotor responses of cat pial arteries⁶. In preliminary experiments, dog and calf pial vessels also demonstrated β -adrenergic-stimulated adenylate cyclase activity, but the degree of stimulation was less than that in the cat.

Figure 1a shows the effects of (-)isoprenaline (Iso), a β -adrenergic agonist, on adenylate cyclase activity in an homogenate of cat pial vessels. In the experiment shown, maximal stimulation by Iso was 160% of control activity and, in other experiments, varied between 150% and 180% of control. Concentrations of Iso as low as 10^{-7} M caused significant stimulation, and half-maximal activity occurred at a concentration (K_d) which varied between 4 and 11×10^{-7} M. (-)Noradrenaline (NA), a mixed β - and α -adrenergic agonist, was less effective than Iso in stimulating enzyme activity (150% of control in pial vessels), whereas (-)phenylephrine, an α -adrenergic agonist, caused only minimal stimulation at comparable concentrations.

Activation of adenylate cyclase activity by Iso and NA was more pronounced in preparations of cat intraparenchymal microvessels (Fig. 1b), varying between 170 and 225% of control activity for Iso and 165 and 220% for NA, at maximum. In the experiment shown, the K_d for Iso was 3×10^{-7} M. In contrast, none of the catecholamines had any effect on the adenylate cyclase activity present in homogenates of cat common and proximal external carotid artery (Fig. 2).

Results similar to those above were obtained with both pial vessels and microvessels from cats which had been perfused systemically to remove intravascular blood. The finding that whole blood, itself, showed little Iso-stimulated enzyme activity, made it unlikely that blood elements contributed to our results. In contrast to the effects observed in vessels, stimulation of whole brain adenylate cyclase by a maximally effective concentration (100 μ M) of Iso was only 128% of control activity (Fig. 2). On the other hand, 100 μ M dopamine (DA) activated the enzyme activity of brain (150% of control) more than that of either pial vessels (130%) or microvessels (124%). Also, further evidence differentiating brain from vessel adenylate cyclase was obtained from the observed effects of GTP, which (at 100 μ M) activated basal enzyme activity of cat brain homogenates by 600% but those of pial vessels and microvessels by only 200%.

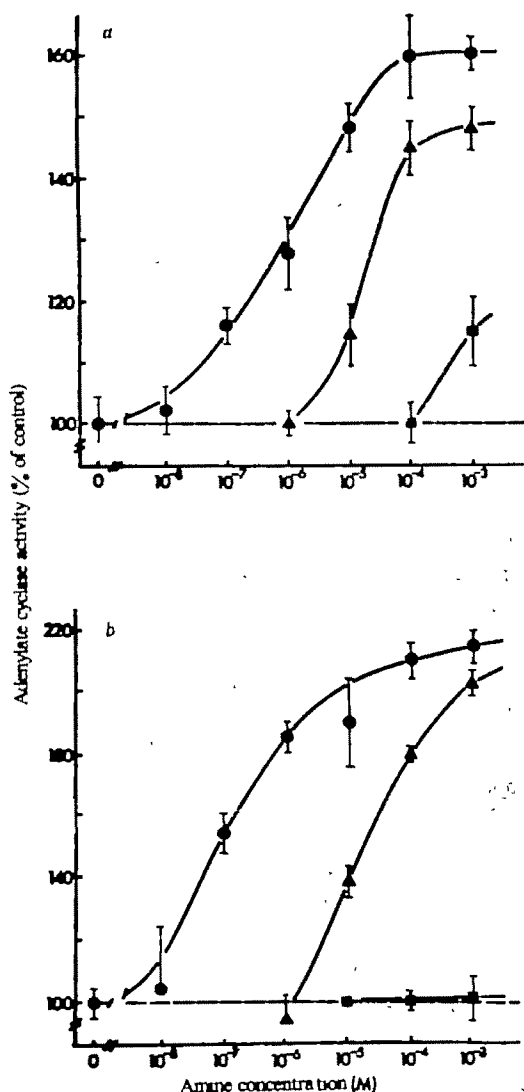


Fig. 1 Effects of (-)isoprenaline (●), (-)noradrenaline (▲) and (-)phenylephrine (■) on adenylate cyclase activity in broken cell preparations of cat cerebral vasculature. *a*, Pial arteries, control activity = 13.8 ± 1.6 pmol per mg protein per min. *b*, Intraparenchymal microvessels, control = 11.5 ± 0.7 pmol per mg protein per min. Values shown are the means ($\pm$ deviation) of replicate samples, each assayed for cyclic AMP in triplicate. For these studies, male cats were anaesthetized with ether and perfused transcardially with 500 ml of phosphate-buffered saline (PBS). Superficial arteries (distal vertebral, basilar, intracranial carotid, proximal portions of anterior, middle and posterior cerebral) were dissected and cleaned of adhering pia, rinsed in PBS, and homogenized (20 mg ml^{-1}) in cold 6 mM Tris-maleate buffer, pH 7.4. To isolate microvessels, the brain was stripped of remaining pia, coarsely minced, and gently homogenized by hand (six vertical strokes, no rotation) in a Teflon-glass homogeniser (0.2 mm clearance) in 60 ml cold 25 mM Tris-maleate buffer, pH 7.4, containing 140 mM NaCl and 0.5% bovine serum albumin (buffer A). After centrifugation at 1,500 g for 15 min, the precipitate was resuspended in 2 vols 0.25 M sucrose and centrifuged (25,000 r.p.m. for 50 min) on a discontinuous 1.0/1.8/2.25 M sucrose gradient. The resulting 1.0/1.8 M and 1.8/2.25 M fractions were diluted in buffer A, centrifuged at 4,000 g, the pellet resuspended in 0.25 M sucrose, and centrifuged (25,000 r.p.m. for 40 min) on a 1.0/1.35/1.5/1.7/2.25 M sucrose gradient. The microvessel fraction (combined 1.5/1.7 M and 1.7/2.25 M interfaces) was washed and collected against a 100- μ m Nitex screen and homogenized (15 mg ml^{-1}) in 6 mM Tris-maleate, pH 7.4. Vessel purity was monitored through the use of light microscopy and enzyme markers¹⁵. Cyclic AMP formation was measured in tubes containing (in 0.3 ml) 80 mM Tris-maleate, pH 7.4, 10 mM theophylline, 6 mM MgSO_4 , 0.1 mM GTP, 1.5 mM ATP, and tissue homogenate (1.0–1.5 mg wet weight), plus test substances as indicated. The reaction (4 min at 30 °C) was initiated by addition of ATP, stopped by heating to 90 °C for 2 min, and then centrifuged to remove insoluble matter. Cyclic AMP was measured as in ref. 16. In these conditions, enzyme activity was linear with time and enzyme concentration, and phosphodiesterase activity was nearly completely inhibited. All experiments were replicated 2–5 times. The results shown in Figs 1 and 2 and Table 1 represent typical experiments.

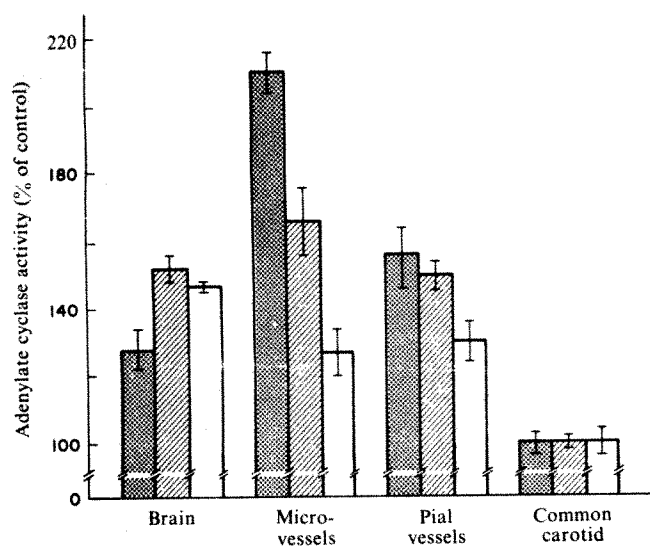


Fig. 2 Comparative effects of 100 μ M (-)-isoprenaline ($\square$), (-)-noradrenaline ($\blacksquare$) and dopamine ($\square$) on adenylate cyclase activity in homogenates of whole cat brain (control activity = 37 ± 1 pmol per mg protein per min) and three preparations of cat vasculature: intraparenchymal microvessels (control = 13.8 ± 0.6 pmol per mg protein per min); superficial pial vessels (control = 12.3 ± 0.9 pmol per mg protein per min); and common and external carotid artery (control = 1.3 ± 0.2 pmol per mg protein per min). The values shown here and in Table 1 are the means ($\pm$ deviation) of replicate samples, each assayed for cyclic AMP content in duplicate.

and 120%, respectively. In contrast to effects on basal activity, Iso stimulation, itself, had an absolute requirement for the presence of exogenous GTP. Other experiments indicated that, unlike adenylate cyclases activated by DA, histamine and serotonin, present in other tissues⁷, Iso-stimulated adenylate cyclase activity in cerebral vessel homogenates was unaffected by calcium chelation with EGTA (calcium did affect basal activity).

The preferential activation of a β -adrenergic receptor by Iso in cerebral vessel homogenates was supported by other experiments using receptor antagonists. Table 1 shows that Iso-stimulated adenylate cyclase activity in a microvessel homogenate was almost completely blocked by the β -adrenergic antagonist, propranolol, but was less affected by the α -adrenergic

antagonist, phentolamine, or the potent DA antagonist, fluphenazine. Similar results were seen in homogenates of pial vessels. Table 1 also demonstrates the non-additive effects of Iso, NA and DA on microvessel adenylate cyclase, results supporting the activation of a single class of receptors.

Taken together, these data demonstrate the presence of a β -adrenergic-sensitive adenylate cyclase in cat intraparenchymal and extraparenchymal cerebral blood vessels. Because of the known association of adenylate cyclase with β -adrenergic receptors in other tissues^{12,13}, identification of this enzyme strongly supports the existence of a β -adrenergic receptor in the mammalian cerebral vasculature. It will be of interest to determine the cellular localisation of β -adrenergic-sensitive adenylate cyclase within the blood vessel wall; a presence in endothelial cells might suggest an involvement in vascular permeability, or a media localisation, an involvement in vasomotor responses. In addition, further characterisation of the cerebral vasculature β -adrenergic receptor, itself, should prove helpful in evaluating and comparing the actions of adrenergic agents (such as β_1 and β_2 selective types) in both intraparenchymal and extraparenchymal cerebral vessels.

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Table 1 Effects of various compounds on adenylate cyclase activity

Agent	Cyclic AMP increase (pmol per mg protein per min)
a Antagonists	
Iso (100 μ M)	11.5 $\pm$ 0.9
Iso (100 μ M) + Prop (10 μ M)	4.6 $\pm$ 1.0
Iso (100 μ M) + Phent (10 μ M)	11.1 $\pm$ 0.6
Iso (100 μ M) + Flu (10 μ M)	11.8 $\pm$ 0.4
Iso (100 μ M) + Prop (50 μ M)	1.0 $\pm$ 0.6
Iso (100 μ M) + Phent (50 μ M)	7.3 $\pm$ 0.3
Iso (100 μ M) + Flu (50 μ M)	7.4 $\pm$ 0.4
b Additivity	
Iso (1 mM)	13.1 $\pm$ 0.1
NA (1 mM)	12.0 $\pm$ 0.5
DA (1 mM)	10.4 $\pm$ 0.4
Iso (1 mM) + NA (1 mM)	12.9 $\pm$ 0.4
Iso (1 mM) + DA (1 mM)	12.9 $\pm$ 0.9

a, Effects of the β -adrenergic antagonist, propranolol (Prop), α -adrenergic antagonist, phentolamine (Phent), and dopamine antagonist, fluphenazine (Flu), on activation of adenylate cyclase activity by (-)-isoprenaline (Iso) in an homogenate of cat cerebral microvessels. **b**, Effects of maximally effective concentrations of Iso, (-)-noradrenaline (NA), and dopamine (DA), alone and in combination, on adenylate cyclase activity in another preparation of microvessels. Qualitatively similar results were obtained in additivity experiments using 10-fold lower amine concentrations. The values shown in **a** are the increases due to Iso over enzyme activity in the presence of antagonist alone. In the absence of added agents, control activity was 13.1 ± 1.1 pmol per mg protein per min in **a** and 17.7 ± 1.1 pmol per mg protein per min in **b**.

Distribution of muscarinic acetylcholine receptors in amphibian cardiac muscle

THE skeletal muscle-nerve junction and many interneuronal synapses are highly specialised and morphologically characterised by a direct apposition of pre- and postsynaptic elements separated by a cleft of no more than 50 nm, localised thickenings of the apposed membranes and an aggregation of small vesicles in the presynaptic process. At several such 'focal' synapses, neurotransmitter receptor molecules are restricted to patches of postsynaptic membrane corresponding to synaptic sites¹⁻³. For example, at the motor endplate of skeletal muscle, acetylcholine receptor packing density in the subsynaptic membrane is about 10^3 times higher than in the extrasynaptic membrane several μ m away⁴⁻⁶. Many neuroeffector junctions such as those in autonomically innervated tissues, however, are much less specialised and lack synaptic membrane apposition and thickenings^{7,8}. In the heart for example, the terminal postganglionic autonomic axons consist of long chains of vesicle-packed varicosities and

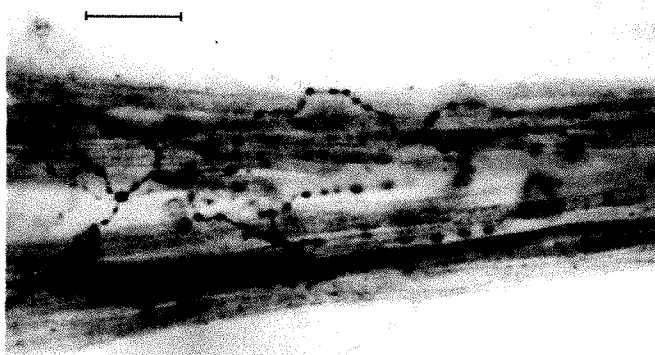


Fig. 1 Two cardiac muscle fibres in a *Xenopus* interatrial septum stained with zinc iodide and osmium. A fresh interatrial septum pinned to a Sylgard-coated cover slip was incubated overnight in ice-cold zinc iodide and osmium. Zinc iodide was made by mixing 2 g Zn dust and 1 g I_2 in 40 ml H_2O . 2 ml of the filtered zinc iodide, 0.5 ml of 2% OsO_4 , and 6 ml of 0.2 M sodium acetate buffer pH 3.9 were mixed to make the zinc iodide and osmium stain (M. Letinsky, personal communication). Scale bar, 20 μm .

form a widely ramifying plexus over the muscle⁹. The varicosities sometimes lie in close apposition to the muscle but may often be several μm away from the nearest target. Even when pre- and postsynaptic membranes are closely apposed, morphological specialisation of the membranes is not normally seen^{7,10-12}. I was interested to determine whether receptor molecules for acetylcholine (ACh) in heart muscle are localised to the relatively infrequent sites of close apposition between nerve and muscle or are more generally distributed. This report demonstrates that muscarinic ACh receptors are uniformly distributed in cardiac muscle fibres in amphibian heart.

Distribution of muscarinic ACh receptors was determined by autoradiography of cardiac muscle labelled with ^3H -quinuclidinyl benzylate (QNB). QNB (Roche) has been shown to bind strongly and with high specificity to muscarinic ACh receptors in mammalian cardiac muscle¹³ and other tissues¹⁴. Similar binding properties of QNB in amphibian heart have recently been found (H.C.H., in preparation). Before autoradiography, conditions for labelling muscarinic ACh receptors with ^3H -QNB were determined by scintillation counting. Pieces of *Xenopus laevis* atrium were incubated with various concentrations of ^3H -QNB for 1.5 h, washed in ice-cold Ringer solution for 10 h and counted. Specific binding, defined as the difference between binding in the presence and absence of 100 nM unlabelled QNB or 1 μM atropine¹³, was saturable with a single apparent K_D of 1.0 nM. Nonspecific binding in the presence of 100 nM unlabelled QNB was nonsaturable. At a saturating concentration of 2.5 nM ^3H -QNB, atrial tissue contains 369 fM specific QNB binding sites per mg Lowry protein and 85 fM mg^{-1} nonspecific binding. Thus, 82% of the total binding is specific in these conditions. Autoradiographic experiments, however, show that most of the nonspecific binding occurs at the ends of muscle fibres which have been damaged or cut. Dissociation of bound ^3H -QNB from receptor was determined using atrial homogenates as described by Fields *et al.*¹³. At 4°C the $t/2$ of dissociation ranged from 20 h to >48 h in five experiments. As tissues processed for autoradiography were washed at 4°C for less than 10 h not more than 20% of the bound ^3H -QNB dissociated during the washing.

Muscarinic ACh receptor distribution was determined by autoradiography of whole mounts of *Xenopus* interatrial septum and sinus venosus. These tissues were chosen because they are very thin sheets, usually not more than 20 μm thick and are thus natural tissue sections¹⁵.

Figure 1 is a micrograph of a portion of interatrial septum stained with zinc iodide and osmium¹⁶ (ZIO) which stains axonal

varicosities black. The diffuse nature of the pattern of innervation is evident. ZIO-stained varicosities measure $1.05 \pm 0.45 \mu m$ in the short dimension by $1.44 \pm 0.48 \mu m$ in the long dimension (mean $\pm$ s.d., $n = 70$). Thus, an average varicosity has a cross-sectional area of $4.8 \mu m^2$. As the density of varicosities on the muscle fibres is $\sim 0.016 \mu m^{-2}$ in the interatrial septum

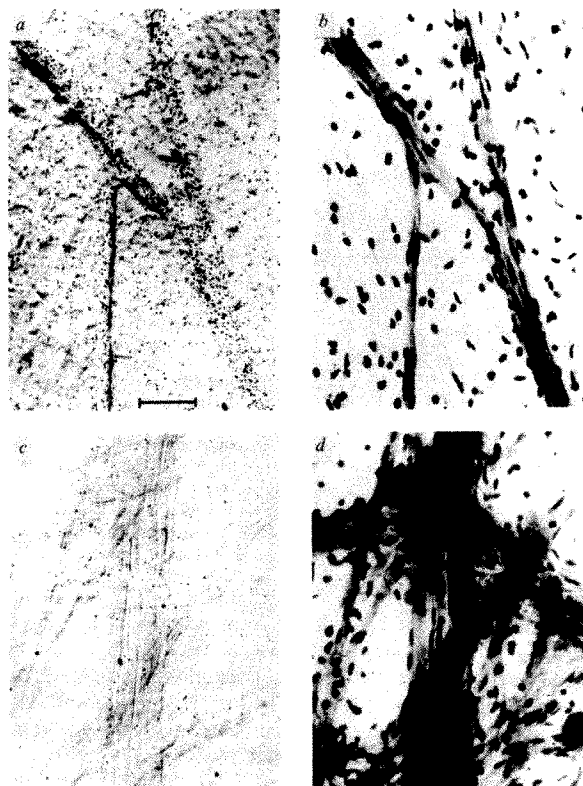


Fig. 2 *a*, Autoradiograph of a fibre in *Xenopus* interatrial septum labelled with ^3H -QNB. The septum was incubated in 2.5 nM ^3H -QNB (NEN, 29.4 Ci $mmol^{-1}$) in frog Ringer solution for 1.5 h, washed with numerous changes of ice-cold Ringer for 2 h, fixed for 30 min in 1% glutaraldehyde buffered with sodium phosphate, pH 7, and washed in Ringer for an additional 7 h. The thinnest portions of the preparation were cut out, placed on a microscope slide with a drop of ice-cold 2% bovine serum albumin in distilled water and rapidly dried in a stream of air at 4°C. A thin layer of carbon was evaporated onto the slide and the slide coated with emulsion by a method similar to that described by Lane *et al.*¹⁷. *b*, Same field as *a* stained with toluidine blue to illustrate cellular composition of the septum. The N-shaped strands are septal myocardial fibres which are embedded in a network of connective tissue cells whose nuclei stain darkly. *c*, Autoradiograph of a preparation incubated in 2.5 nM ^3H -QNB and 100 nM unlabelled QNB to demonstrate nonspecific binding. *d*, Same field as in *c* stained with toluidine blue. Autoradiographs were exposed for 14 d. These fields are representative of seven preparations. Scale bar, 50 μm .

(manuscript in preparation), it is estimated that at most 8% of the muscle surface is synaptic; since some of the varicosities are located several μm from the muscle, focal synaptic area is probably less than 8%.

Figure 2*a* is an autoradiograph of a muscle fibre labelled with ^3H -QNB in the interatrial septum. Silver grains are distributed over the entire surface of the muscle fibre. Most fibres in a given preparation exhibit a similar grain density, although occasional fibres are seen which have few grains. Silver grains are seen at much lower density over non-muscle tissue including connective tissue, endothelial cells and neurones. As the high density of silver grains is localised to the muscle fibres, little or no diffusion

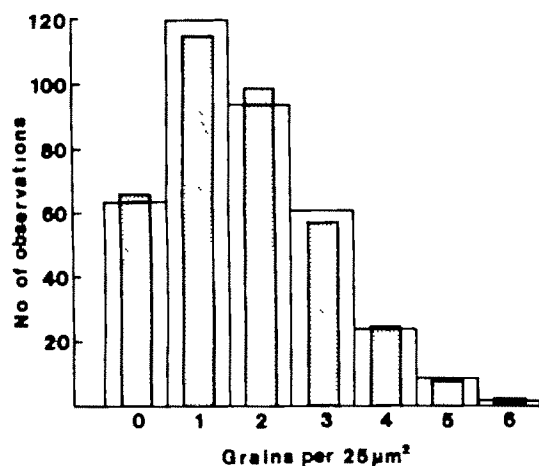


Fig. 3 Frequency distribution of silver grains of ^3H -QNB-labelled fibre. The number of $25\text{-}\mu\text{m}^2$ areas containing different numbers of silver grains are shown as a histogram (open bars). Data from cell shown in Fig. 2a. Average grain density is 1.72 grains per $25\text{-}\mu\text{m}^2$. This value was used for calculating the expected Poisson distribution (stippled bars). The calculated χ^2 value was 1.02 . Similar results were obtained using unit counting areas of $10\text{-}\mu\text{m}^2$ and $100\text{-}\mu\text{m}^2$.

of QNB during autoradiography appears to have occurred. Preparations which are incubated in ^3H -QNB in the presence of 100 nM unlabelled QNB have grain densities which are not different from background (Fig. 2c) except near the ends of cut fibres.

As silver grains are produced by random radioactive decay, a uniform distribution of QNB binding sites on the fibre would show as a random distribution of silver grains in the autoradiograph. To determine whether silver grains were randomly distributed, grains were counted in $25\text{-}\mu\text{m}^2$ areas of the muscle fibre. A frequency-grain count histogram was constructed and compared to the Poisson distribution expected if grain distributions were random. Figure 3 shows that grain distribution closely approximates a Poisson process (χ^2 goodness of fit shows that the observed and expected distributions are not statistically different ($P < 0.02$)).

Muscarinic ACh receptors in the heart function to decrease the force and rate of spontaneous contraction. To be effective, therefore, the action of ACh must extend over several cycles of the beat. Purves¹⁸ has pointed out that the slow kinetics of the muscarinic response is well suited for temporal summation and continuity of ACh action over several cardiac cycles. Further, I propose that the effective modulatory influence of ACh requires spatial continuity of ACh action; that is, because probably every cell in the sinus venosus and atrium can be a pacemaker cell, focal application of ACh to a small region would be ineffective in modulating overall heart rate or force. The diffuse pattern of innervation and uniform distribution of ACh receptors is ideally suited for spatial continuity of ACh action. The arrangement of presynaptic release sites is such that transmitter is in effect 'bath applied' to the muscle and the target responds to transmitter over its entire surface. Also, the slow time course of ACh action may be due in part to diffusion of transmitter across the receptor-populated surface of the muscle.

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Progesterone derivative binds to cardiac ouabain receptor and shows dissociation between sodium pump inhibition and increased contractile force

DIGITALIS and related steroid glycosides exert two well established actions: augmentation of the contractile force of cardiac muscle, the basis for the clinical use of these drugs; and inhibition of Na-K-ATPase, a ubiquitous membrane enzyme concerned with maintenance of transcellular ionic gradients. There is much evidence to support the disputed view that the inotropic effect of the cardiotonic steroids is a direct result of Na-K-ATPase inhibition^{1,2}. In a previous study³ a large number of steroids, drugs and other substances were screened for potential activity in a radioreceptor assay for cardiac glycosides. High affinity saturable binding of ^3H -ouabain to a particulate fraction from dog heart was highly specific and antagonised only by cardioactive glycosides or their aglycones and certain steroids related to hydroxyprogesterone. The most potent progestins active in the binding assay shared certain structural features: a 17α -acetoxy moiety and unsaturation or substitution in the B ring. Of the 17α -acetate progestins available for testing in the radioreceptor assay, chlormadinone acetate (CMA), 3,5-diene-3-one- 17α -ol-6-chloro-pregn-17-acetate, was the most active. In this report we describe results of studies with CMA designed to ascertain other potential digitalis-like actions. Digitalis-sensitive processes include ion and sugar flux in rat hemidiaphragm⁴ and guinea pig atrium⁵ and Na-K-ATPase activity in several tissues⁶.

In the radioreceptor assay CMA is about 1/50th as potent as ouabain in displacing specifically bound ^3H -ouabain (Fig. 1). (If inorganic phosphate is used in place of ATP + Na^+ in the assays, CMA is about 1/20th as potent as ouabain.) Several related progestins, medroxyprogesterone acetate, megestrol acetate, and cyproterone acetate were 4-10 times less potent, whereas progesterone and 17α -hydroxyprogesterone acetate were about 100 times less potent than CMA. All other steroids tested, including 17α -hydroxyprogesterone and a large number of 17α -substituted pregnanes, androstanes and estranes, were inactive.

In both beating and resting atria, concentrations of CMA ($0.5\text{-}5.0 \times 10^{-5}\text{ M}$) markedly increased internal Na^+ , indicating inhibition of the Na^+ pump (Fig. 2). Lower concentrations had a slight stimulatory effect on the pump, reflected in lower Na^+ levels. Changes in internal K^+ (not shown) were reciprocal to those in Na^+ concentrations. The same biphasic pattern has been described for ouabain in cardiac^{5,6} and skeletal muscle^{4,7}. The biphasic effect of CMA on the influx of the non-metabolised glucose analogue, 3-O-methyl-D-glucose (Fig. 2) was also

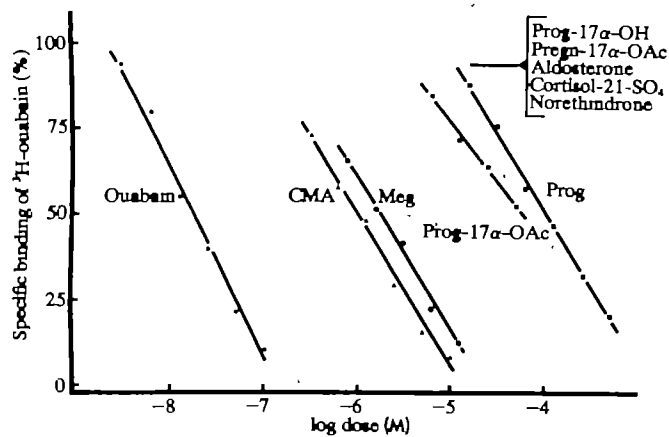


Fig. 1 Competitive binding of steroids in a ^3H -ouabain (11 Ci mmol^{-1} , Amersham) radioreceptor assay. *a*, Chlormadinone acetate (Lilly); *b*, megestrol acetate; Prog, progesterone; Preg: pregnenolone. Fresh or frozen heart ventricle from adult male dogs was minced with scissors and 20 g homogenised in a Polytron for 1.5 min in 160 ml of 10% sucrose in 10 mM Tris-HCl buffer, pH 7.4. The homogenate was filtered through a $210 \mu\text{m}$ nylon mesh screen and centrifuged at $110,000g$ for 30 min. The pellet was suspended in 160 ml of sucrose-Tris buffer, stirred for 1 h at 4°C and recentrifuged as above. The pellet was resuspended in buffer and used immediately or stored frozen in convenient aliquots. The buffer is slightly modified from that used by Schwartz *et al.*⁹ and contains 150 mM NaCl, 1 mM EDTA, 1.25 mM MgCl_2 and 50 mM Tris-HCl buffer (pH 7.4); ATP to yield a final concentration of 1.25 mM was added just before use. Competing test substance or unlabelled ouabain in 0.1 ml was mixed with 0.1 ml ^3H -ouabain in buffer to yield 9 nM of label, 0.6 ml buffer and 0.2 ml of freshly prepared or frozen suspension equivalent to 12.5 mg tissue wet weight and incubated at 37°C for 60 min with shaking. Incubation was terminated by rapid cooling of the assay tubes in an ice bath and centrifuging at $2,700g$ for 20 min. The resulting supernatant containing unbound ^3H -ouabain was discarded by aspiration. The membrane pellet was dissolved in 0.3 ml of 2M KOH by heating in a 70°C water bath for 10 min, and 0.2 ml transferred to a vial containing 10 ml of scintillation medium for ^3H counting. Steroids were insoluble in the buffer and were dissolved and diluted in ethanol. In the latter case incubation medium was added to the tube and vortexed after evaporation of the ethanol under a nitrogen stream. Unlabelled ouabain was added in the same way. Reproducibility of results with actively competing steroids indicated that the compounds were taken up by the buffer phase. Identical results were obtained in a few experiments where the alcohol stock solution was added directly to the incubation medium.

analogous to the effect of ouabain in resting atria reported previously^{5,6}. Sugar transport in resting muscle has been shown to vary in parallel with the intracellular level of sodium⁴⁻⁶ which governs Ca^{2+} influx by sarcolemmal $\text{Na}^+-\text{Ca}^{2+}$ exchange, and to reflect, therefore, the activity of the Na^+ pump. However, sugar transport is also affected by Ca^{2+} fluxes accompanying muscular contraction⁸ and depends on the rate and force of myocardial contraction. Accordingly, in beating atria the inotropic effect of ouabain is paralleled by a large rise in sugar transport⁶ (Fig. 2, broken line) which peaks at the concentration causing maximal inotropic effect ($0.5\text{--}1.0 \times 10^{-6} \text{ M}$). In contrast, CMA elicited no such contraction-dependent rise, and its effect on sugar transport in beating atria was like that in resting muscles and paralleled the changes in internal Na^+ . The effects of CMA on sugar transport are thus consistent with a lack of inotropic effect, together with a digitalis-like effect on the sodium pump.

In microsomes from guinea pig brain, CMA inhibited Na-K-ATPase, but not Mg-ATPase, 95% inhibition occurring at $4 \times 10^{-4} \text{ M}$. CMA was tested for potential inotropic activity on six isolated guinea pig atria at concentrations from 10^{-9} to $5 \times 10^{-5} \text{ M}$. In no instance was there any increase in contractile force; at high concentrations a decrease in contractility was

usually noted. In contrast, ouabain at $5 \times 10^{-7} \text{ M}$ consistently enhanced contractile force severalfold, the effect becoming apparent within 2 min and increasing over the 3-min observation period. In Fig. 3 typical records show that CMA neither exerted an action of its own on the beating heart nor interfered with the inotropic action of ouabain. In a separate series of experiments, cyproterone acetate also failed to affect contractility but consistently inhibited the pump.

Thomas *et al.*¹¹ reported a lack of strict proportionality between Na-K-ATPase inhibition and cardiotoxic activity of

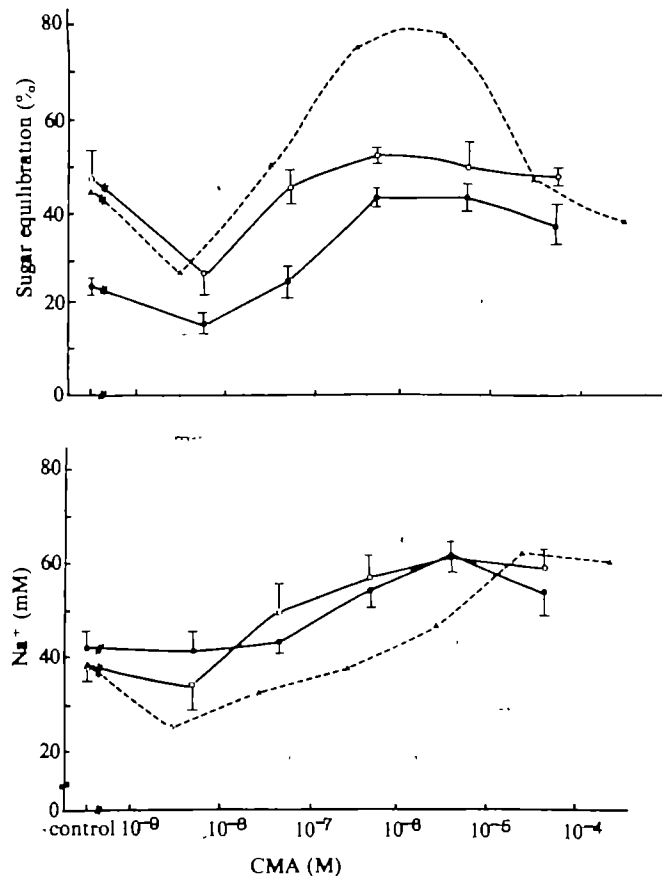


Fig. 2 Effect of CMA on internal Na levels and sugar transport in guinea pig atria. ●, Resting atria; ○, beating atria, 75 min^{-1} . Isolated guinea pig left atria were prepared and perfused as described¹⁰ with ^{14}C -labelled 3-O-methyl-D-glucose (5.0 mM), tracer amounts of ^3H -inulin and CMA in the concentrations indicated on the abscissa. The ordinates show, respectively, per cent equilibration of 3-methylglucose in the intracellular water space, reached after 15 min perfusion, and intracellular concentration of Na^+ , determined by flame photometry, at the end of perfusion; both values were corrected for inulin space. Tissues were exposed to CMA for 15 min before and during perfusion with sugar. Stock solution of CMA in 95% ethanol was added dropwise to the buffer containing 4% 'fatty acid-free' serum albumin (Sigma). Concentrations of CMA in the buffer up to 10^{-4} M yielded clear solutions which were then used to replace the total volume of the organ bath. The final ethanol concentration was 0.13% and appropriate vehicle controls produced no detectable effects. Radioactivity was determined by liquid scintillation spectrometry¹⁰. Vertical bars indicate $\pm \text{s.e.m.}$ For comparison, effects of ouabain in beating atria are included (Δ) (redrawn from ref. 6). Statistical analysis (Student's *t* test) sugar transport: increased in beating against resting ($P < 0.01$) at all concentrations up to $5 \times 10^{-7} \text{ M}$ CMA; decreased at $5 \times 10^{-9} \text{ M}$ against control in beating ($P < 0.02$) and resting ($P < 0.001$). Increased at $5 \times 10^{-7} \text{ M}$ against $5 \times 10^{-9} \text{ M}$ in beating ($P < 0.005$), and against $5 \times 10^{-9} \text{ M}$ or control in resting ($P < 0.001$). Na^+ content: increased against control at $5 \times 10^{-8} \text{ M}$ ($P < 0.05$) and all higher concentrations ($P < 0.001$) in beating and at $5 \times 10^{-7} \text{ M}$ ($P < 0.02$) and $5 \times 10^{-6} \text{ M}$ ($P < 0.001$) in resting. No significant difference between resting and beating at any concentration.

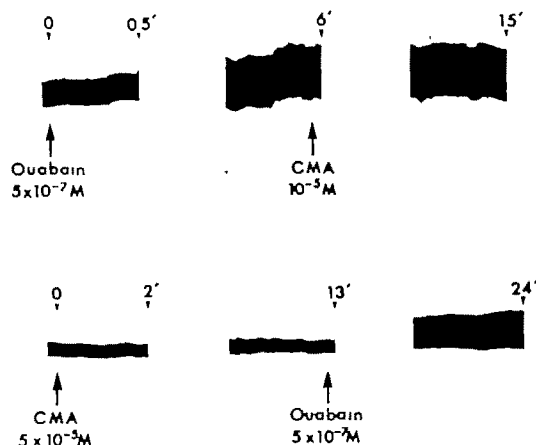


Fig. 3 Contractility of the isolated guinea pig atrium as affected by ouabain and CMA. The left atrium of male guinea pigs was suspended (resting tension 1.5 g) at 37 °C in a 10-ml bath containing Krebs-bicarbonate buffer, pH 7.4, bubbled with 95% O₂/5% CO₂, and isometric contractions recorded on a polygraph with the use of a Grass force-displacement transducer. The atrium was stimulated at 2–3 Hz, 15 V, pulse duration 0.2 ms. CMA was added to the bath in a few μ l of ethanol, dimethyl sulphoxide, or Cremophore El (Sigma) with appropriate controls. The steroid was also solubilised in albumin-containing medium, in which CMA consistently inhibited the sodium pump.

some digitaloid derivatives. Our findings with CMA extend this to demonstrate complete lack of cardiotonic activity in a compound which significantly inhibits Na-K-ATPase in broken cells and Na⁺ pumping in intact cells and, moreover, competes with ouabain for specific binding sites. The data on sugar transport, serving as indicator of Na⁺ pumping and contractile activity, further support this dichotomy. These results indicate that sodium pump inhibition, even of the highly specific 'digitaloid' type, does not necessarily lead to increased contractility of cardiac cells. Furthermore, pump inhibition by CMA presumably occurs as a consequence of binding to the digitalis acceptor sites on the enzyme. This conclusion is based on the observation that CMA can displace practically all of the specifically bound ³H-ouabain, including, presumably, that portion of the cardiac glycoside which binds to and inhibits Na-K-ATPase.

The failure of CMA to antagonise the cardiotonic action of digitalis suggests that the progestin does not compete with digitalis at receptor sites coupled to events leading to increased contractility. Thus, only a very small proportion of digitalis specifically bound to cardiac membranes seems to be concerned with cardiotonic functions. It is proposed that digitalis binds with high affinity and high specificity to certain species of binding acceptor sites, also accepting active progestins, and including sites on Na-K-ATPase. In addition, digitalis may combine with another site, having little or no affinity for the progestins but concerned with the inotropic response.

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Do laser diffraction studies on striated muscle indicate stepwise sarcomere shortening?

ILLUMINATION of striated muscle with a laser beam and measurement of the ensuing diffraction angles is a widely used method for the determination of sarcomere lengths. Pollack *et al.*¹ recently presented results showing that during internal shortening of single muscle fibres the angle of the diffracted first order beam changes in stepwise fashion. As the most likely explanation for this finding they suggested that the majority of the sarcomeres in the field illuminated by the laser beam, after having started shortening, suddenly cease shortening for several milliseconds before continuing the shortening process. The authors point out that this possibility would impose serious complications on the cross-bridge theory as presently envisaged^{2,3}, and in fact, on any proposed molecular theory of contraction. On the basis of diffraction studies using an experimental arrangement similar to that of Pollack *et al.* we now propose that the steps in the records of sarcomere length during contraction are an expression of slight differences in sarcomere length existing in myofibrillar clusters within the muscle fibre and therefore do not conflict with present contraction theories.

The intensity of light diffracted by striated muscle is strongly dependent on the angle ω between the beam of incident light and the fibre axis. This can be shown by rotating a single frog skeletal muscle fibre around an axis perpendicular to the fibre axis and perpendicular to the axis of a 100- μ m wide HeNe laser beam (Spectra Physics Model 155, $\lambda = 0.6328 \mu$ m). During this rotation we recorded the intensity of diffracted light for each order at the respective diffraction angles using a photodiode (PIN 25, UDT Inc.). Figure 1 presents results from such ω -scans (from +15° to -25° at a speed of 2° per s) obtained with an anterior tibial fibre (diameter $\approx 120 \mu$ m) at striation spacings set to a mean of 2.0 μ m (a) and 2.6 μ m (b). The intensity of light diffracted in the first order to the left is represented by dotted lines, and that diffracted to the right by solid lines. Such spectra show a distribution of light intensity over a range of some 20° with a halfwidth of about 10°. Sharp lines are superimposed on the continuous intensity distribution. The number and position of these lines is different for each illuminated fibre site. In general, maximum intensity does not occur at normal light incidence ($\omega = 0^\circ$). This intensity behaviour of diffracted light can be explained with the assumption that even a single fibre is too big to act as a plane grating. Rather volume effects seem to determine the diffraction process⁴.

The A-I-band pattern of adjacent myofibrils is arranged in register so that segments of high and low refractive index provide the fibre with a three-dimensional lattice of one-dimensional periodicity. Light microscopy shows that the register is not perfect in two respects. First, the segments (or idealised: lattice planes) may be inclined at a small angle η against the plane normal to the fibre axis. Second, this angle of inclination is not the same throughout the fibre diameter and along the illuminated fibre length. The myofibrils are rather arranged in clusters of variable size whose planes are inclined at small angles to each other. Thus, the lattice of the fibre resembles that of an imperfect crystal.

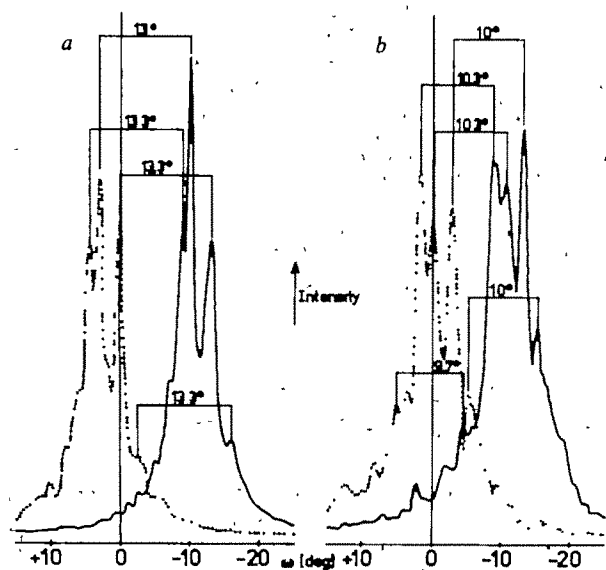


Fig. 1 Intensity of light diffracted in the first order to the left (dotted lines) and to the right (solid lines) as a function of the angle ω between incident laser beam and muscle fibre axis (linear scales, relative units). Sarcomere length set to 2.0 μm in *a* and to 2.6 μm in *b*.

Light diffraction by an imperfect volume lattice can be described using the Bragg formalism⁵. Only those lattice planes contribute to the diffraction process for which the condition $2d \sin \theta = k\lambda$ is fulfilled (d , lattice constant $\approx$ sarcomere length; θ , glancing angle between incident light and lattice plane; k , order of diffraction, λ , wavelength of light). Therefore, with normal light incidence the diffracted light is not generated by all myofibrils illuminated by the laser beam, but only by the small proportion whose planes happen to be inclined against the fibre axis at the Bragg angle ($\eta = \theta$). The Bragg formalism allows the diffraction process to be regarded as if the incident light is 'reflected' from the lattice planes, and each set of planes is able to reflect the light to the left or right if the glancing angle is appropriately set. This is why spectra of corresponding left and right orders from the same illuminated field are very similar in shape. Corresponding lines from the same myofibrillar cluster can easily be correlated as in Fig. 1. The Bragg condition requires that their positions in a plot of intensity against ω be separated by twice the Bragg angle. From the separation of the line positions, therefore, the sarcomere length of the corresponding myofibrillar cluster can be calculated.

The above points have been extensively discussed in an earlier paper⁴, but we now present evidence that the separation of corresponding lines is not exactly the same for all lines in a left-right pair of spectra. The most likely explanation for this is that different myofibrillar clusters in the fibre do not possess the same sarcomere length. As indicated in Fig. 1*a*, the lines with the largest amplitude are 13° apart, corresponding to a sarcomere length $d = 2.03 \mu\text{m}$, while most other line pairs are 13.3° apart, corresponding to $d = 1.99 \mu\text{m}$. Similarly, at the longer sarcomere length in Fig. 1*b*, the line separation varies between 9.7 and 10.3°, corresponding to $d = 2.72$ and $2.56 \mu\text{m}$. In other fibres similar variations were found, and in general the variation was at least 0.1 μm at $d = 2.5 \mu\text{m}$. Variability of the sarcomere length can also be determined from the angular dispersion of diffracted beams and variations of the same order of magnitude (2–3%) have been reported^{6,7}. These variations were, however, so far assumed to be distributed over the illuminated fibre volume in a more or less random way. We stress that the diffracted beams when viewed on a frosted glass screen show a microstructure, as described by Cleworth and Edman⁶, as if composed of several beams which show little dispersion, but

which are diffracted at slightly different angles. This microstructure and the results obtained from ω -scans (Fig. 1) suggest that sarcomere length dispersion stems from differences in the sarcomere length in the various myofibrillar clusters which themselves have a much more homogenous sarcomere length.

When a muscle fibre shortens, the Bragg condition requires that left and right hand spectra move apart along the ω -axis (Fig. 1*b* $\rightarrow$ *a*). The spectra also change their shape in an unpredictable way because different myofibrillar clusters will happen to fulfill the Bragg condition as sarcomere length decreases. If during such shortening the incident beam is first diffracted by a cluster with shorter sarcomere length and at a later time by a cluster with longer sarcomere length, the length signal in a diffractometer will behave as if the fibre ceases shortening or even lengthens, although both clusters shorten. This is schematically indicated in Fig. 2 in which for the sake of clarity this effect is exaggerated. Further, the figure represents only two clusters, one initially of $d = 2.2 \mu\text{m}$ and with an inclination η of 8.3°, the other of $d = 2.4 \mu\text{m}$ and with $\eta = 7.9^\circ$. The Bragg condition requires $\theta = 8.3^\circ$ for 2.2 μm and $\theta = 7.6^\circ$ for 2.4 μm . Thus, only the shorter cluster can diffract and the diffractometer indicates 2.2 μm . When both clusters shorten by 0.1 μm leaving their inclination η unchanged, at the new sarcomere lengths the Bragg condition requires $\theta = 8.7^\circ$ for $d = 2.1 \mu\text{m}$ and $\theta = 7.9^\circ$ for $d = 2.3 \mu\text{m}$. Only the longer cluster can now diffract and the diffractometer indicates 2.3 μm , that is, a lengthening, although both clusters have shortened. In a sarcomere length-time plot such as those recorded by Pollack *et al.*¹ such transitions could appear as a cessation of shortening. The duration of this cessation is about 4 ms in their records. This would correspond to a difference in sarcomere length between clusters of about 0.05 μm and therefore is well within the range of differences that we found by rotating the fibre (Fig. 1). If the Bragg condition is fulfilled initially by a cluster with greater sarcomere length and later by one with smaller d , a transition will appear as an acceleration. Accelerated shortening also seems present in the records of Pollack *et al.*

Since for a given angle of laser beam incidence (such as $\omega = 0^\circ$) the first order beams to the left and right are diffracted by completely different myofibrillar clusters, transitions from one cluster to the other during shortening should be uncorrelated when recorded with the left and right first order beams. We have repeated the experiments of Pollack *et al.* with an apparatus described previously⁸, recording internal shortening alternately with the left and right beam (Fig. 3). Results from five subsequent twitches are superimposed to show reproducibility. Although the diffraction process used for the records in Fig. 3*a* and *b* occurs in the same fibre volume, the left beam consistently

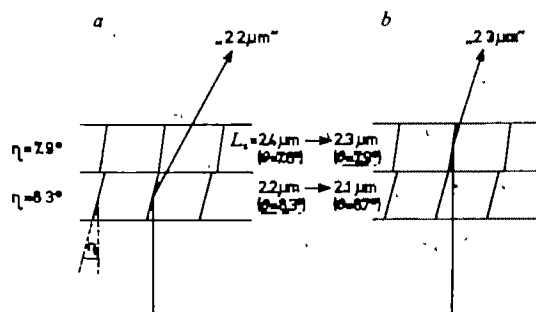


Fig. 2 *a*, Schematic drawing of a muscle fibre having two myofibrillar clusters of different sarcomere length 2.2 (lower) and 2.4 μm (upper). The striations have inclinations η against planes perpendicular to the fibre axis of 8.3° (lower) and 7.9° (upper). Only the lower cluster fulfils the Bragg condition (for $\lambda = 0.63 \mu\text{m}$), therefore the diffractometer indicates a sarcomere length of 2.2 μm . *b*, Both clusters shorten by 0.1 μm leaving their inclinations unchanged. Now only the upper cluster fulfils the Bragg condition, therefore the diffractometer indicates 2.3 μm . For clarity all angles are doubled in the drawing.

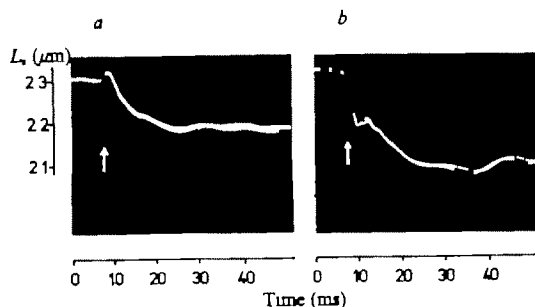


Fig. 3 Records of the time course of sarcomere length during internal shortening of a frog semitendinosus fibre. Panels *a* and *b* were obtained from exactly the same fibre volume recording the left (*a*) and right (*b*) first order beam. Traces obtained from five subsequent twitches are superimposed.

indicates a lengthening while the right beam indicates an accelerated shortening at the same time, 8 ms after the stimulus (arrows).

Although our experiments do not exclude the possibility of stepwise sarcomere shortening, they allow an interpretation of the stepwise changes during shortening of the angle of the diffracted beams which does not impose complications on molecular theories of contraction. The results suggest that the determination of sarcomere length from diffraction angles is not as simple and unproblematic as often believed. In particular, the sarcomere length determined at $\omega = 0^\circ$ may not be at all representative for the majority of the illuminated sarcomeres.

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Evidence for the low molecular weight nature of scrapie agent

THE nature of the aetiological agent of scrapie disease, a transmissible encephalopathy naturally affecting sheep and goats, remains a perplexing problem of fundamental biological importance. Recent studies on brain tissue from outbred Syrian hamsters infected with a strain originating from the Chandler strain of mouse-adapted scrapie have led to a report of a DNA component essential for scrapie infectivity as suggested by sensitivity to DNase¹. The data presented here describe a purification procedure resulting in scrapie infectivity which migrates during polyacrylamide gel electrophoresis as a defined area of infectivity in a region characteristic of low molecular weight nucleic acid. This electrophoretically migrating species of the scrapie agent retains the characteristic disease properties by intercerebral inoculation of crude hamster brain homogenates. Infectivity isolated from this region was resistant to treatment with a combination of SDS and proteinase K followed by

ethanol precipitation. These data support the suggestion that the scrapie agent may be an infectious low molecular weight DNA.

Bioassay and electron microscopic examination of post-ribosomal high speed supernatant (HSS) subcellular fractions from hamsters clinically affected with scrapie disease revealed substantial amounts of scrapie infectivity and an absence of discernible membrane structures². Furthermore, 10% of that infectivity remained in the supernatant fraction after centrifugation at 100,000g for 44 h in sucrose-free buffer. Many of the properties of the scrapie agent—thermal stability, resistance to UV irradiation, nucleases, and proteinases—have been attributed to the intimate association of the scrapie agent with host cell components³. Accordingly, scrapie infectivity in the relatively less complex HSS fractions was more sensitive to heat and UV irradiation than in membrane-rich fractions⁴. However, exposure of the HSS fractions to the more specific degradation of nucleases and proteinases resulted in no apparent loss of infectivity. These results suggest that while discernible membrane structures were no longer associated with the scrapie agent, complexes might still offer protection of scrapie infectivity.

In an attempt to partially purify scrapie infectivity from host-cell components, the HSS was sequentially precipitated with 20, 40, 60 and 80% ammonium sulphate. Bioassay data indicated that scrapie infectivity was associated with all precipitated fractions and that the scrapie specific activity was not increased with respect to protein (Table 1). However, similar procedures applied to purified nucleic acids suggested that precipitation with 20 or 40% ammonium sulphate might result in preparations with low concentrations of host nucleic acids (Table 2), thus increasing scrapie specific activity with respect to cellular nucleic acid.

The 0-40% ammonium sulphate precipitate was electrophoresed on cylindrical 2.2% polyacrylamide gels containing 0.5% agarose and 0.1% SDS for 2 h at 6 mA per gel⁵. Absorbance profiles at 260 and 280 nm were identical when either scrapie-infected or uninfected samples were electrophoresed (Fig. 1b). Bioassay of eluates from gel sections indicated that a significant fraction of the scrapie infectivity penetrated the gel matrix and migrated with a characteristic mobility (Fig. 1c). Scrapie infectivity from gel eluates has been shown to be resistant to degradation by RNases and proteinases but sensitive to DNases with a decrease in infectivity (LD_{50} per ml) of three log units¹. As the hamster-scrapie bioassay system is only sensitive to changes in 90% of the total infectivity resulting in a change of one log₁₀ unit, concentration of three log₁₀ units of scrapie infectivity in one region of the gel over that found in other gel regions indicated a substantial level of purification by electrophoresis.

These results suggest that scrapie infectivity may be associated with a population of complexes of heterogeneous size and composition, from which the putative minimal infectious unit could be released or purified by ammonium sulphate precipitation and polyacrylamide gel electrophoresis. Whether the separation of this form of the scrapie agent from the HSS was due to partitioning of an existing minimal form of the scrapie agent or to a salt-dependent release of scrapie infectivity is not yet known.

Table 1 Ammonium sulphate precipitation of scrapie infectivity from HSS fractions

Ammonium sulphate (% at 0 °C)	Scrapie infectivity (log ₁₀ LD ₅₀ per ml)	Specific activity (log ₁₀ LD ₅₀ per µg protein)
0	8.7	6.4
0-20	8.0	5.9
20-40	8.0	5.1
40-60	8.0	5.6
60-80	8.0	5.1
80 supernatant	5.5	3.9

Table 2 Ammonium sulphate precipitation of purified radioactively labelled nucleic acids

Ammonium sulphate (% at 0 °C)	Percentage of acid-precipitable counts rRNA	DNA and tRNA
0-20	11%	11%
20-40	18%	12%
40-60	100%	16%
60-80	100%	29%

Electrophoresis of tRNA, rRNA and the citrus exocortis viroid (CEV) as RNA molecular weight markers and fragments from a *Hae*III digest of ΦX174 (RF) as DNA molecular weight markers indicated that RNA molecules of 23,000–70,000 and double-stranded DNA molecules of 48,000–130,000 molecular weight migrated to the region from which high scrapie activity could be eluted (Fig. 1a). The small size of the scrapie agent suggested by the electrophoretic mobility is compatible with the estimated target size deduced from inactivation by ionising irradiation^{6,7} and a small DNA eluted from hydroxyapatite⁸. Nucleic acid markers of low molecular weight migrate in a similar way to that of scrapie infectivity; it must be cautioned that the scrapie preparation may not be assumed to be a purified nucleic acid, and that association of scrapie DNA with one or more macromolecules could affect the migration pattern.

The apparent low molecular weight and increased sensitivity to degradative treatment upon purification resemble the properties of viroids⁹, the pathogenic RNA molecules responsible for several plant diseases. However, application of procedures for the extraction of the citrus exocortis viroid¹⁰ to infected brain tissue have resulted in a complete loss of infectivity¹¹. Possible explanations for the loss of infectivity are (1) that the nucleic acid must be associated with another component in order to be infectious, (2) that the nucleic acid is infectious, but only at low levels, or (3) that recovery of the infectious nucleic acid is dependent on the extraction procedure.

To examine the nucleic acid nature of the scrapie agent and the necessity of auxiliary components further, scrapie infectivity eluted from section 3 of polyacrylamide gels (Fig. 1c) was incubated with 0.5% SDS and 50 µg ml⁻¹ proteinase K for the deproteinisation of DNA¹², followed by ethanol precipitation and bioassay. This procedure resulted in no loss of infectivity. Examination of the gel eluate fraction under the electron microscope has revealed no particles suggestive of conventional viruses. These data further support the suggestion that the agent responsible for scrapie disease may be an infectious low molecular weight nucleic acid.

However, the possibility that the infectious unit may be nucleic acid in association with another macromolecule to form a complex unlike the regular geometric structure of viruses cannot be excluded. A nucleosome-like structure composed of 140–200 base pairs of DNA in a 10–11S nucleoprotein complex might exhibit similar migration properties in polyacrylamide gel electrophoresis, as well as variable sensitivity to DNase. A model similar to the nucleosome with DNA surrounding a protein molecule might explain the resistance of scrapie infectivity to degradation by proteinases. A further parallel might be drawn to adenovirus-5 DNA which is infectious, but with a 100-fold lower efficiency of transfection than that of the native DNA-protein complex. This DNA-protein complex is excluded from entering agarose or agarose-acrylamide composite gels during electrophoresis, while pronase-treated DNA migrates characteristically¹³, as does scrapie infectivity.

Thus the electrophoretic mobility of the minimal scrapie infectious unit is characteristic of a low molecular weight molecule, possibly of a free nucleic acid. Definition of the exact size and structure must await detection of the scrapie agent by physical procedures.

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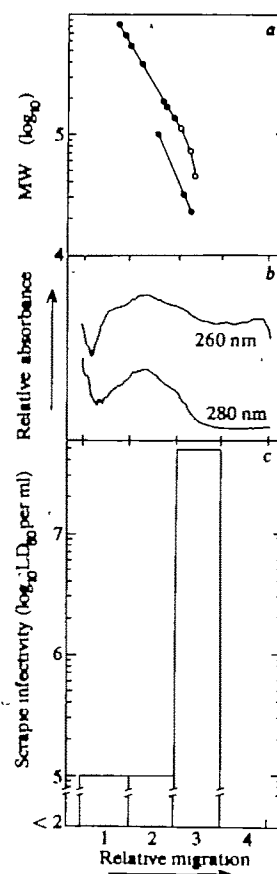


Fig. 1 Polyacrylamide gel electrophoresis of a 0–40% ammonium sulphate precipitated fraction of HSS. *a*, Migration of RNA (●) and DNA (○) molecular weight standards. *b*, Relative absorbance at 260 and 280 nm. *c*, Scrapie infectivity in eluates from 2.25-cm gel sections after the top 0.05 cm had been discarded.

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Guanine nucleotides distinguish between two dopamine receptors

BEHAVIOURAL¹ and neurophysiological² data suggest the existence of more than one dopamine receptor in the brain³. Differences in drug specificity of the dopamine-sensitive adenylate cyclase and dopamine receptors labelled by the butyrophenones, ³H-haloperidol or ³H-spiroperidol, indicate that the cyclase and the binding sites may, in part, involve distinct receptors^{4,5}. Lesion studies directly demonstrate the existence of physically distinct dopamine receptors in the corpus striatum⁶. Kainic acid microinjections, which selectively destroy intrinsic neurones in the corpus striatum, almost totally deplete the dopamine-sensitive adenylate cyclase while producing only a 40–50% decline in ³H-haloperidol or ³H-spiroperidol binding. Most of the remaining ³H-haloperidol or ³H-spiroperidol binding is lost following cerebral cortex ablation, which removes a major neuronal input to the corpus striatum, indicating that these binding sites are localised to axons and terminals of the cortico-striate projection⁶. As the dopamine-sensitive adenylate cyclase is not reduced by cerebral cortex ablation, the cortico-striate dopamine receptors do not seem to be linked to adenylate cyclase. Whether or not the ³H-butyrophenone binding sites destroyed by kainic acid represent the same dopamine receptors as those linked to adenylate cyclase has not been clear. Guanine nucleotides regulate binding at several hormone and neurotransmitter receptors, especially those associated with adenylate cyclase^{7–13}. Previously, we^{14–16} and others¹⁷ showed that dopamine receptor binding of ³H-agonists and ³H-antagonists can be regulated by guanine nucleotides. We now report that kainic acid lesions abolish the sensitivity of dopamine receptor binding to guanine nucleotides. Thus, ³H-spiroperidol and ³H-apomorphine binding sites involve two dopamine receptors, only one of which is regulated by guanine nucleotides.

After anaesthesia with Equithesin (0.6 ml, Jensen-Salisbury), Sprague-Dawley rats (160 g) were positioned in a David Kopf small-animal stereotaxic apparatus and a 0.3-mm Hamilton cannula was inserted into the striatum through a burr hole in the calvarium (coordinates: 7.9A; 2.6L; 4.8V). Kainic acid (Sigma), 2 µg in 1 µl of artificial cerebrospinal fluid titrated to pH 7.4, was infused and the scalp was then apposed with sutures. Two to five weeks after kainic acid injection, the corpus striatum ipsilateral to the lesion and the contralateral unlesioned striatum, and striata from unlesioned animals were assayed for ³H-spiroperidol and ³H-apomorphine binding, essentially as previously described¹⁴. Freshly homogenised striata (100 volumes of 50 mM Tris buffer with 0.1% ascorbic acid, pH 7.1, at 30 °C disrupted with a sonicator) were centrifuged twice at 50,000g

for 10 min with rehomogenisation of the intermediate pellet in fresh buffer. The final pellet was resuspended in buffer mix (5 mg ml⁻¹). Incubation tubes in triplicate received ³H-apomorphine or ³H-spiroperidol, tissue suspension and buffer to 1 ml. Tubes were incubated for 10 min at 37 °C and then rapidly filtered under vacuum through Whatman GF/B filters with three 5-ml rinses of ice-cold buffer. The filters were counted in 9 ml Formula 947 (NEN) after 12 h extraction at 4 °C at efficiencies of 37–44%. Saturable or specific binding was measured as the excess over blanks containing 1 µM and 10 µM *d*-butaclamol for ³H-spiroperidol and ³H-apomorphine assays respectively. Blank values were 20% and 40% of total ³H-spiroperidol and ³H-apomorphine binding, respectively.

As reported earlier, GTP lowers the binding of the agonist ³H-apomorphine with an IC₅₀ of 5–10 µM (refs 14–16) (Table 1). GDP and Gpp(NH)p (the non-metabolised analogue of GTP) have the same potency in eliciting this effect, but GMP, ATP, ADP and AMP are completely inactive.

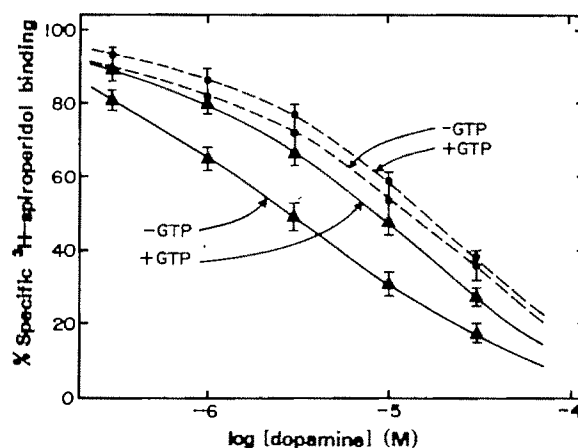


Fig. 1 The effect of striatal kainate lesion on GTP alteration of the potency of dopamine in competing for striatal ³H-spiroperidol binding. Each point is the mean of 10 determinations. Solid lines, control; dashed lines, kainate lesion.

GTP does not reduce the total binding of the antagonist ³H-spiroperidol. However, as found previously, GTP decreases the dopamine inhibition of ³H-spiroperidol binding^{14–17}. The IC₅₀ concentration for dopamine (2 µM) is increased about threefold (to 6.4 µM) in the presence of 50 µM GTP. This effect is specific in that it is exerted with the same potency by GDP and Gpp(NH)p but not by GMP, ATP, ADP or AMP. However, inhibition of ³H-spiroperidol binding by antagonists such as fluphenazine and haloperidol is unaffected by GTP¹⁶.

Kainic acid lesions, in conditions which destroy intrinsic striatal neurones as verified by biochemical and anatomical examination in this laboratory⁶, but which spare glia and neurones whose cells originate in other brain regions, reduce specific ³H-spiroperidol binding by 54 ± 3%. In lesioned animals dopamine is 4–5-fold less potent an inhibitor of ³H-spiroperidol binding than in control animals. However, the residual binding after kainate lesions still involves dopamine receptors, as drug specificity studies indicate a greater potency for dopamine than for noradrenaline or serotonin in inhibiting ³H-spiroperidol binding, and isoprenaline is inactive.

Strikingly, after kainate lesion, GTP no longer affects the potency of dopamine as an inhibitor of ³H-spiroperidol binding (Fig. 1). This loss of effect of GTP suggests that the residual binding involves a distinct dopamine receptor site which is not regulated by guanine nucleotides. To ascertain whether two such distinct dopamine receptors were labelled by ³H-agonists,

Table 1 Kainate lesion abolishes influence of GTP on striatal ³H-apomorphine binding

	³ H-apomorphine binding (pmol per g tissue wet weight)	
	Unlesioned side	Lesioned side
– GTP	18.5 ± 0.46	6.62 ± 3.2
+ 50 µM GTP	9.90* ± 0.15	5.73 ± 2.5
% Reduction by GTP	47.0 ± 1	9.0 ± 5

Kainate lesions and ³H-apomorphine binding were carried out as described in the text. Data are means ± s.e.m. for groups of 3 rats. This experiment was repeated twice in groups of 3–4 rats each. Per cent reduction data were determined by computing for each rat the ratio of binding in the presence and absence of GTP.

* Differs from unlesioned side without GTP $P < 0.001$

we evaluated the binding of ^3H -apomorphine (Table 1). GTP ($50\ \mu\text{M}$) reduces ^3H -apomorphine binding in normal rats by 47%, but no longer does so following kainate lesion, which lowers specific ^3H -apomorphine binding to 36% of control. Thus, following kainate lesions both residual ^3H -apomorphine and ^3H -spiroperidol binding is resistant to GTP.

Thus, we have found that guanine nucleotide regulation of both ^3H -agonist and ^3H -antagonist binding to dopamine receptor is lost following kainic acid lesion. This suggests that the dopamine receptors contained on the intrinsic neurones destroyed by kainic acid are regulated by guanine nucleotides, whereas those localised to the cortico-striate neurones are not. As guanine nucleotides regulate binding of receptors linked to adenylate cyclase and as the dopamine cyclase is also contained on intrinsic neurones, we conclude that the ^3H -spiroperidol and ^3H -apomorphine binding sites contained on the intrinsic neurones destroyed by kainate may involve the cyclase-linked dopamine receptors referred to as D-1 dopamine receptors^{17,18}. The notion that the cyclase-linked dopamine receptor is regulated by GTP is supported by the direct demonstration of GTP influences on the dopamine-sensitive adenylate cyclase of striatal homogenates¹⁹. By contrast, the cortico-striate dopamine receptors are not linked to adenylate cyclase and may be designated D-2 dopamine receptors.

The drug specificity of ^3H -spiroperidol binding differs from that of the dopamine-sensitive adenylate cyclase. Previously, we proposed that ^3H -agonists and ^3H -butyrophenone antagonists label distinct agonist and antagonist preferring states of dopamine receptors⁴. We suggest that ^3H -spiroperidol and ^3H -apomorphine binding to intrinsic striatal neurones destroyed by kainate label distinct agonist and antagonist preferring states of the cyclase-linked dopamine receptors. This would account for the finding that the drug specificity of the ^3H -apomorphine binding sites resembles that of the dopamine-sensitive adenylate cyclase more closely than that of ^3H -spiroperidol binding¹⁴.

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New type of twisted mesophase in jellies and solid films of chiral 12-hydroxyoctadecanoic acid

SOME optically active substances are known to form a unique state of matter, the twisted mesophase, in which a supramolecular helical structure gives rise to some striking optical phenomena¹. This type of structure was first found in melts of single compounds and later in solutions of some biopolymers. The only lyotropic twisted mesophases reported so far are the cholesteric (twisted nematic) type. However, we describe here a new type, found in the thermally-reversible jellies formed by chiral 12-hydroxyoctadecanoic acid (12HOA) with CCl_4 or aromatic solvents; solid films obtained by drying the jellies on quartz supports retained the twisted structure. Microscopy and circular dichroism (CD) indicated that this mesophase had a supramolecular helical structure, and X-ray examination of the dried solid films indicated that it was not a nematic type, but probably smectic.

Crystalline samples of 12HOA were prepared as described previously². Solutions of chiral 12HOA in CCl_4 set to an almost transparent jelly above $13\ \text{mmol l}^{-1}$ at 20°C , while solutions of racemic 12HOA showed no gelation until they deposited crystals from a saturated solution. The thin preparations of the jellies observed under crossed polarisers in a polarising microscope exhibited a birefringent pattern with a spherulitic or Schlieren texture of the sort observed for some liquid crystals. This characteristic appearance suggests that the jellies are a lyotropic mesophase.

The jellies exhibited a weak but definite CD in a range of wavelengths in which they absorbed no light, and the sign was negative for the D-enantiomer and positive for the L-enantiomer, irrespective of solvents used. Figure 1 shows a typical

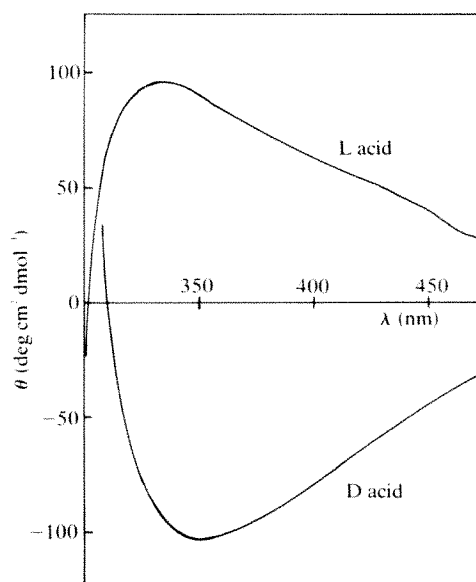


Fig. 1 Enantiomeric CD bands of jellies of 12HOA- CCl_4 ($25.7\ \text{mmol l}^{-1}$ for the L acid, $35.7\ \text{mmol l}^{-1}$ for the D acid). These spectra were measured using a 10 mm-thick cell on a JASCO J-20A spectropolarimeter at room temperature. The location of the CD maximum was dependent on solvents (for example 350 nm for CCl_4 , 480 nm for benzene), but insensitive to change of temperature. The intensity and the location of the peak varied slightly for different samples.

example of the enantiomeric CD bands for jellies of D- and L-12HOA. Little linear dichroism was observed when jellies were examined by the procedure of Tunis-Schneider and Maestre³. Therefore we ascribed the observed CD to the preferential reflection of circularly polarised light of one sense by the jellies. The same optical phenomenon¹ has been observed for cholesteric liquid crystals and explained by assuming a supramolecular helical structure with the twist determined by the chirality of the constituent molecules.

Enantiomeric induced CD (ICD) could also be observed for some achiral molecules dissolved in jellies of chiral 12HOA. Figure 2 shows the ICD bands (superimposed on the CD curve of the jelly) and electronic spectra between 330 and 395 nm for anthracene dissolved in a jelly of D-12HOA. Interestingly, the ICD bands show a single sign and follow closely the electronic spectral bands; such ICD characteristics have been obtained for lyotropic cholesteric mesophases of poly- γ -benzyl-L(or D)-glutamate (PBG)⁴ and other polypeptides, and more recently for those of *N*-acylamino acids⁵.

In view of these CD and ICD results, it is possible that a supramolecular helical structure exists in jellies of 12HOA. Then the 350 nm CD band maximum would correspond to the 'pitch band' and the observed ICD would be caused by the dissymmetrical field of the helical structure, although it is weak compared with the effect in cholesteric systems⁴. This proposed helical structure is associated with the jelly structure in which hydrogen bonding is involved⁶, because melting of the jelly by heating resulted in the loss of all the optical properties mentioned above.

We found that all the optical phenomena described here manifested themselves more clearly in thin semi-transparent solid films (about 50 μ m) obtained by drying jellies of chiral 12HOA on quartz supports. When the films were dried, the CD increased in intensity and the peak was shifted to the shorter wavelength side. These results indicate that the original supramolecular helical structure in jellies is fully retained in such solid films. Similar behaviour has been found with the cholesteric mesophase of polypeptides⁷.

Microscopy with a controlled hot stage showed that, when such solid films were heated, the mesomorphic texture became obscure at about 348 K, and then needle-like microcrystals formed: CD was no longer observed. Finally the microcrystals melted at 353 K (melting point of chiral 12HOA).

X-ray diffraction patterns for these solid films obtained by drying were similar to those for the crystalline powder, although slightly more broad. A long spacing of 46.7 Å was obtained and interpreted as the distance between double plane layers of amphiphilic molecules of 12HOA. This suggests that the mesophase structure retained in these solid films, and possibly also the structure of the mesophase itself, is not nematic, as has been shown for the cholesteric mesophase of PBG, but smectic. In the layer structure with a twisting, the molecular long axes would have to be tilted relative to the layer planes. The length of the crystallographic *c* axis of octadecanoic acid which is an analogue of 12HOA, that is, the extended length of the bimolecule, has been reported to be 49.38 Å for the B form⁸ and 50.7 Å for the C form⁹. These values are greater than 46.7 Å. This result supports the tilt possibility. It was impossible, however, to prepare orientated samples of jellies and solid films which exhibited regularly-spaced retardation lines or iridescent colours like those observed for the cholesteric mesophase of polypeptides. It seems reasonable to consider that the twisted mesophase structure exists locally as small domains which disperse with randomly-orientated helical axes within jellies or solid films. This would explain the small size of the observed CD band compared with the effect in cholesteric mesophases.

Electron microscopy has shown that both chiral 12HOA (ref. 2) and PBG (ref. 9) formed by precipitation from certain solvents produce helically twisted fibrils with handedness determined by the chirality of the molecule. Because biologically important molecules are essentially chiral, it may be biologically significant that some chiral molecules form helically

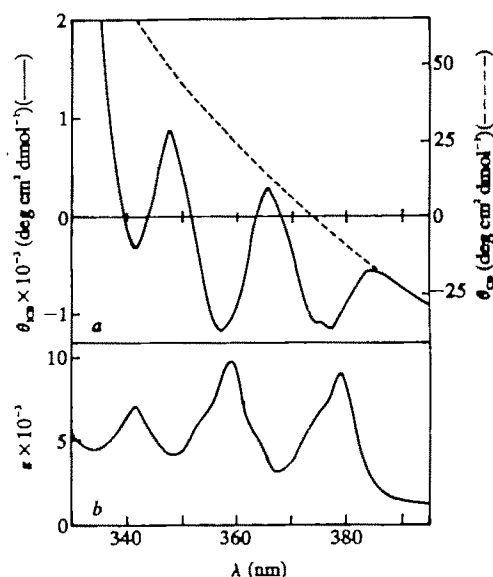


Fig. 2 a, ICD spectral bands (—) for anthracene (8.8 mmol l^{-1}) superimposed on the CD curve (---) of the jelly (D-12HOA- CCl_4). b, Electronic spectra of anthracene. Enantiomeric ICD bands were obtained for a jelly of the L acid. On addition of anthracene, the CD maximum of the jelly itself was shifted to the longer wavelength side ($\sim 450 \text{ nm}$).

organised systems in different stages, as has been shown for 12HOA and polypeptides.

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Erratum

In the letter 'Maximum density of random placing of membrane particles' by L. Finegold and J. T. Donnell, *Nature* **278**, 443-445, the bottom line of the legend to Table 1 should be included in the table under 'Circular particles'.

Corrigendum

In the letter 'Deficient production of tyramine and octopamine in cases of depression' by M. Sandler *et al.*, *Nature* **278**, 357-358, the units of the third column of Table 2 should read nmol instead of μ mol.

matters arising

Origin of the Metazoa

THE recent article by LaBarbera¹, in which the late Precambrian appearance of multicellular organisms is related to the development of shallow-water marine environments late in the Proterozoic, requires some examination of the evidence from which his conclusions have been drawn. LaBarbera bases his arguments on Hargraves² model for the evolution of the Earth through the Precambrian. Hargraves' model has been shown by Windley³ to be totally incompatible with the geological evidence as it relates to processes taking place on the Earth's surface.

LaBarbera's statement that "during the first three-quarters of the Precambrian . . . , shallow water environments (defined here as less than 200 m depth) were very rare, and those that did exist were the result of volcanic or dynamic mountain-building processes and were geologically transitory" cannot stand unchallenged.

While it can be argued that, apart from the 3,000 Myr Pongola sequence⁴, most shallow-water environments recognised in Archaean sediments⁵⁻⁹ may have been geologically transitory, it cannot reasonably be disputed that from about 2,500 Myr ago, shallow-water environments occurred frequently, were developed extensively and were geologically persistent. Examples are too numerous to list here¹⁰, but a few outstanding and clearly documented cases from the Early and Middle Proterozoic from widely separated areas are the sediments of the Athapuscow Aulacogen¹¹ and Kilohigok Basin¹² of Canada, the Transvaal Basin of South Africa¹³, and the Nabberu¹⁴ and McArthur Basins^{15,16} of Australia. All the major Proterozoic basins contain stromatolitic carbonate sediments, most of which are interpreted as being of shallow-water marine origin¹⁷. Thus LaBarbera's comment that the shallow-water fossils "are restricted to a surprisingly few localities" cannot be sustained. Furthermore, these basins frequently contain additional sedimentological evidence which, taken together, indicates deposition in shallow-water environments; for example, numerous breaks in sedimentation, with desiccation, formation of evaporites¹⁵, oolitic, pisolitic and intraclastic beds, and

tidal channel and associated continental marginal deposits. In fact, in the Proterozoic record, it is deep-water sediments that are rare, and not shallow-water ones.

A further point which must be challenged is LaBarbera's contention that early life evolved in deep-water environments, and that its most primitive manifestations must have been planktic. However, the assemblages¹⁸ which he quotes as being the "fossil record for most of the Precambrian" are all associated with stromatolitic carbonates, and they are all benthic. The organisms lived at the sediment-water interface, or just below it, and trapped or precipitated carbonate sediments by their metabolic activities. Some of the described microfossils may have had a planktic phase in their life cycle, or isolated species (for example, *Eosphaera*¹⁹ from the Gunflint Iron Formation, Canada) may have lived in surface waters—but in all cases, the water depth was shallow to very shallow. Clear evidence for oceanic microplankton appears only in the latest Precambrian.

Two recently described assemblages from Middle Proterozoic shales^{20,21} contain large spheres that may be planktic; but petrographic and palaeogeographic studies show these shales to be products of shallow-water environments. It should be added, however, that oceanic plankton has poor preservation potential in deep water unless it has very chemically resistant walls²².

Thus the major point that LaBarbera had hoped to demonstrate for the evolution of multicellular organisms is geologically untenable. A number of other recent models²³⁻²⁷ are more compatible with the geological evidence.

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LABARBERA REPLIES—Muir, Walter and Jackson (and others¹) have apparently misinterpreted the purpose of my article² despite the explicit statement in the first paragraph that it was an "attempt to evaluate some of the implications of Hargraves' model of the geophysical evolution of the Earth during the Precambrian for the evolution of the metazoan phyla". The statement cited by Muir *et al.* concerning the existence of shallow-water environments through much of the Precambrian was not my conclusion, although failure to quote the entire sentence from my article makes it sound as though it were (missing from their quote is the phrase, "The primary implication of Hargraves' model is that . . .").

Muir *et al.* take two other quotes out of context, and by doing so change their original meanings. The complete relevant section reads, "The fossil record for most

of the Precambrian is restricted to algal stromatolites and associated microfossils which are acknowledged to be of shallow water origin. However, these fossils are restricted to a surprisingly few localities...". Nowhere do I either state or imply that Precambrian deep-water deposits are common in an absolute sense or more common than Precambrian shallow-water deposits. Nor, as is obvious from the full quote, do I deny the shallow-water origin of stromatolites. The point of this section (perhaps too tersely made) was that one cannot presume full knowledge of the history of life on an entire planet over approximately 3,500 Myr when the fossil evidence is restricted to a single (shallow-water) environment from approximately 30 sequences³.

My article² was not a defence of Hargraves' model⁴ but an exploration of some of its implications. I welcome the contributions of Windley⁵ and Knoll⁶, who have attempted to separate fact from fancy, to this discussion. However, the extent to which Hargraves' model is applicable to Precambrian geological history is still being evaluated^{7,8} and some observations^{8,9} have yet to be adequately explained in the context of more traditional models.

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Mutagenesis of Chinese hamster cells is facilitated by thymidine and deoxycytidine

THE presence of thymidine and/or deoxycytidine in cell cultures has been reported by Peterson *et al.*¹ to modify the mutagenic potential of methylation by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. It has long been proposed that *O*⁶-alkylguanines are potential promutagenic lesions in alkylated DNA² and this has been supported by *in vitro* experiments with bacterial RNA polymerase³. From our own *in vitro* experiments⁴ using *Escherichia coli* polymerase I acting on methylated poly(dC-dG) templates, we have concluded that any miscoding (that is, mutations, shown by the incorporation of dTMP) arising from the presence of *O*⁶-methylguanine in the template is

competitive with the normal (correct) incorporation of dCMP. Furthermore, the amount of miscoding observed depends on the relative concentrations of the DNA precursors dTTP and dCTP used in the assay. Such competitive miscoding by *O*⁶-methylguanine leads to the possibility of an alteration in the level of miscoding by factors which influence the size of the deoxynucleoside 5'-triphosphate DNA-precursor pools within the cell. It is therefore possible that agents which alter the size of these pools may enhance, or reduce, the mutagenic potential of alkylating agents. Although factors governing mutation within cultured mammalian cells are extremely complex, we believe that our observations may in part explain the results obtained by Peterson *et al.*

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Synergistic chelation therapy or mixed ligand complexes for plutonium and cadmium poisoning?

PROGRESS in the development of chelating therapeutic drugs has been disappointing, especially when one considers the variety of diseases which respond to trace element regulation¹. The unparalleled success of two simultaneously administered ligand drugs at eliminating plutonium, reported by Schubert and Derr², is therefore most impressive.

Conceptually, the use of such double ligand treatment is not new; it has been advocated for the removal of both lead³ and copper⁴. However, Schubert's remarkable biological data emphasise the need to understand how a combination of chelating agents act. We suggest that there are alternative explanations besides the formation of mixed ligand complexes proposed in ref. 2, in which both drugs are complexed on to the metal ion.

In this context, biological transport is by passive diffusion⁵ so there are at least six requirements, which a chelating agent must ideally satisfy. It should (1) bind the metal strongly enough to compete with biological ligands, especially proteins; (2) discriminate against the relatively abundant calcium and zinc cations *in vivo*; (3) be sufficiently lipophilic to penetrate membranes and hence reach the site of

heavy metal deposition; (4) form a lipophilic complex in the body compartment where the metal has accumulated; (5) change to a hydrophilic complex in plasma so the metal is eliminated in urine, not redistributed into other tissues; (6) have a low toxicity (this has implications for each of the preceding requirements).

A single ligand cannot easily meet these conditions, particularly those dealing with lipophilicity. For example, the commonly used polyaminocarboxylic acids are confined to extracellular space and rely on naturally occurring ligands to leach the poisonous metal out of the tissues^{2,5}. On the other hand, two dissimilar drugs can be chosen to satisfy collectively all the above-mentioned requirements. Synergistic chelation therapy occurs when one drug mobilises the metal while the other traps it in plasma to be excreted in the urine.

Low molecular weight transition metal complexes in biological systems almost always occur at concentrations well below the level of analytical detection⁶. Thus, we advocate that computer simulation studies of speciation in biological fluids^{2,6} be extended to plutonium and cadmium to establish whether Schubert's drugs bind to the metal ion simultaneously *in vivo* as he suggests, or whether they function separately.

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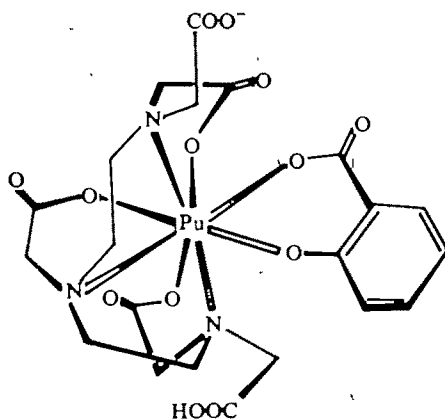
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SCHUBERT AND DERR REPLY—The use of double ligand therapy for decontamination of metals and therapy of metal poisoning is discussed in several references cited in our necessarily brief report. Combinations of chelating agents for decontamination were used in Hamilton's laboratory at Berkeley¹ assuming, as is usual^{2,3}, that pharmacokinetic differences would produce enhanced effects. In some instances, mixed ligand chelates which might have formed were either too weak and/or used chelants readily metabolised, such as citrates³. The results we aimed for and achieved⁴, required deliberate use of chelant combinations which form mixed ligand chelates with stabilities orders of magnitude greater than single chelants. From

the known chemistry of mixed ligand chelate formation, as we describe elsewhere⁵, the choice of suitable combinations is straightforward. For example, Martell *et al.*⁶ demonstrated, using potentiometric titrations, that Th(IV) formed very stable mixed ligand chelates with EDTA, salicylate and catechol (10^{11} – 10^{13} times more stable than EDTA alone). As expected, Pu(IV) reacted similarly.

We reject the suggestion, unsupported by experimental evidence, that alternative explanations besides mixed ligand complexes are responsible for our results. Our experiments^{4,5}, including ultrafiltration, solvent extraction, and *in vivo* reversal of salicylate antipyresis by Zn and Cu EDTA, provided experimental evidence for mixed ligand chelate formation. The proposed mixed ligand chelate of Pu with DTPA and salicylate is depicted by the following structure:



The requirements for biological transport emphasised by May and Williams have long been known. It is not necessarily true, however, that highly charged polyaminopolycarboxylic acids are confined to extracellular spaces. A very small but adequate fraction of these chelants penetrate intracellular space as shown by sustained excretion of metals following single treatment^{7,8}. Charged metal ions, for example, chromate, can also penetrate cell membranes and reach cell interiors⁹. May and Williams' proposal that synergistic chelation therapy occurs when one drug mobilises the metal and the other traps it in plasma is inapplicable to our systems, as compounds such as salicylate and catechol by themselves do not react with Pu(IV) in *in vivo* conditions.

Although computer simulation studies of speciation in biological fluids have a certain value, they are unnecessary in our therapeutic investigations, as even simple non-computer calculations readily show that the very strong mixed ligand chelates used by us bind >99% of the total metal. Indeed, this is shown in their own calculations¹⁰, even with single chelants, and computer calculations used previously¹¹ by one of us. The ability of complexing

agents to bind metals in the blood stream does not necessarily coincide with the ability to remove metals deposited in the tissues¹².

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Evolution of the 3', 5' internucleotide bond

DHINGRA AND SARMA¹ posed the question "Why do nucleic acids have 3', 5' phosphodiester bonds?". They concluded from a detailed NMR investigation of the conformations of dinucleoside monophosphates that 2', 5'-linked oligomers are unlikely to form helical structures that are stabilised by base-stacking. Thus 2', 5'-linked nucleic acids would be less suitable for participation in present day biochemical operations that involve helix formation. Dhingra and Sarma considered a polymer as a set of repeating dimer structures and did not consider the perturbations that could be introduced by the presence of a complementary strand. There is, of course, evidence that 2', 5'-linked oligomers can complex with 3', 5'-linked complementary polymers, although the melting temperatures of these complexes do tend to be lower than in the case of an all 3', 5'-linked complex².

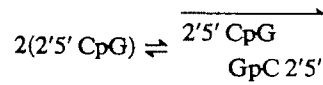
We considered this same question some years ago, and predicted³ that the 2', 5' bond in a helical oligonucleotide would be more susceptible to non-enzymatic chain breakage than would the 3', 5' bond. A large difference in kinetic stability was subsequently demonstrated experimentally for a triple helix of oligoadenylates and poly U in mildly basic solution⁴. Thus during the early chemical evolution of nucleic acids, it seems reasonable to suppose that there was a strong selective pressure for the 3', 5' bond, and against the 2', 5' bond.⁵

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SARMA AND DHINGRA REPLY—Since the publication of our paper in *Nature* we have carried out extensive NMR studies in 100% water on self-complementary 2'5'-dinucleoside monophosphates, that is



to monitor the Watson–Crick hydrogen bonding protons. The data showed that 2'5'-linked dimers have little proclivity to form Watson–Crick hydrogen bonded miniature double helices under conditions in which their 3'5' analogues have been shown¹ to form such complexes. The study has revealed that the availability of a complementary dimer does not introduce enough perturbations to the intrinsic stereochemistry of 2'5' systems to induce the formation of miniature double helices. In this connection, it is important to note that in the reported crystal structure of 2'5' ApU no miniature double helices have been observed², contrary to the crystal structure findings on 3'5' ApU and 3'5' GpC^{3–5}, but like 3'5' UpA⁶.

R. H. SARMA
M. M. DHINGRA

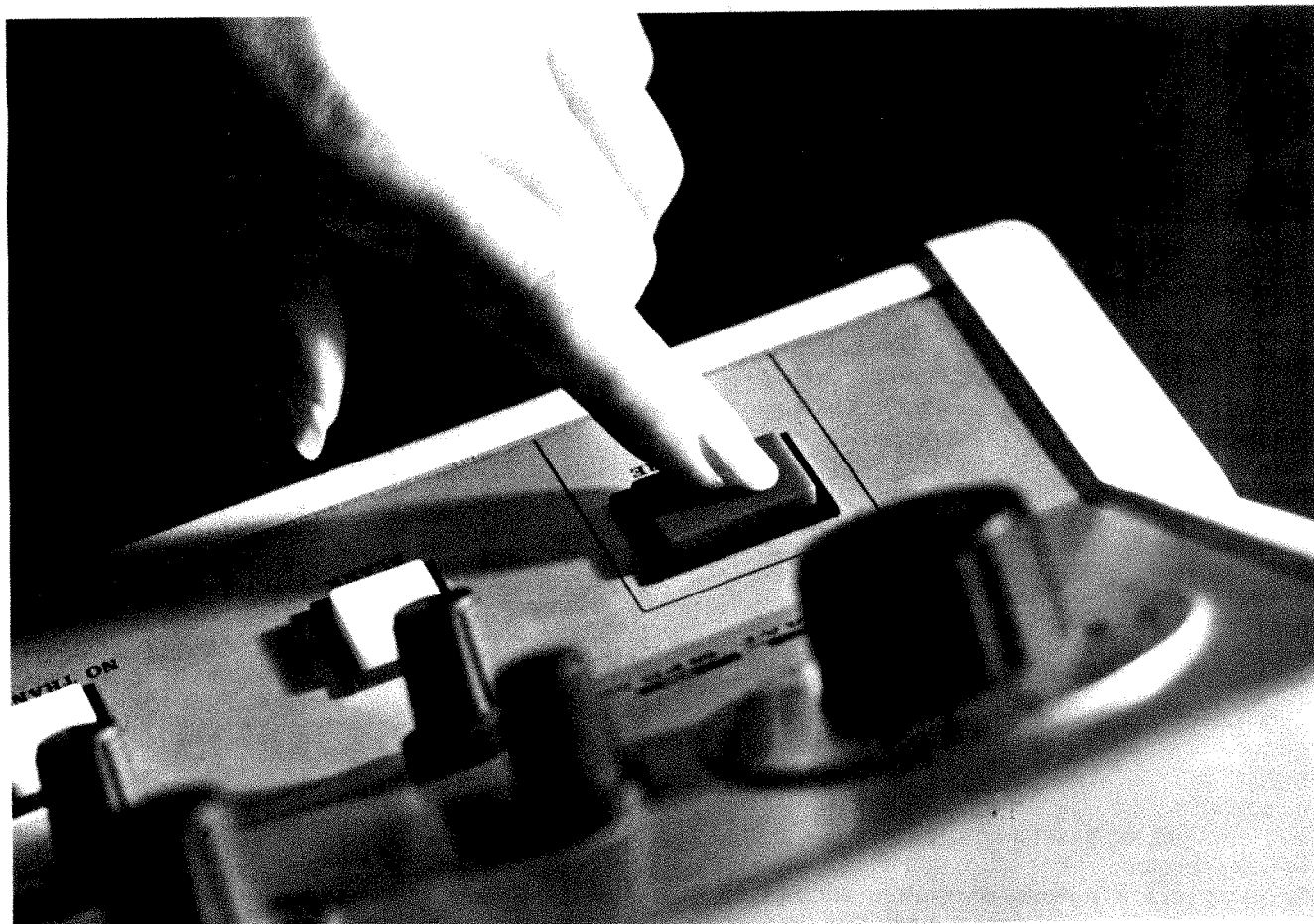
Institute of Biomolecular Stereodynamics
and Department of Chemistry,
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Albany, New York 12222

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Matters Arising

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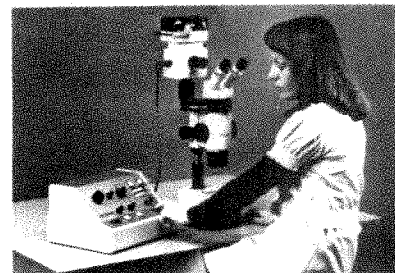


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Bibliography of Ab Initio Molecular Wave Functions

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W. G. Richards, P. R. Scott,
E. A. Colbourn, and A. F. Marchington

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reviews

Historical cetology

J. H. S. Blaxter

The Natural History of the Whale. By L. Harrison Matthews. Pp. 219. (Weidenfeld and Nicolson. London, 1978.) £12.50.

THE *World Naturalist Series* (not to be confused with the *New Naturalist Series*) continues with a book on whales by the editor of the series. There are nine chapters—on Cetology from its Beginnings, Cetacean Diversity, Food and Feeding, Breeding and Growth, Swimming and Diving, Migration, Communication and Echolocation, Behaviour and Social Relations, Parasites, Diseases and Enemies—followed by a list of living species, a bibliography and index.

The book is very readable and is especially strong on the historical aspects of research on whales. Unlike so many subjects where the older literature tends to become obsolete, modern cetology depends very much on the earlier work. One of the reasons for this lies, alas, in the appalling over-exploitation of whales, so that many species are protected and some may be near extinction. Thus, material, often available in the past only through the whaling industry, is no longer obtainable. The discoveries made have been impressive, especially when one considers the difficulty of handling such large specimens, the problem of rapid decomposition before they can be dissected and the need, often, to play 'second fiddle' to the whale-catchers. Much of the more recent discoveries on whale physiology and behaviour come from the smaller cetaceans such as the dolphins which are fairly easy to catch and keep in captivity. The building of oceanaria has also made it possible to watch and train larger cetaceans such as the Killer Whale. Speculation about echolocation, feeding habits, buoyancy mechanisms and the problems of deep diving have come from the study of so-called functional anatomy.

This all makes fascinating reading. Everything is on a massive or record scale. The blue whale is the largest living animal; whales make migrations of 28,000–30,000 kilometres and can 'sprint' at 20 knots; sperm whales can dive to 3,000 feet and become entangled in submarine cables; the Antarctic whales in their heyday ate 150 million tons of krill annually; some

whales have tape-worms which are 50 feet long!

I found the style of the book rather uneven. The author expects a fairly high standard of knowledge from the reader when discussing some of the anatomy, especially that of the skull, yet elsewhere, for example, he discusses the genetic code, DNA and the philosophy of immortality (pages 76 and 137). In general the diagrams, though clear, are inadequately labelled and have no scale given. There are 16 pages of black-and-white plates which cannot fail to fascinate the reader. One aerial photograph of a school of white whales in the Canadian Arctic showing adults and calves is especially interesting.

The author is decidedly acerbic about some of his predecessors and of some later investigators. On page 28 he gives very short shrift to *Nessiteras rhombopteryx* P. Scott (1975) the "non-animal of Loch Ness" (reported in *Nature* 258, 466, 1975). On page 101 he castigates the owners of pets who recycled whale meat as dog-droppings on city pavements. He is rightly uncompromising in his criticism of anthropomorphism in our interpretation of cetacean behaviour. For example, the explanation of the apparent saving of

human swimmers in distress by dolphins lifting them to the surface almost certainly lies in the lifting of newly-born calves by their mothers to the surface for their first breath of air. Other instances of relationships between cetaceans and man such as the riding of ships' bow-waves can easily be explained as advantageous to the animal, which saves energy as it utilises the bow-wave to push itself along. Although the author is not in possession of the results of naval research on the use of dolphins for underwater warfare, he thinks their intelligence has been overestimated and their potential is limited. If dolphins were really intelligent would they not take every opportunity "to pull a human swimmer in difficulties down to death" considering that man has been a ruthless destroyer of dolphins for centuries?

Dr Harrison Matthews' book is quite different in style from two recent books on sea mammals reviewed in *Nature* (270, 641, 1977). It deals only with cetaceans and in greater detail. I would rate it as reading for any biologist or well-informed laymen. □

J. H. S. Blaxter is Senior Principal Scientific Officer at the Dunstaffnage Marine Research Laboratory, Oban, Argyll, UK.

Summary of rheological activity

Mechanics of Non-Newtonian Fluids. By W. R. Schowalter. Pp. 300. (Pergamon: Oxford, 1978.) £17.50.

THIS text, directed toward graduate students and others beginning research in rheology, begins with a fine motivational chapter 1 and then launches into a five-chapter development of mathematical and physical foundations in terms of tensor analysis. This segment, rigorous but rather dry for students, would require elaboration by an instructor to be fully successful.

The following three chapters are designed to describe rheometric and related flows; Noll's simple-fluid concept is used throughout. Chapter 7 introduces the idea of viscometric flows and provides several illustrations. The equations of continuity and motion for incompressible fluids, in the three major coordinate systems, are

appended. Measurements of the viscometric functions are described in chapter 8, in which numerous flows are studied: plane and cylindrical Couette flow, axial motion in open and annular tubes, jet methods (jet swell, thrust on tube), and the Jackson-Kaye elevated cone procedure. A survey of experimental difficulties is given separately, including end effects, viscous heating, and hole error. Chapter 9 emphasises stretch-history concepts, specialising to extensional flow and perturbations from viscometric flows.

Model-building is encountered in chapters 10 and 11. Elementary models—purely viscous, generalised Newtonian, and linear viscoelastic (differential and integral) fluids—are found in chapter 10. For the first time molecular concepts are introduced, through the dilute-solution polymer dynamics theory of Zimm. The latter treatment is

somewhat patchy and needs to be read with the five chapter appendices to be coherent. The appendices describe Gaussian springs, Brownian motion, hydrodynamic interaction, stress tensor construction, and finally full solution of the eigenvalue problem. (It is unfortunate, however, that the relaxation time τ_r is implied on p155 to represent the response of the p/h sub-molecule rather than the p/h normal mode.) Chapter 11 generalises this material in various ways, including a description of the Oldroyd approach and the use of non-linear time derivatives in modelling. Commentary on many of the models is so brief that a trek to the cited literature is really required.

Chapter 12 on applications comes close to several engineering problems. Again, the treatment is a survey, but this reader found it quite successful: boundary layer theory, converging flow, falling sphere, extensional motion, flow in packed beds, transients, secondary motion, stability theory, extruder analysis, orthogonal rheometer and

inclined open channel. Chapter 13 concludes the book with another excursion into the microscopic, 'Suspension Rheology'. Both rigid and deforming particles are considered, with most detail given to dilute emulsions.

Arising from notes used by Professor Schowalter in over ten years of teaching, the book contains homework problems appended to 11 of the 13 chapters and ample references. Few data are given—only three figures show data points, although there are many schematic drawings—and notation is cumbersome in places. However, the writing style is clear and contains a fine flavour of good humour, and relatively few misprints have crept in. One is advised to ignore the purple and lavender cover and use this text as a guide to the wide variety of rheological activity during the modern transition of 1950–75.

Michael C. Williams

Michael C. Williams is Professor in the Department of Chemical Engineering at the University of California, Berkeley.

Atomic absorption spectroscopy

Atomic Absorption, Fluorescence and Flame Emission Spectroscopy: A Practical Approach. By K. C. Thompson and R. J. Reynolds. Pp. 319. (Charles Griffin: London and High Wycombe, UK, 1978.) £15.

THE second edition of this book, first published in 1970, includes coverage, in varying detail, of the very extensive advances which have been made in atomic spectroscopy in the intervening years. In some areas, however, the extent of the coverage leaves something to be desired. There is commendable emphasis on safety, of which the reader should take careful note: unfortunately, burner explosions are not unknown.

The book can be considered in two main sections, one being principally chemical and the other instrumental. The chemical section covers very thoroughly the areas of applications, chemical techniques and interferences in atomic absorption spectroscopy (AAS), although some of the instrumental procedures described tend to be somewhat dated. For instance, the inexperienced reader would be forgiven for thinking that digital readout is a rare feature, whereas, in fact, it is incorporated in nearly all present-day instruments. It is also noticeable that strong emphasis is given to the relatively little used hydride technique,

whereas the very important area of flameless electrothermal AAS is relegated to the chapter on "Some further techniques"; and it should be mentioned that some authorities claim considerably lower detection limits for

Molecular models

Molecular and Crystal Structure Models. By A. Walton. Pp. 201. (Ellis Horwood: Chichester, UK, 1978.) £9.

MAKING models of the physical world is the stuff of science. Extending and refining these models is what we are doing when we do research. Nowhere is the concept of models more evident than in chemistry. Both at the detailed level of molecular orbitals and, more commonly, in the consideration of three dimensional structure we consider molecules to be representable by models which we can imagine actually constructing as tangible objects.

Dr Walton's book surveys most of the commercially available molecular models and contains advice on do-it-yourself versions. The survey is not exhaustive (an exhaustive survey would soon date), but it is sufficiently wide to be a useful first step for anyone looking for a particular feature.

Models showing special features or behaving in special ways are commonly constructed in research and this book will provide useful tips for that would-be modeller. It is, however, to

some elements than those given in the table on pages 33 and 34.

On the instrumental side, it seems that the authors have a bias against double-beam instruments and the reader is advised to be cautious in accepting the advice given. It is also felt that the guidance on performance parameters could be clearer for the inexperienced user. Again, it is noticeable that only brief mention is made of microprocessor-based instruments, although these now represent a high proportion of those available.

Flame emission and atomic fluorescence spectroscopy are covered in a separate chapter, primarily from the point of view of comparison with AAS. In this respect, an unfortunate weakness is the fact that the very important and competitive (with AAS) technique of plasma emission is dismissed in five lines.

A very extensive bibliography is given at the end of each chapter, but it might have been appropriate to mention that a complete atomic absorption bibliography is available. Overall, the book can be recommended to both new and more experienced users of AAS from the chemical standpoint but with reservations on the instrumental side.

M. A. Ford

M. A. Ford is Technical Director of Perkin-Elmer Limited, Beaconsfield, UK.

the teacher that it will most appeal, providing as it does descriptions of most of the off-the-shelf models, and photographs of many. In it one should be able to find the (non-ideal) model which is a good compromise and which can be provided within the departmental budget for a class of students.

Apart from being inexhaustive, the book suffers from, inevitably, describing each model in a different way. It is particularly unfortunate that it was not possible to have photographs of every model type. This makes it difficult to use for searching out models of a particular type, but it does make it readable. The principal deficiency is, however, the lack of information on consumer-response—is it easy for students to see the point the lecturer is making? Do the students like building these models and do they understand the principles that they are supposed to demonstrate? What are their deficiencies? Perhaps the only answer, as every teacher wishes to emphasise different aspects, is to use this book (with its extensive cross-referencing) to narrow down the choice and then try the models oneself.

S. D. Dover

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Sedimentary environments

Ancient Sedimentary Environments. Second edition. By R. C. Selley. Pp. 287 (Chapman and Hall: London, 1978). Hardback £8; paperback £4.95.

THIS is an inexpensive book, in which the essential features of the major sedimentary environments are succinctly described. In a book of 287 pages, the treatment does not attempt to be detailed and each synthesis is supported by a generally well chosen list of references. The references, which form 17% of the text, are one of the strengths of the book, for they provide either the student or the experienced geologist with basic references up to 1977. Chapters cover, sedimentary environments and facies; river deposits; wind-blown sediments; lake deposits; deltas, linear clastic shorelines; mixed clastic; carbonate shorelines; shelf deposits; carbonate and terrigenous; reefs; flysch and turbidites; pelagic deposits, and sedimentary models, mythical and mathematical. As might be expected, the emphasis reflects the author's wide field experience, but the omission of glacial sediments is surprising.

The opening chapter contains definitions and discussions of a sedimentary environment and facies. Although brief, the discussion is a clear statement of important basic concepts. For instance—"One of the main problems of determining the origin of ancient sediments is that, though essentially reflecting depositional environments, they also inherit features of earlier erosional and non-erosional phases"—a truism which gives rise to many problems in interpretation.

The author points out that it is only sedimentary structures (and palaeocurrents) which unequivocally indicate the depositional environment. The five defining parameters of a sedimentary facies—geometry, lithology, sedimentary structures, palaeocurrents and fossils—are examined in an instructive way, flavoured with many cautions derived from experience.

In many cases, environmental interpretation must be based on borehole or seismic data, so the inclusion is very appropriate of a general section on the use of vertical grain-size profiles from geophysical logs and the use of the down-hole dipmeter.

The whole text is intended as a practical guide to "environmental interpretation [which] is at best an imprecise art, rather than a deterministic scientific discipline", though the author's treatment is a good preparation for

travelling along the latter route. Generally, each chapter has a main section covering one or more case histories, followed by sections giving wider views of the particular sedimentary facies, its economic significance, subsurface recognition and finally a list of references. Numerous clearly presented diagrams reduce the need for lengthy descriptions.

By its very nature, the book is a compilation of generalisations, but these are made by a perceptive geologist who continuously reminds his readers that the exceptions are the

really interesting problems. The whole presentation is admirably clear and concise, making it a most suitable book for a second-year university course. Geologists at all levels will gain much information from the eminently readable and witty style, but the most important impact is the encouragement of a discriminating and critical sifting of geological evidence. At such an economical price, the book is a good investment.

D. Hamilton

D. Hamilton is Senior Lecturer in Geology at the University of Bristol, UK.

Physiology of fungal colonies

Practical Fungal Physiology. By P. M. Robinson. Pp. 122. (Wiley: Chichester, UK, and New York, 1978.) £3.50.

ONE can only agree with the author's opening statement in the preface to this book that "The potential of fungi as experimental material is not always fully exploited". This book provides a series of experiments designed to introduce a student to some basic aspects of fungal physiology. There are nine chapters and the suggested experiments are described in each chapter within an overall account of the cellular processes involved. Topics dealt with include spore germination, hyphal growth, fungal nutrition, morphogenetic substances, staling, colony morphology, mycostasis and continuous culture. The experiments described in most chapters are very basic and have been designed to utilise a minimum of expensive equipment. There are references to original papers in the relevant areas and figures are included to

illustrate the expected results of certain experiments. Experiments are described in flowsheet form and adequate details are provided for media recipes, inoculation times and general experimental protocols.

Overall, this simplified approach should provide a reasonable introduction to the physiology of the fungal colony. It is, perhaps, unfortunate that many of the experiments involve aspects of fungal physiology of whose cellular and biochemical mechanisms we have little understanding. This limitation is compounded by the omission of experiments involving well understood phenomena such as the phototropic responses of *Phycomyces*.

Despite these criticisms the book should prove useful to school and university teachers looking for ideas to be included in an introductory course on mycology. Also, if the book helps move the teaching of mycology away from the classical taxonomic approach, then it is to be welcomed.

Keith Gull

Keith Gull is Lecturer in Microbiology at the University of Kent, Canterbury, UK.

Molecular dynamics

Molecular Structure and Dynamics. By W. H. Flygare. Pp. 696. (Prentice-Hall: Englewood Cliffs, New Jersey, and Hemel Hempstead, UK, 1978.) £18.25.

ONE can tell from the front leaf of the dustcover that this is a crowded treatise. There is the ^{13}C -NMR spectrum of a methyl group with its free induction decay, the ESR spectrum of the cyclopentyl radical, the Raman spectrum of CO_2 , the low resolution IR spectrum of CH_2Cl_2 , the radial wave function of the 1^{-5s} orbital, a coordinate system for electron scattering from a triatomic molecule and another relating laboratory and molecular axes, and the X-ray structure factor for

metallic potassium. If the back leaf had been used to show the electron density difference plot of HF, the splitting pattern of a ^2D state in an octahedral field (with spin-orbit interaction), the Doppler spectrum of interstellar formaldehyde and the symmetry elements of CH_4 , then one could have dispensed with the statement of contents.

The book would be suitable for a BSc honours course in chemical physics or for a postgraduate course for spectroscopists. It is a well written book and I found little to quarrel with in either the contents or their presentation.

I must, however, admit to a preference for shorter books on a narrower front. Seven hundred pages is indigestible and I cannot envisage ever starting such a book and getting to the end. Of course, there are books on my shelf that I use just for reference, and per-

haps this will be added to their number. Professor Flygare clearly hopes for more—for example, by challenging the reader with problems at the end of each chapter.

The difficulty with an epic work is that one dare not be discursive or one cannot get it all between two hard covers. Let me take, for example, the section on scattering in this book. The fifty pages devoted to this topic is sufficient to include topics such as vibrational and rotational inelastic scattering or the relationship between the potential energy surface for a reaction and the energy distribution in the products.

Mitochondrial and plastid heredity

Organelle Heredity. By N. W. Gillham. Pp. 602. (Raven. New York, 1978.) \$64.35.

NON-MENDELIAN HEREDITY has been the Cinderella of genetics ever since Correns discovered it exactly 70 years ago. It has long been complicated, confused and experimentally intractable. It is still complicated, but powerful new techniques, ably described in this new book by one of their chief wielders, are rapidly reducing the confusion.

For over a decade it has been clear that there are two fundamentally different causes of non-Mendelian heredity. One of these, the direct inheritance of preformed cell structure—seemingly independently of DNA—is well established only for the ciliate cortex. The more widespread cause is the existence of DNA inherited other than in a Mendelian fashion. Sometimes such DNA is nuclear—B-chromosomes are the most obvious example—but more usually it is cytoplasmic. Although there are numerous scattered cases where cytoplasmic DNA constituting the genome of intracellular viruses or cellular endosymbionts causes non-Mendelian inheritance, cytoplasmic DNA occurs regularly only in plastids and mitochondria; the genetics of these, not of organelles in general, is the subject of Gillham's excellent book.

The book is the most thorough treatment yet of mitochondrial and plastid genetics, and deserves to be read widely by geneticists, biochemists, cell biologists and advanced students. Although it excludes evolutionary discussions, it will be invaluable to all interested in organelle evolution. Fifteen of the twenty-one chapters concentrate on transmission genetics, the four on baker's yeast and the four on *Chlamydomonas* forming the heart of the book.

There is no mention of the Born approximation or of semi-classical quantisation (apart from the Bohr atom). The book would not be sufficient in itself for a postgraduate course on molecular dynamics and would be a little thin even for an advanced undergraduate course on this topic.

Perhaps I am being petty. It is in the main a fine work which I shall find very useful. I doubt, however, that it will be adopted as a course book in many British universities. J. N. Murrell

J. N. Murrell is Professor of Chemistry at the University of Sussex, Brighton, UK.

Nuclear transmission genetics was substantially worked out within a single decade of Morgan's first concentration on *Drosophila*. Yet, although Ephrussi and Sager made yeast and *Chlamydomonas* the *drosophilas* of cytoplasmic heredity decades ago, we still do not understand it. This is because until the past decade the characteristic uniparental inheritance of organelle genes, and the scarcity of different mutants, precluded traditional genetic analysis of segregation and recombination. Much of the recent progress stems from strictly genetic advances—the ability to obtain biparental inheritance and to induce and isolate a greater variety of mutants. This has led to an impressive array, often bewildering to the outsider, of new mapping techniques; and it is valuable to have them so clearly laid out in one place.

The discovery of organelle DNA has also been important, especially on the psychological side as a booster of the intellectual respectability of the field. Wherever possible Gillham integrates the molecular and genetic approaches, but where this cannot be done, as in the conflicting data on the inheritance of *Chlamydomonas* chloroplast DNA, he simply describes the basic data. His account of the conflicts between Sager's work and his own are admirably balanced. Particularly valuable are the comparisons between the yeast and *Chlamydomonas* work. Although yeast is simpler and more intensively studied, in the long run *Chlamydomonas* will be ideal for studying the interactions between biparentally inherited nuclear DNA, 'maternally' inherited plastid DNA and its 'paternally' inherited mitochondrial DNA. ('Maternal' and 'paternal' are peculiarly inappropriate for *Chlamydomonas* which is isogamous and has an organelle like the acrosomal tubule of sperm on its so-called 'maternal' gametes.)

Mitotic segregation prevents enumeration by the *cis-trans* test of the polypeptides coded by organelle DNA,

but genetic techniques can help in assigning specific gene products to nuclear or organelle DNA. Gillham ably describes the combined genetic and biochemical study of mutants, the use of interspecific hybridisation and the analysis of mammalian cell hybrids in tissue culture. DNA hybridisation studies of mules and hinnies have shown mitochondrial DNA to be maternally inherited, as in fungi. Here, however, the mechanism may simply be dilution of sperm mitochondria by the vast excess of female ones in the egg. In fungi, many of which are isogamous like *Chlamydomonas*, this cannot be so; curiously, twelve years after I discovered chloroplast fusion in *Chlamydomonas* the cytological fate of mitochondria in fungal zygotes is still unknown, although in 1978 Gaffal demonstrated mitochondrial fusion in zygotes of the alga *Polytoma*. As Gillham points out, cytology is lagging behind genetics and biochemistry.

Although emphasising genetics, Gillham also summarises present biochemical, cytological and physiological knowledge of the structure and functions of organelle DNA. The first chapter is an up-to-date outline of the structure and function of plastids and mitochondria. It is followed by one on the structure and replication of mitochondrial and chloroplast DNA, including brief accounts of the underlying techniques. The two chapters on the RNA- and protein-synthetic machinery of plastids and mitochondria, and their biogenesis, describe the evidence that organelle RNA is coded by organelle DNA; the majority of proteins, on the other hand, including nearly all the ribosomal ones, are coded in the nucleus and synthesised outside the organelles.

The remaining two chapters discuss the biosynthesis of the mitochondrial inner membrane and the thylakoids. Here also most of the proteins are coded by the nuclear DNA; only a handful are yet known to be coded by the organellar plasmids. I suspect that the bulk of this DNA—like nuclear DNA—does not code for proteins. The pace of advance of both the genetic and the biochemical methods Gillham so clearly describes is such that we shall soon know; things too recent to be included are constantly being discovered, like the presence of split genes in both mitochondria and plastids.

This well produced book, with its ample references, is a milestone in the history of genetics.

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- PICK, Jr., Herbert L., LEIBOW TZ, Herschel W., SINGER, Jerome E., STEINSCHNEIDER, Alfred, and STEVENSON, Harold W. (edited by). Psychology: From Research to Practice. Pp.xi + 390. ISBN-0-306-31132-1. (New York and London: Plenum Press, 1978.) £11.93.
- PINCUS, Jonathan H., and TUCKER, Gary J. Behavioural Neurology. Second edition. Pp.xix + 284. ISBN-0-19-502306-4. (Oxford, London and New York: Oxford University Press, 1978.) np.
- POSNER, Michael I. Chronometric Explorations of Mind. (The Experimental Psychology Series.) Pp.xiii + 271. ISBN-0-470-26491-8. (Hillsdale, NJ.: Lawrence Erlbaum Associates, Publishers, 1978. Distributed by Haisged Press, a Division of John Wiley and Sons, New York and Chichester.) £10.50.
- THOMPSON, Robert. A Behavioural Atlas of the Rat Brain. Pp.ix + 80. ISBN-0-19-502269-6. (Oxford and New York: Oxford University Press, 1978.) £6.75 net.

announcements

Awards

The following have been elected Fellows of the Royal Society:

Professor John Robert Laurence Allen, Professor of Geology in the University of Reading; **Professor Michael Farries Ashby**, Professor of Engineering Materials in the University of Cambridge; **Professor Evelyn Martin Lansdowne Beale**, Technical Director of Scicon Computer Services Limited; **Professor John Maxwell Cowley**, Professor of Physics at Arizona State University; **Professor Bernard Crossland**, Professor, and Head of the Department, of Mechanical and Industrial Engineering in the Queen's University, Belfast; **Professor Durward William John Cruickshank**, Professor of Chemistry in the University of Manchester Institute of Science and Technology; **Dr Michael Joseph Crumpton**, Head of the Division of Biochemistry at the National Institute for Medical Research, London; **Professor John Hilton Edwards**, Professor, and Head of the Department, of Clinical Genetics and Professor of Human Genetics in the University of Birmingham; **Dr Michael Elliott**, Senior Principal Scientific Officer at Rothamsted Experimental Station; **Professor Michael Anthony Epstein**, Professor, and Head of the Department, of Pathology in the University of Bristol; **Professor William Charles Evans**, Professor, and Head of the Department, of Biochemistry and Soil Science in the University College of North Wales, Bangor; **Dr Raymond Freeman**, University Lecturer in Physical Chemistry in the University of Oxford; **Professor Richard Lavenham Gardner**, Henry Dale Research Professor of the Royal Society at the University of Oxford; **Professor Alastair Graham**, Emeritus Professor of Zoology in the University of Reading; **Dr Cyril Hilsum**, Chief Scientific Officer in the Physics Group at the Royal Signals and Radar Establishment, Malvern; **Professor David Alan Hopwood**, John Innes Professor of Genetics in the University of East Anglia and Head of the Genetics Department of the John Innes Institute; **Dr Keith Usherwood Ingold**, Associate Director of the Division of Chemistry in the National Research Council of Canada, Ottawa; **Dr Edward Irving**, Geophysicist in the Department of Energy, Mines and Resources, Ottawa, Canada; **Professor Geoffrey**

Melville Jones, Professor of Physiology and Director of the Defence Research Board Aviation Medical Research Unit, McGill University, Montreal, Canada; **Professor Devendra Lal**, Director of the Physical Research Laboratory, Ahmedabad, India; **Professor Michael Franz Lappert**, Professor of Chemistry in the University of Sussex; **Professor Ian Grant Macdonald**, Professor of Mathematics at Queen Mary College in the University of London; **Dr Digby Johns McLaren**, Director-General of the Geological Survey of Canada, Ottawa; **Professor Robert McCredie May**, Professor of Jersey; **Patrick Anthony Merton**, Reader in Human Physiology in the University of Cambridge; **Professor Brenda Milner**, Professor of Psychology at the Montreal Neurological Institute, Canada; **Professor Kenneth Murray**, Professor of Molecular Biology in the University of Edinburgh; **Dr Denis Noble**, Lecturer in Physiology in the University of Oxford; **Professor Martin John Rees**, Plumian Professor of Astronomy and Experimental Philosophy in the University of Cambridge; **Professor Paul Henry Roberts**, Professor of Applied Mathematics in the University of Newcastle upon Tyne; **Professor Milton Robert James Salton**, Professor of Microbiology at New York University; **Professor Harry Smith**, Professor, and Head of the Department, of Microbiology in the University of Birmingham; **Dr Godfrey Harry Stafford**, CBE, Director of the Rutherford Laboratory, Chilton, Didcot; **Professor John Griggs Thompson**, Rouse Ball Professor of Mathematics in the University of Cambridge; **Professor David James Thouless**, Professor in the Department of Engineering and Applied Science, Yale University, Connecticut; **Dr Winifred Anne Tutin**, Principal Scientific Officer of the Freshwater Biological Association, Ambleside, and Honorary Reader in Botany in the University of Leicester; **Dame Janet Maria Vaughan**, DBE, formerly Principal of Somerville College, Oxford; **Professor David Alan Walker**, Professor, and Head of the Department, of Biology in the University of Sheffield; **Professor Janet Vida Watson**, Professor of Geology at the Imperial College of Science and Technology in the University of London; **Professor Olgierd Cecil Zienkiewicz**, Professor, and Head of the Department, of Civil Engineering in the University College of Swansea, Wales.

The Council of The Institute of Physics has made the following awards for 1979: Bragg Medal and Prize to **Sister Maureen Hurst** of La Retraite High School, London; Charles Chree Medal and Prize to **Professor J. T. Houghton** of the University of Oxford; Charles Vernon Boys Prize to **Dr D. C. Robinson** of the Culham Laboratory; Duddel Medal and Prize to **Dr J. R. Sandercock** of the Laboratories RCA, Ltd, Zurich; Glazebrook Medal and Prize to **Dr T. G. Pickavance**, formerly of the Rutherford Laboratory; Guthrie Medal and Prize to **Professor D. H. Perkins** of the University of Oxford; Maxwell Medal and Prize to **Dr. C. H. Llewellyn Smith** of the University of Oxford; Thomas Young Medal and Prize to **Professor C. Cohen-Tannoudji** of the Collège de France, Paris.

The Clough Medal for 1978-79 has been awarded to **Professor M. H. P. Bott**, Department of Geological Sciences, University of Durham, in recognition of distinguished contributions towards the elucidation of geological structures by geophysical means in and around Scotland and the North of England.

The Clough Award for 1978-79 has been conferred on **Dr D. J. Fettes** of the Institute of Geological Sciences, Edinburgh, in appreciation of his work on the metamorphism of the Dalradian rocks of Scotland.

The William Hopkins Prize of the Cambridge Philosophical Society has been awarded to **Dr D. P. McKenzie**, of King's College, for his work on the theoretical geophysics of the solid earth, with particular reference to plate tectonics.

The Society for the History of Alchemy and Chemistry, has awarded the 1978 Partington Prize to **Dr Reinhard Löw** of the Institut für Philosophie der Universität München.

The Kurt P. Richter Prize has been established for meritorious research in the area of psychoneuroendocrinology. This essay prize, the sum of \$1,000 will be awarded annually for the best manuscript or essay submitted by a scientist or physician under 35 years of age. Write to: Dr Fleur L. Strand, Secretary, International Society of Psychoneuroendocrinology, New York University, Washington Square, New York, New York 10003.

The Trustees of the Lady Tata Memorial Trust invite applications for Awards (both predoctoral and postdoctoral) for research on leukaemia, in the Academic Year beginning October 1979. Write to the Secretary of the (European) Scientific Advisory Committee, Lady Tata Memorial Trust, Chester Beatty Research Institute, Fulham Road, London SW3, UK.

The Douglas Bomford Trust announces the offer of a Travelling Scholarship in Agricultural Engineering to be awarded in the year 1979-80. Write to The Institution of Agricultural Engineers, West End Road, Silsoe, Bedford, UK.

One or two Unilever European Fellowships will be awarded for the academic year 1979-80, for either (a) research in biochemistry in a laboratory in continental Europe by British citizens resident in Great Britain or (b) work in Britain by European nationals. Applicants should hold a PhD degree or offer evidence of having equivalent qualifications or experience. The Fellowships will normally be within the UK University Lecturer scale. Write to the Executive Secretary, The Biochemical Society, 7 Warwick Court, London WC1, UK.

The 1979 Nikon's Small World Photomicro/macrography Contest is open to everyone interested in scientific and industrial photography. The subject matter is unrestricted; any type of specimen is acceptable. Any photomicro/macrography technique may be used, including phase contrast, polarised light, fluorescence, dark field, interference, and reflected light. This year's deadline will be 2 July 1979, and the first-prize award is a \$1,500 vacation trip or \$1,500 worth of Nikon instruments or photographic equipment. For a copy of the official contest rules and entry form, write to Ms Susie Weiss, Nikon's Small World Competition, Nikon Incorporated, Instrument Division, 623 Stewart Avenue, Garden City, New York 11530.

The Roussel Prize is given every two years to a chemist or a biochemist whose work has been chosen as the most outstanding by an international committee of distinguished scientists in the field. The next Prize (\$20,000) is scheduled for April 1980, and will be concerned with outstanding work on Chemistry or Biochemistry of Steroids and other Squalenoids published before 30 September 1979. Candidates may be of any nationality and from any laboratory. They should be introduced by a person of high scientific standing and supported by two other referees. Write to Professor J. Mathieu, Centre de Recherches, Roussel Uclaf, 93230 Romainville, France.

Meetings

2-6 April, **Appropriate Energy Technology for Development**, Glasgow (Dr J. Twidell, Dept of Applied Physics, University of Strathclyde, Glasgow, UK).

17 April, **Pain—New Trends in Research and Management**, New York (Eugene and Estelle Kone, American Pain Society, 280 Knollwood Drive, New Haven, Connecticut 06515).

19 April, **Gas Injection into Liquid Metals**, Newcastle upon Tyne (Dr A. E. Wraith, Dept of Metallurgy and Engineering Materials, The University, Newcastle upon Tyne, UK).

23-27 April, **Remote Sensing of the Environment**, Ann Arbor (The University of Michigan Extension Service, Dept of Conferences and Institutes, 412 Maynard St, Ann Arbor, Michigan 48109).

8-11 May, **International Symposium on Personal Protective Equipment**, Torremolinos (Servicio Social de Higiene Y Seguridad del Trabajo, Instituto Territorial, Apartado de Correos 615, Sevilla, Spain).

15 May, **Mechanisms of Ion Selectivity**, Aberdeen (17 May, Cambridge) (Faraday Division, The Chemical Society, Burlington House, London W1, UK).

22 May, **Transport across Interfaces**, Bristol (Faraday Division, The Chemical Society, Burlington House, London W1, UK).

18-19 June, **Virus-Lymphocyte Interactions: Implications for Disease**, Cleveland (Max R. Proffitt, Dept of Immunology, Cleveland Clinic Foundation, Cleveland, Ohio 44106).

2-3 July, **Laboratory Safety in Higher Education**, Manchester (Mrs S. Leclercq, The Chemical Society, Burlington House, Piccadilly, London W1, UK).

2-6 July, **Management Studies for Production Chemists**, Slough (Mrs S. Leclercq, The Chemical Society, Burlington House, Piccadilly, London W1, UK).

4-6 & 10-13 July, **4th National Oceanographic Symposium**, Johannesburg & Cape Town (Symposium Secretariat S.189, CSIR Conference Division, PO Box 395, Pretoria 0001, South Africa).

6-18 July, **Automation in Industrial and Clinical Chemistry**, London (Scientific Symposia Ltd, UTP House, 33-35 Bowling Green Lane, London EC1, UK).

9-13 July, **Automatic Methods of Analysis**, Swansea (Mrs S. Leclercq, The Chemical Society, Burlington House, Piccadilly, London W1, UK).

10 July, **Remote Measurement of Sea-State and Oceanic Winds**, Birmingham (Royal Meteorological Society, James Glashier House, Grenville Place, Bracknell, Berkshire, UK).

17 July-19 August, **Techniques of Molecular Biology**, Santiago (Dr Jorge E. Allende, Casilla 6671, Santiago 7, Chile).

15-22 July, **Workshop on Bioethics and Public Policy**, New York (Institute of Society, Ethics and the Life Sciences, 360 Broadway, Hastings-on-Hudson, New York 10706).

19-20 July, **Heat Treatment—Methods and Media**, Birmingham (The Meetings Secretary, The Institute of Metallurgical Technicians, Northway House, Whetstone, London N20, UK).

23-25 July, **Isotopes: Essential Chemistry and Applications**, Surrey (Mrs S. Leclercq, The Chemical Society, Burlington House Piccadilly, London W1, UK).

13-24 August, **The Scientific and Mathematical Foundations of Engineering Acoustics**, Massachusetts (Office of the Summer Session, Room E19-356, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139).

15-17 August, **20th Canadian High Polymer Forum**, Quebec (Dr Ian McEwan, CIL Paint Research Laboratory, 1330 Caslefield Ave, Toronto, Ontario, Canada M6B 4B3).

Reports & Publications

Other countries—February

United States Department of the Interior. Geological Survey, Bulletin 1455: *Climatic Implications of Two Pollen Analyses from Newly Recognized Rocks of Latest Pliocene Age in the Washburn Range, Yellowstone National Park, Wyoming*. By Gerald M. Richmond, William Mulleenders and Mady Coromano. Pp. iii+13. (Washington, DC: US Government Printing Office, 1978.) [192]

Dictionary of Biomedical Acronyms/Dictionnaire d'Acronymes Biomedicaux, Anglo-Américains. Par Jacques Dupuyrat. Pp. 109. (Paris-La Défense 2: Librairie SCM, Place des Reflets, Centre Buref, 1978.) [202]

Geological Survey of Canada. Bulletin 253: *Alban Foraminifera of the Yukon Territory*. By T. P. Charnay. Pp. 62 (11 plates). (Ottawa: Geological Survey of Canada, 1978.) \$9 [202]

Unesco Technical Papers in Marine Science, No. 29: *Committee for the Preparation of CLOFETA/Comité de Préparation du CLOFETA Report of the First Meeting*, Paris, 16-18 January 1978/Rapport de la Première Réunion, Paris, 16-18 Janvier, 1978. Pp. 55. (Paris: Unesco, 1979.) [212]

Postharvest Food Losses in Developing Countries (Board on Science and Technology for International Development, Commission on International Relations) Pp. viii+206. (Washington, DC: National Academy of Sciences, 1978.) [222]

World Health Organization Technical Report Series, No. 629: *The Application of Advances in Neurosciences for the Control of Neurological Disorders—Report of a WHO Study Group*. Pp. 83. (Geneva: WHO, London: HMSO, 1978.) Sw fr.9 [222]

American Museum of Natural History 109th Annual Report, 1977/1978. Pp. 44. Report of the Scientific and Education Departments, July 1977 through June 1978. Pp. 38. (New York: American Museum of Natural History, Central Park West at 79th Street, 1978.) [232]

United States Department of the Interior: Geological Survey. Professional Paper 813-P: *Summary Appraisals of the Nation's Ground-Water Resources—Alaska*. By Chester Zeno and Gary S. Anderson. Pp. vi+28. Professional Paper 932-B: *Eocene Rocks, Fossils, and Geological History, Teton Range, Northwestern Wyoming*. By J. D. Love, Estelle B. Leopold and D. W. Love. Pp. iii+40+4 plates. (Washington, DC: US Government Printing Office, 1978.) [262]

Research in Norway, 1978. Pp. 84. (Oslo: Research in Norway, Munchbeigt, 29, 1979.) [272]

Australia. Department of National Development Bureau of Mineral Resources, Geology and Geophysics Bulletin 170: *Sedimentology and Holocene History of a Tropical Estuary (Broad Sound, Queensland)*. By P. J. Cook and W. Mayo. Pp. x+206. (Canberra: Australian Government Publishing Service, 1977.) [272]

United States Department of the Interior: Geological Survey. Water-Supply Paper 2054: *Effectiveness of Sediment-Control Techniques Used During Highway Construction in Central Pennsylvania*. By Lloyd A. Reed. Pp. A+57. (Washington, DC: US Government Printing Office, 1978.) [282]

newly on the market

Atomic absorption system. Pye Unicam. With microprocessor data handling and a completely new video furnace programmer, Pye Unicam's atomic absorption system SP9 is flexible yet extremely easy to use. Available for both flame and flameless operation, the system SP9 incorporates many extra features such as silica-coated optics.

Circle No. 42 on Reader Enquiry Card.



The Pye Unicam furnace autosampler

Tunable laser source. Glen Creston introduce the EDSI, which is a compact, bench top tunable laser source which covers the range 400–700 nm. It is easy to operate, air cooled and plugs into any 13 A socket. The output from the dye is 30 μ J at 25 hZ, peak power 10 kW or greater and spectral line width approximately 1 nm. The unit is suitable for a wide variety of spectroscopic applications and, in view of its relatively low cost, it is also ideal for teaching purposes.

Circle No. 43 on Reader Enquiry Card.

Tube gel chamber. Miles. A newly redesigned tube gel chamber for electrophoresis has been introduced by Miles Laboratories. Versatile and compact, the Canalso tube gel chamber is suitable for electrophoresis in uniform or gradient gels, isoelectric focusing, and isotachopheresis.

Circle No. 44 on Reader Enquiry Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Enquiry Card bound inside the cover.

Portable printing calculator. Decimo. The Vatman mini video from Decimo is a portable 10-digit fast print and display model which is fully rechargeable and supplied complete with adaptor. Features include: fully independent 4 key memory; sigma for auto entry to memory; percentage key; facility for working on display only and whilst in this mode feature for printing figure shown on display. Dimensions 220 $\times$ 105 $\times$ 55 mm.

Circle No. 45 on Reader Enquiry Card.

Wound probe system. Radiation Monitoring Devices. A small, highly sensitive probe system for detecting radiation in wounds caused by nuclear accidents has been introduced by Radiation Monitoring Devices. The RMD wound probe system WPS-1 uses a high efficiency cadmium telluride probe, which requires no photomultipliers, high voltage, or liquid nitrogen cooling, to detect γ radiation in wounds. Sensitive to energies as low as the 17 keV of plutonium, the standard wound probe incorporates a 3.5 mm diameter cadmium telluride crystal. In the field it can be battery powered and used with a miniature counter.

Circle No. 46 on Reader Enquiry Card.

Haemoglobin A₁ test kit. Bio-Rad Laboratories has developed a convenient and precise haemoglobin A₁ by column test that requires minimal preparation and only one column elution. The test measures the amount of glycosylated haemoglobin (HbA₁) in whole blood. Blood (100 μ l) is mixed with an efficient lysing agent. An aliquot of the haemolysate is then applied to a cation exchange resin in a disposable column. The percentage of HbA₁ is easily determined spectrophotometrically. The test kit comes complete with reagents and columns sufficient for 100 assays.

Circle No. 47 on Reader Enquiry Card.

Liquid scintillation system. Packard. The tri-carb 2660 is a liquid scintillation system which is computer controlled. Fifteen measurement programs are stored on a flexible diskette for random access. The system requires no user interaction other than loading samples. Programs can be user defined. Sample activity is computed from efficiency correlation parameters automatically established by the system. The servo-tray sample changer is multi-user orientated.

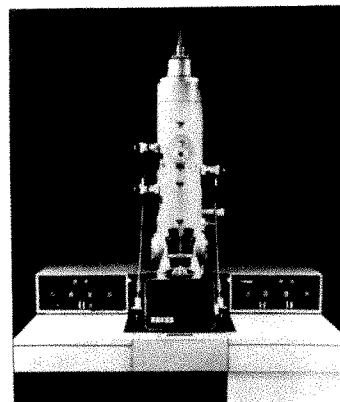
Circle No. 48 on Reader Enquiry Card.

Multi-detector γ counter. LKB-Wallac. A high throughput multi-detector λ counter has been introduced by LKB-Wallac to supplement their range of λ counters for use in biochemistry and clinical chemistry. The 1260 Multi-Gamma has 12 detectors and a built-in microcomputer, which because of its rapid spline function program, allows users to produce radioimmunoassay concentration results even more rapidly than other multi-detector instruments which do not use microcomputers. Using the 'rapid load' sample tray a set of 120 RIA samples can be counted, the standard curve plotted, and concentrations, with error limits, can be calculated in 16 minutes.

Circle No. 49 on Reader Enquiry Card.

Electron microscope. The Zeiss EM 109 has a 3.44Å resolution, a high degree of specimen protection from excessive irradiation and the elimination of contamination. The improvements in this electron microscope include a camera system that is totally outside the vacuum, an automatic focusing system for maximum specimen protection, and a vacuum system providing ultra-clean specimen conditions. Also with the new Micro-Dose Focusing system, the specimen area to be studied can be selected at low intensity. A push of a button shifts the image to a non-critical area adjacent to the selected detail, automatically switches to high magnification, making precise focusing easy for the operator and protecting the critical specimen area from unnecessary exposure to the electron beam. After selection of the desired magnification, the electron beam switches automatically back to the area to be studied without losing focus.

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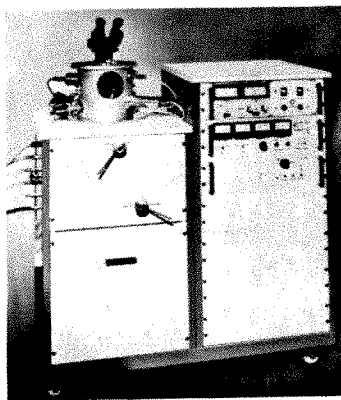
The Zeiss EM 109

Richest field adapter. Celestron International announces a new product called a Richest Field Adapter. This device is used on Celestron's existing line of telescopes to quadruple the brightness of faint deep sky objects and also double the actual field-of-view through the C5, C8 or C14 telescopes. The new 20-mm Erfle Ocular is also introduced which gives a wide apparent field of approximately 75°. Works extremely well on all Celestron telescopes.

Circle No. 51 on Reader Enquiry Card.

Ion beam processing unit. Ion Tech have introduced a new surface preparation version of the very successful Super Microlap. This new ion beam thinning unit possesses all the features of the advanced Super Microlap which will thin copper specimens at 50 µm per hour. In addition this new model B305W has a servo motor drive and control system for rocking a wide beam saddle-field ion source over a rotating scanning electron microscope specimen. The B305W can be supplied either as a module for retro-fitting to an existing pumping system or can be supplied installed as an integral package with the Ion Tech B500 high vacuum pumping system.

Circle No. 52 on Reader Enquiry Card.



The Ion Tech B305W

Filter units. Millipore. Millex-GS single-use filter units, from Millipore, provide the pharmacist and technician with the ideal means of sterilisation and clarification of small-volume ophthalmic solutions. Containing a 0.22 µm, low-extractable, cellulose-ester membrane filter, Millex-GS removes particulates, fibres and microorganisms from extemporaneously prepared solutions and in conjunction with a sterile syringe, needle and bottle, comprises the complete system for aseptic dispensing of volumes up to 250 ml.

Circle No. 53 on Reader Enquiry Card.

Photoresist illuminators. Oriel Scientific announces a new range of high performance photoresist illuminators providing collimated UV light with a beam uniformity of better than $\pm 5\%$. Applications include processing thin and thick films, printed circuit boards, optical encoders, LCD displays, plasma panels, graticule manufacture and UV curing. The illuminators are available in convenient arrangements giving horizontal or vertical beams for contact, proximity or projection printing.

Circle No. 54 on Reader Enquiry Card.

Rotary table for photoresist illuminators. Oriel. The Oriel model 8422 rotary table with dual alignment fixtures allows exposure of one mask while the operator unloads and loads the previously exposed substrate. This system can be used for contact or proximity printing and will accept various size masks and substrates. Accurate alignment is made easy with micrometer settings for X Y Z on θ . Two micrometers can also be positioned in various locations along the X axes to accommodate various size substrates.

Circle No. 55 on Reader Enquiry Card.

Liquid chromatograph. Schoeffel. A new, full-featured high performance liquid chromatograph—the Series 750—has been introduced by the Schoeffel Instrument Division of Kratos. The new system is suitable for high pressure analytical separations and medium flow semi-preparative separations. Because the system is modular, the chromatographer may initially acquire a basic system and later add to that system. The system features a dual piston, high pressure pump, a high pressure gradient programmer, a column oven, and a multi-wavelength UV detector. The components may be used independently or together as a complete system.

Circle No. 56 on Reader Enquiry Card.

Pyrogen test kit. Microbiological Associates. The Limulus amoebocyte lysate test is a means of rapidly detecting the presence (or absence) of endotoxins and radiopharmaceuticals. A clear result is achieved in an hour or less. In addition, the LAL test has more than ten times the sensitivity of the rabbit test. Now Microbiological Associates is able to offer from stock the only licensed kit, to allow the user to eliminate any risk of cross contamination and to provide a full set of controls. Lysate is predispensed in pyrogen-free vials and the user need only add 0.2 ml of sample, using one of the pyrogen-free pipettes supplied (one for each test), and place in a heating block for up to an hour. The controls are included.

Circle No. 57 on Reader Enquiry Card.

Automatic powder diffractometer. Philips. A new version of the Philips APD 10 automatic powder diffractometer has now been introduced. This up-dated unit retains the essential features of the former model, but with a revised layout to optimise system modularity and permit more convenient operation. With mini-computer control and a wide range of software options, the APD 10 enables routine analyses to be carried out far more rapidly than is possible with manual diffractometers. Precision is also ensured by the incorporation of automatic calibration corrections.

Circle No. 58 on Reader Enquiry Card.



The new Philips APD 10

Liquid chromatograph. Shandon Southern. A high performance liquid chromatograph, has been developed at the University of Edinburgh in collaboration with Shandon Southern. Within minutes it can separate minute quantities of the different chemical components of complex chemical mixtures. The chromatograph works by pumping a small sample of the mixture of interest under high pressure (up to 5,000 lb per square inch) through a 5-mm bore stainless steel tube. The tube contains a special absorbent through which the different components of the mixture migrate at different speeds. Thus a 'time-profile' is produced on the instrument's read-out system as the sample is expelled from the column, revealing the presence and measuring the quantities of the various components in the original mixture.

Circle No. 59 on Reader Enquiry Card.

Industrial fluidised baths. Techne. A new series of industrial fluidised baths providing a working depth of up to 48 inches and a working diameter of up to 24 inches is available from Techne Inc. Called models IFB-111, -121, -131, and -510, the units use fluidised aluminium oxide (a safe, dry, non-toxic, stable material) as a heat transfer medium up to 600 °C.

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Entrée en fonction: 1er septembre 1979. Date-limite de l'inscription: **1er mai 1979**. Les candidatures (curriculum vitae, plan de recherche, publications) doivent parvenir au Doyenat de la Faculté des Sciences, Collège propédeutique 1015 Lausanne (Suisse).

Tout renseignement complémentaire peut y être obtenu.

W30(A)

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Further particulars and application forms available from the Establishment Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. Closing date April 30, 1979. 341(A)

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ASSOCIATE PROFESSOR/
LECTURER IN LAND USE;
5. PROFESSOR/
ASSOCIATE PROFESSOR/
LECTURER IN WILDLIFE
CONSERVATION AND
MANAGEMENT;
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7. PROFESSOR/
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Salary scales: Professor, £55,460 per annum; Associate Professor, £54,030 to £54,550 per annum; Lecturer, £51,500 to £53,500 per annum (excluding cost of living allowance). (£51=£1.23 sterling.) The British Government may supplement salaries in range £3,894 to £4,542 per annum (sterling) for married appointees or £1,830 to £2,310 per annum (sterling) for single appointees (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages, various allowances; superannuation scheme; annual overseas leave.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail to the Personnel Secretary, University of Khartoum, P.O. Box 321, Khartoum, Sudan, by May 8, 1979.

Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. 289(A)

UNIVERSITY OF WARWICK LECTURESHIP IN VIOLOGY

Applications are invited for a new post tenable from October 1, 1979 in the Department of Biological Sciences. Teaching will be within the degree courses taught in the Department, the precise area depending upon the interests and experience of the candidate and requirements of the Department. The successful applicant will be appointed to the Virology Research Group led by Professor D. C. Burke; and will be expected to undertake research which complements one or more of the existing projects. Applications from candidates from all branches of virology will be welcome. Initial salary will be within the first four points of the Lecturer scale: £3,883 by (£4,631) to £7,754 p.a. (under review). Further details and application forms from the Academic Registrar, University of Warwick, Coventry CV4 7AL quoting Ref. No. 35/2A/79. Closing date for receipt of applications May 4, 1979. 366(A)

AUSTRALIA MINISTRY FOR THE ARTS NATIONAL MUSEUM OF VICTORIA

The National Museum of Victoria founded in 1854, is a state government funded museum covering the fields of Zoology, Mineralogy, Paleontology, and Anthropology with a special research interest in Southeastern Australia. The museum is under the control of the National Museum of Victoria Council and staff are employed by the Public Service Board of Victoria. There are eleven curatorial departments with a total scientific staff of 26. Research is supported by one of the largest natural history reference libraries in Australia. The collections contain significant holdings in most animal groups.

Applications are invited from person who possess appropriate qualification for the following position:

CURATOR OF CRUSTACEA

Class "SO-5", Second Division
\$A18,251

The appointee will be responsible to the Director to carry out appropriate taxonomic research, to maintain and curate the departmental collection answer public and professional enquiries and contribute, as required to the educational and exhibition programmes of the museum.

The successful applicant will possess an approved higher degree in Science with a major in Zoology and provide evidence of wide research experience appropriate to the collections, together with ability to maintain collection and advise on museum exhibits.

The successful applicant will, subject to passing a prescribed medical examination, be appointed as permanent officer of the Victorian Public Service. Conditions of service include four weeks' annual leave with holiday pay loading, liberal sick leave, maternity and paternity leave, eligibility for long service leave after completion of ten years' service and a generous contributory superannuation scheme.

Applications in writing, quoting Position No. L08/25/0151/8 and stating qualifications, experience and date of birth, must reach the Secretary, Public Service Board of Victoria, Treasury Place, Melbourne, 3002 Victoria, Australia, by not later than 9.30 a.m. on Wednesday, May 16, 1979.

VICTORIAN PUBLIC SERVICE
W24(A)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF BIOCHEMISTRY TECHNICIAN (ELECTRON MICROSCOPE)

To operate a J.E.M. T7 electron microscope and associated equipment providing a service to several research groups. Candidates should possess H.N.C. or equivalent as a minimum qualification and several years experience, but applications from candidates with lower qualifications will be considered. Salary within a range up to £3,720 p.a. (under review) according to qualifications and experience. Application forms may be obtained from The Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quoting Ref: RV/549/N. 364(A)

DESIGNER (SCIENCE PROJECTS) OPEN UNIVERSITY PRODUCTIONS

Are you interested in the presentation of science through television? There is an opportunity to work on the design and preparation of scientific and technological experiments and demonstrations for Open University television programmes. Applicants must have mechanical workshop skills and should have experience of electronics to HNC level or equivalent. Ability to work in a wide range of scientific areas, e.g. Chemistry, Biology, Geology and Physics is essential.

Based at Alexandra Palace, North London, but it is anticipated that Open University Productions will move to Milton Keynes in 1981.

Salary (under review): £5,890 p.a. (may be higher if qualifications exceptional) by £225 to £7,015 p.a. Relocation expenses considered.

Telephone or write immediately for an application form, enclosing addressed envelope and quoting reference 2183/NA, to **Appointments Department, BBC, London W1A 1AA. Telephone: 01-580 4468, ext. 4619.**

BBC tv

332(A)

Section Head- Inhalation Toxicity

ICI Central Toxicology Laboratory

This Laboratory provides advisory and experimental services to ICI Limited, for the control of toxic hazards in manufacturing processes and in other fields of growing importance, such as the development of pesticides and food additives. Emphasis is placed on improving and assessing methods for studying the biological effect of foreign compounds and upon understanding mechanisms of toxicity. The provision of toxicological information, prior to product clearance and registration in many countries, is a major activity undertaken by the Laboratory on behalf of the ICI group.

We currently have a vacancy for someone to head our Inhalation Toxicity Section. The job holder will be responsible for managing approximately 18 people who are involved in the provision of a facility to assess any potential health risks through inhalation of chemicals and particulates. The job holder is expected to contribute to the overall management of the Laboratory, and to formulate the correct technical policy for the Section whilst ensuring that results produced are reliable.

The successful candidate will have post-doctoral experience of research on the pulmonary system together with proven ability in managing multi-disciplinary project teams. It is unlikely that any candidate below 30 years of age will have accumulated sufficient experience.

The Laboratory is two miles south of the village of Alderley Edge on the main road (A34) between Manchester and the Potteries on a 300 acre site which the Laboratory shares with ICI's Pharmaceuticals Division. Alderley Edge is less than 20 miles from Manchester and in addition to the well-wooded Cheshire countryside which surrounds it, Alderley is within easy reach of the Derbyshire Peak District, the Lake District and the North Wales Coast. Staff working at Alderley live over a wide area but mainly in the Macclesfield and Alderley Edge/Wilmslow area.

In addition to salary ICI also operate a Productivity Bonus and Profit Sharing Scheme.

Anyone interested in applying for this vacancy (either male or female) should telephone or write (quoting reference IT/SH/SCC) to:-



Miss S C Carson,
Personnel Officer,
ICI Ltd, Central Toxicology Laboratory,
Alderley Park, Nr Macclesfield, Cheshire.
Phone - Alderley Edge E82711, extension 144.

Overseas candidates should only apply if they have a planned visit to the UK within the next two months. Closing date for applications. 23 April, 1979.

353(A)

PLANT BREEDING INSTITUTE MARIS LANE, TRUMPINGTON CAMBRIDGE CB2 2LQ HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER CYTOGENETICS DEPARTMENT

A plant cell biologist, with experience in the preparation and culture of protoplasts and the regeneration of these into mature plants is required to participate in a programme assisted by the National Research Development Council to investigate transformation of plant cells using bacterials and other plasmids. The Officer will be expected to collaborate with other members of a team developing plasmids. It is expected that this Officer will be responsible for the plant cell cultures of the laboratory. Additional experience in molecular biology would be a distinct advantage.

Candidates should have a 1st or Upper 2nd Class Honours Degree in an appropriate biological science and at least two years' relevant postgraduate experience in cell biology.

The appointment will be as Higher Scientific Officer (£4,101 to £5,448 per annum) or Senior Scientific Officer (£5,154 to £6,898 per annum). At least four years relevant postgraduate experience is required for appointment as Senior Scientific Officer. Non-Contributory Pension Scheme.

Applications with curriculum vitae and the names and addresses of three referees should be sent to the Assistant Secretary (Estab.), quoting reference N.R.D.C. 50, by April 27.

Further details are available on request. 359(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF PURE AND APPLIED ZOOLOGY

Applications are invited
for the post of
DEMONSTRATOR
in the above Department.

The successful candidate will be required to assist with teaching in the honours degree in Zoology and Agricultural Zoology and to assist with research. Candidates should have a good honours degree in Zoology, Agricultural Zoology or Biology and should preferably also have, or be about to submit for, a Ph.D. degree. Preference may be given to applicants with research interests in applied insect physiology/biochemistry or agricultural entomology but applicants with other research interests in zoology or in applied zoology will also be considered. The appointment will be made for a fixed period of three years with effect from October 1, 1979.

Salary at an appropriate point on the 1B scale for Other Rel... Staff (£3,384 to £5,604) (under review), according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 56/4/D. Closing date for applications: May 14, 1979.

299(A)

Whittington Hospital Research Assistant

(Basic Grade Biochemist Scale)
(£3,840 - £5,263 depending on experience in
National Health Service)

You will work in the Department of Chemical Pathology to join a team studying lipid peroxidation and other free-radical processes in biological and clinical material. Rotation into the main hospital service laboratory can be arranged so that the post may be suitable for training in clinical chemistry and acceptable for the probationary period of the basic grade biochemist grade.

The post is funded by the N.E. Thames Regional Health Authority for one year in the first instance but may be renewable for another two years. Short-listed candidates will be invited to visit the department.

Application forms from Hospital Personnel Department, Whittington Hospital, Highgate Hill, London N.19. Tel: 01-272 9873. Completed forms should be returned by 19th April, 1979. 316(A)

CAMDEN & ISLINGTON
AREA HEALTH AUTHORITY (TEACHING)

316(A)



CSIRO AUSTRALIA

Research Scientist Division of Textile Physics Ryde NSW

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: Fabric Mechanics.

General: The Division carries out research and development in fibre physics, measurement technology applied to raw wool, and the physics of yarns and fabrics.

Duties: The appointee will carry out research in textile fabric mechanics with a view to understanding the bases of fabric performance, and therefore to improving properties such as wrinkling, drape, abrasion resistance and dimensional stability.

Qualifications: A relevant PH.D. degree or its equivalent together with subsequent research experience. Demonstrated ability to make and maintain appropriate industrial contacts would be an advantage.

Salary: Research Scientist or Senior Research Scientist. \$A15,422 to \$A22,405 p.a.

Tenure: Indefinite with superannuation.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 465/438 should reach:—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 4th MAY, 1979.

Applications in U.S.A. and Canada should be sent to:—

The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A. 368(A)

THE BRITISH INDUSTRIAL BIOLOGICAL RESEARCH ASSOCIATION

GENETIC TOXICOLOGIST

THE BIBRA Research Laboratories are seeking a Genetic Toxicologist to initiate and lead a research team investigating problems relating to abnormalities of the genetic apparatus, and to assume responsibility for the laboratory's short-term carcinogenicity programme.

This is a senior position for a person with established scientific credentials and the employment package will reflect the responsibility.

Applications with a curriculum vitae should be addressed to:

The Secretary
The British Industrial Biological
Research Association
Woodmansterne Road
Carshalton, Surrey SM5 4DS.

321(A)

THE OPEN UNIVERSITY



Research Assistant in Biology

A research assistant is required for a project to investigate the role of physiological adaptation in populations of *Drosophila*.

Candidates should have a good first degree preferably in Genetics, and some experience in the handling of *Drosophila*.

The post is available initially for one year with the possibility that the grant may be renewed.

Commencing salary will be within the range £3,384—£3,883 per annum with USS benefits.

Application forms and further particulars are available, by postcard request please, from The Recruitment Office (JD3721/2). The Open University, P.O. Box 75, Walton Hall, Milton Keynes, MK7 6AL or telephone Milton Keynes 63404; there is a 24 hour answering service on 63868.

Closing date for applications: April 19. 291(A)

INORGANIC CHEMISTRY LABORATORY UNIVERSITY OF OXFORD

An S.R.C. postdoctoral appointment in organo-transition metal chemistry is available from August 1, 1979 for one/two years. The research would be suitable for those with a Ph.D. in either inorganic or organic chemistry or in X-ray crystallographic methods. Send enquiries or applications with curriculum vitae and names of referees to Dr M. L. H. Green (Tel: 0365 57387), Inorganic Chemistry Laboratory, South Parks Road, Oxford OX1 3QR. 306(A)

PLANT BREEDING INSTITUTE MARIS LANE, TRUMPINGTON CAMBRIDGE CB2 2LQ HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER CYTOGENETICS DEPARTMENT

A molecular biologist is required to participate in a programme assisted by the National Research Development Council designed to transform genetically plant cells. In particular, this Officer will be responsible for the construction of vectors to facilitate the selection and detection of transformed plant cells. Consequently, experience in plasmid molecular biology and in DNA biochemistry is essential for this position. The Officer will be expected to collaborate with investigating and preparing plant cells for transformation.

Candidates should have a 1st or Upper 2nd Class Honours Degree in an appropriate biological science and at least two years postgraduate experience in molecular biology or related fields of biochemistry.

The appointment will be as Higher Scientific Officer (£4,101 to £5,448 per annum) or Senior Scientific Officer (£5,154 to £6,898 per annum). At least four years' postgraduate experience is required for appointment as Senior Scientific Officer. Non-Contributory Pension Scheme.

Applications with curriculum vitae and the names and addresses of three referees should be sent to the Assistant Secretary (Establishment), quoting reference NRDC 52, by April 27.

Further details available on request. 358(A)

NATURAL ENVIRONMENT RESEARCH COUNCIL British Antarctic Survey Advanced Ionospheric Sounder Project PERIOD APPOINTMENTS

The British Antarctic Survey has vacancies for a Physicist and an Electronics Engineer to participate in a major new research project to study the auroral ionosphere, using a recently-developed computer and microprocessor controlled high frequency radar known as the Advanced Ionospheric Sounder (AIS). The AIS is to be deployed at Halley station (76°S 27°W), Antarctica, and its operation will involve co-operation with University and Government laboratory groups both in UK and abroad. Successful candidates will be required to install, operate and maintain the equipment in Antarctica and will be encouraged to contribute to the research programmes. Applicants must be aged between 21 and 35, physically fit and male.

The Engineer, probably a graduate, will contribute to the evolution of the AIS system. He will be responsible for the system hardware and should also understand and be able to modify the software. The appointment will be for a period of approximately 33 months, commencing in the Summer of 1979. The successful candidate will undergo training, based in Cambridge, until sailing for Antarctica in October 1980 to spend one Antarctic winter at Halley station. He may be offered a further contract of one year to complete outstanding work in the UK.

The Physicist should be a graduate who has (or expects to achieve) a first or upper second honours degree in physics or computer science. He will direct the day-to-day scientific programme in the field and carry out research. The appointment will be for a fixed period of five years starting in Summer 1979. The successful candidate will be trained in Cambridge before sailing for Antarctica in October 1980 to spend two winters at Halley station. Suitable candidates can register for a higher degree at the University of Leicester and the remainder of the period is to enable the research to be written up at the University.

For further details of conditions of service and salary please write giving full details of qualifications and experience to:

The Establishment Officer,
British Antarctic Survey,
Madingley Road,
Cambridge CB3 0ET.

Closing date for applications: April 26, 1979.

Please quote reference BAS 10. 293(A)

Geology and Geophysics UNIVERSITY OF CALIFORNIA, BERKELEY

An Assistant Professorship in Geology is a vacancy to be filled during the 1979-80 academic year in the Department of Geology and Geophysics. Candidates must have an expertise which will contribute to an integrated programme of field and laboratory research and teaching. Individuals with interdisciplinary qualifications, experience and interests are encouraged to apply. Research emphasis may span such fields as metamorphic geology, structure-stratigraphy-sedimentology, or tectonics. The position will involve teaching and research supervision in the field. The Ph.D. degree is required.

Applications should be submitted by May 15, 1979 to:

T. V. McEvilly, Chairman
Geology and Geophysics
University of California
Berkeley, Ca 94720

and should include the names of three academic referees.

The University of California is an Affirmative Action employer. W34(A)



SOUTHAMPTON THE UNIVERSITY

DEPARTMENT OF PHYSICS

Applications are invited for the post of

LECTURER

in the Department of Physics. Candidates should have an active research interest in Quantum Physics and experience with the use of fields and groups. The person appointed will be expected to interact effectively with the Theoretical High Energy Physics group and to play a full part in the Department's programme of teaching in theoretical physics.

Salary scale £3,883 to £7,754 per annum plus U.S.S. The initial salary will depend on qualifications and experience. Further particulars may be obtained from Mrs E. C. P. Sears, Staffing Section, The University Southampton SO9 5NH to whom applications (7 copies from United Kingdom applicants) should be sent not later than May 18, 1979. Please quote reference number 1803/A. 253(A)

CHELSEA COLLEGE UNIVERSITY OF LONDON UNIVERSITY OF GHANA TEMPORARY LECTURESHIP IN GEOPHYSICS

An Inter University Council supported link scheme has existed since 1976 between the Geology Departments of Chelsea College and University of Ghana. The scheme includes the post of Lecturer in Geophysics under the Home Base Scheme and applications for this post are invited. The appointment is as Lecturer in the Geology Department, Chelsea College or a period of four years but the first three will be spent on secondment to the University of Ghana. Duties here will include undergraduate teaching of pure and applied geophysics and research in seismology, including the operation of a seismograph station that has been recording since 1976, and aspects of applied geophysics. Salary scale while at the home base is £3,883 to £7,754 per annum (under review) plus £502 London Allowance.

At the University of Ghana the salary scale Cedis 6,420 to 9,780 (£1=Cedis 5.50 approx.) would be supplemented under the British Expatriate Supplementation Scheme where appropriate. This scheme provides salary supplementation in the range £6,018 to £7,068 married and £3,504 to £4,296 single (under review), plus appointment grant, allowances for family passages, education allowance and children's holiday visit passages etc.

Further details and application forms from the Personnel Officer, Chelsea College, Friese Greene House, Chelsea Manor Street, London SW3 2TW. Applications to be received by May 18, 1979. 369(A)

INSTITUTE OF CANCER RESEARCH: JUNIOR TECHNICIAN required to join a research group at the Chester Beatty Research Institute, Fulham Road, S.W.3, investigating the metabolism of polycyclic hydrocarbons to derivatives that react with nucleic acids. Applicants for the post, which offers an opportunity of learning a variety of biochemical techniques, should have two 'A' levels in science subjects or O.N.C. and, preferably, some laboratory experience. Salary in scale £2,247 to £3,015 per annum depending on age and experience. Applications, in duplicate, with the names of two referees to the Secretary, Institute of Cancer Research, 34 Munster Place, London SW7 3NU, quoting ref. 301/B/66. 282(A)

SYNCHROTRON RADIATION EXPERIMENTS *Experimental Scientists*

The Daresbury Laboratory, an establishment of the Science Research Council situated in the north Cheshire countryside, is constructing a purpose built Synchrotron Radiation Source.

The source will begin to operate in April 1980 and will provide beams of intense electro-magnetic radiation ranging from the infra red through the visible and UV to the x-ray region. Research programmes involving Daresbury and University research workers will cover a very broad range of activities in Physics, Chemistry, Biology and related subjects.

DUTIES

Applications, from either sex, are invited from experimental scientists who are interested in collaborating with and/or providing technical support to the research programmes of the synchrotron radiation users. Successful candidates will join the Laboratory in-house group of scientists and technical staff and will be involved in the design, construction and installation of equipment for the research programmes and in the collection and analysis of data. An on-line data acquisition network is under construction and candidates interested in contributing to this latter aspect of the work are also invited to apply. Successful applicants must be prepared to work in a demanding multidisciplinary research environment and be capable of contributing new ideas to the development of the research facilities. The SRS will operate continuously over long periods and members of the in-house group will need to adapt their hours of working to the SRS running schedule.

QUALIFICATIONS

It is expected that successful candidates will have attained a good honours degree (or equivalent qualification) in a relevant field. In addition, experience in such fields as x-ray optics and spectroscopy, VUV spectroscopy, photoelectron and photon spectroscopy, photochemistry and photobiology is desirable for posts at the higher level.

POSTS AVAILABLE

Appointments will be made at both SO and HSO level on salary scales £2,839-£4,415 and £4,101-£5,648 respectively. It is possible that some fixed term research associate posts may also be available on a salary scale £3,695-£6,216. All the salary scales quoted are under review.

The Laboratory also offers a generous annual leave allowance, a non-contributory superannuation scheme and a flexible working hours scheme.

Closing Date: 1st May 1979

Please write, or telephone Warrington 65000 Ext. 467 for an application form, quoting reference No. DL/667/T to:

The Personnel Officer

DARESBURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

298(A)

PARASITOLOGY RESEARCH

Pfizer Research in the U.K., which is part of an integrated international organisation, is located at Sandwich on the Kent Coast. Here in modern, well equipped facilities, which include a 300-acre research farm, about 500 scientists and support staff are engaged in the discovery and development of novel compounds for use in human and veterinary medicine.

We now require an innovative biologist to join the Parasitology department of our Agricultural Products R & D division to strengthen our research effort in the areas of endo- and ectoparasitic diseases of animals.

The appointment calls for a man or woman with up to three years post-doctoral research experience, who as well as being a creative scientist is also a good motivator and communicator, since our multi-disciplinary project management system requires close collaboration between specialists in various scientific disciplines.

Ideally, candidates will have a Ph.D in parasitology or entomology and should have specialist knowledge of physiology or biochemistry with an interest in the application of their subject to disease control. They should have a flexible attitude to and be interested in a long-term career in research.

The starting salary will reflect qualifications and experience and there is a good bonus potential. Pension, salary continuance and death benefit schemes are in operation and there is generous assistance with relocation expenses. Flexible hours are worked and the social/recreational facilities are first class.

Pfizer

Applications, which will be treated in confidence, giving details of qualifications, experience age etc., should be sent to:—
D. W. Sells
Personnel Manager
Pfizer Central Research
Sandwich, Kent.

362(A)

AN FORAS TALUNTAIS LAND RECLAMATION RESEARCH

Applications are invited for a RESEARCH POST in an expanding land reclamation programme, with particular emphasis on drainage. The post will be in the Western Research Centre at Creagh Station, Ballinrobe, Co. Mayo. The appointee will be engaged in the investigation, design, construction and evaluation of land reclamation works. The post will also involve work organisation and control, the use of machinery and materials and cost-benefit analyses.

Essential: An honours University Degree, or equivalent, in Engineering, Agricultural Engineering or Agricultural Science.

Desirable: (1) Suitable postgraduate training and/or experience.

(2) An interest and/or background in the Earth Sciences and Hydrology.

Applications also considered from 1979 Final Degree candidates.

Appointment up to and including Senior Research Officer level depending on qualifications and experience. Marriage and childrens' allowances. Non-contributory pension scheme and provision for widows' and orphans' pensions.

Application forms and further particulars by telephone request from Personnel Department, An Foras Taluntais, Headquarters, 19 Sandymount Avenue, Ballsbridge, Dublin 4. Telephone: 01-688188. Latest date for receipt of completed forms: Wednesday, April 25, 1979. W32(A)

MEDICAL RESEARCH COUNCIL CYCLOTRON UNIT has a vacancy for a SCIENTIST

to join a team working on the radiobiology of tumours and normal tissues, the aim being to improve the treatment of cancer. The successful candidate will investigate various aspects of the response of experimental tumours to fast neutrons, hyperthermia and chemotherapeutic agents. The work will involve animal and cell studies and the department has excellent facilities for both.

Knowledge of radiation biology and tumour biology will be an advantage although some training can be given. The minimum qualifications are a first or upper second class degree in an appropriate subject and a Ph.D. or equivalent. Several years' relevant postdoctoral research experience would be advantageous. This position offers a unique opportunity for someone with ability and enthusiasm for research of this nature.

Salary, according to age and experience, in the range £6,649 to £8,204 (inclusive of London weighting) plus six weeks annual leave.

Please write, giving qualifications and curriculum vitae, to:

Miss Alison Pires, M.R.C. Cyclotron Unit, Hammersmith Hospital, Duane Road, London W12 0HS. Tel: 01-743 4594, extn. 103. 319(A)

PLANT BREEDING INSTITUTE

MARIS LANE, TRUMPINGTON
CAMBRIDGE CB2 2LQ
SCIENTIFIC OFFICER
CYTOGENETICS DEPARTMENT

A Scientific Officer is required to provide support for a research programme on transformation of yeast and higher plants. The programme will include the construction of plasmid vectors suitable for selection and detection in yeast and plant cells. The officer should therefore have had some training in molecular genetics or biology or relevant areas of biochemistry.

Candidates should have an Honours Degree or equivalent in Biochemistry or an appropriate biological science, or an ordinary degree with relevant experience.

The appointment will be as a Scientific Officer (£2,839 to £4,415 per annum). The point of entry on the scale being determined in relation to qualifications and experience. Non-Contributory Pension Scheme.

Applications with curriculum vitae and the names and addresses of three referees should be sent to the Assistant Secretary (Establishment), quoting reference CYT 54, by April 27. Further details are available on request. 356(A)

UNIVERSITY OF NOTTINGHAM DEPARTMENT OF BIOCHEMISTRY AND NUTRITION Applications are invited for the post of DEMONSTRATOR

in the Food Science Laboratories of this Department which is accommodated at the University's School of Agriculture at Sutton Bonington.

This will be a two year appointment and the initial salary will be £3,384 per annum (under review).

Further particulars and forms of application, returnable not later than April 18, 1979, from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Ref. No. 679. 284(A)

UNIVERSITY OF CAPE TOWN

Applications are invited for the following posts:

SENIOR LECTURER IN

ORGANIC CHEMISTRY

This post falls vacant on July 1, 1979. Closing date for applications May 31, 1979.

LECTURERS IN

MICROBIOLOGY

Due to retirement of present staff members and expansion of the department, three lectureships will be vacant from January 1, 1980. Interest and experience in one or more of the following fields will be a strong recommendation: bacterial biochemistry; industrial microbiology; microbiology; genetics; molecular biology and virology. The posts may also be filled on a contract basis for two or three years with the possibility of either renewal or conversion into a permanent post. Closing date for application May 25, 1979.

GENERAL: Appointment to the above posts will be made, according to qualifications and experience, on salary scales ranging as follows:

FOR SENIOR LECTURER:

From R10,144 to R13,489 p.a.

FOR LECTURER:

From R7,554 to R11,007 p.a.

(Improvement to these salary scale is imminent).

Applicants should submit a curriculum vitae stating present salary, experience, research interests and publications if any, the date duty could be assumed, and the names and addresses of three referees.

Further information concerning the positions and general conditions of service should be obtained from the Registrar, Room 10, University of Cape Town, Private Bag 1, Rondebosch 7700, South Africa.

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion, or colour. Further information on the implementation of this policy is obtainable from the Registrar. W25(A)

UNIVERSITY OF NAIROBI KENYA

Applications are invited for the posts of

LECTURER

IN DEPARTMENT OF GEOGRAPHY

Applicants should hold postgraduate qualifications in Geography preferably at Ph.D. level. The Department seeking to appoint geographers with research interests and competence specifically in any of these areas: Biogeography (especially in the geography of soils), Climatology, Economic geography, Transport geography, Urban geography and the Geography of resources (with special interest in Remote Sensing). The appointees will also be expected to participate in field courses and in the teaching of practical geography to the different classes of undergraduates and undertake supervision of postgraduate students in the specialised areas besides undertaking research. Salary Scale: K£2,016 to K£3,312 p.a. (K£1=£1.32 sterling). The British Government may supplement salaries in range £4,278 to £4,818 p.a. (sterling) for married appointees (£2,730 to £3,156 p.a. (sterling) for single appointees (reviewed annually and normally free of all tax) and provide children's education allowance and holiday visit passages. F.S.S.U. family passages; various allowances. Detailed applications (2 copies) including a curriculum vitae and naming referees should be sent by airmail by May 10, 1979 to the Registrar (Recruitment and Training), University of Nairobi, P.O. Box 30197, Nairobi Kenya. Applicants resident in U.K. should also send 1 copy to the University Council, 90-91 Tottenham Court Road, London W1P 0D. Further particulars may be obtained from either address. 370(A)

UNIVERSITY OF WALES UNIVERSITY COLLEGE OF SWANSEA

RESEARCH DEMONSTRATOR

Applications are invited for the vacancy of Research Demonstrator in the Department of Geology. Applicants should either be in possession of a B.Sc. or have such a degree near to completion. A background in petrology, geochemistry and/or structural geology would be preferred.

The appointment, which will be for one year in the first instance renewable on an annual basis for a maximum of three years, will be on a scale up to £4,382 per annum, together with J.S.S./U.S.D.P.S. benefits.

Further particulars and application forms (two copies) may be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea SA2 8PP, to whom they should be returned by Friday, April 17, 1979. 338(A)

UNIVERSITY OF ZAMBIA

Applications are invited for the following posts in the School of Medicine.

- ASSOCIATE PROFESSOR/
SENIOR LECTURER/
CHIEF TECHNICIAN IN
DEPARTMENT OF PHYSIOLOGY,
- SENIOR LECTURER IN
DEPARTMENT OF ANATOMY,
- PROFESSOR/
ASSOCIATE PROFESSOR/
SENIOR LECTURER IN
DEPARTMENT OF MEDICINE,
- PROFESSOR/
ASSOCIATE PROFESSOR/
SENIOR LECTURER/
IN DEPARTMENT OF
OBSTETRICS AND
GYNAECOLOGY,
- PROFESSOR/
ASSOCIATE PROFESSOR/
SENIOR LECTURER IN
DEPARTMENT OF
PAEDIATRICS,
- CHIEF TECHNICIAN
(CYTOLOGY) IN DEPARTMENT
OF OBSTETRICS AND
GYNAECOLOGY

Salary scales: Professor K8,616 to £9,135 per annum, Associate Professor, £8,232 to K8,616 per annum, Senior Lecturer, K7,608 to K8,016 per annum, Chief Technician K4,416 to K5,136 per annum. A medical differential of \$1,200 per annum may be paid to clinical appointees (£1 sterling = £1.61). The British Government may supplement salaries as follows: Non-clinical academic staff, in range £4,350 to £5,952 per annum (sterling) or married appointees or £1,968 to £3,366 per annum for single appointees, clinical academic appointees—in range £4,566 to £6,042 per annum (sterling) or married appointees or £1,980 to £3,456 per annum (sterling) for single appointees; Chief Technician, by £4,182 per annum (sterling) for married appointees or £1,986 per annum (sterling) for single appointees, and provide children's education allowances and holiday visit passages. Family passages, baggage allowance, superannuation, medical aid scheme, regular overseas leave.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees should be sent by airmail to Registrar, University of Zambia, P.O. Box 237, Lusaka, Zambia, by May 7, 1979.

Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address 290(A)



Wellcome

Department of Experimental Immunobiology

Immunologist

£5000 - £7800*

We seek a post-doctoral scientist to work on a programme concerned with the development of new immunological adjuvants and the analysis of the mechanisms of adjuvant action.

The appointment will be for a period of 2-3 years in the first instance and applicants should be able to provide evidence of an innovative approach to immunobiological research.

Senior Technician

£3350 - £4750*

We seek a senior technician to work in a research team involved with immunological studies in immunostimulation and new vaccine development.

Previous experience in biochemistry and immunology and a knowledge of methods involved, including working with animals, would be an advantage.

The Laboratories are situated in pleasant parkland surroundings within easy reach of Central London. Conditions of employment are excellent and include subsidised canteen, pension scheme, 4 weeks' holiday, generous relocation expenses where appropriate, and sports and social facilities.

*Salary will be in the range indicated with a review on May 1, 1979.

For an application form please write, giving brief details, to the Personnel Manager, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.



330 (A)

JUNIOR TECHNICIAN/ TECHNICIAN

(Ref. 198/199/79)

required to assist with clinical studies on serum factors with tumour marker potential. Initially, the work will involve routine assays including radioassays. Previous experience in immunological techniques an advantage. ONC/HNC/Degree required. Salary, depending on age, qualifications and laboratory experience within range £2,800 to £3,300 or £3,500 to £4,900. For application form and job description, please telephone Mrs Nelson, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2 on 01-242 0200, ext 305 345(A)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL

(University of London)

RESEARCH TECHNICIAN

required for research programme in the Child Health Department. Duties involve programmes in infant nutrition, biochemical genetics, endocrinology and lipid metabolism in children. HNC or degree in biochemistry or related subject required. Emoluments up to £3,995 per annum (under review) according to experience. Informal enquiries to Dr N. Carter (01-672 1255, Ext 4171). Application forms available from the Establishment Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. 339(A)

EDITORIAL SERVICES CONTROLLER

We need an experienced, literate and adaptable Editorial Controller to work in our Higher and Further Education division, managing a wide variety of typescripts in subjects ranging from undergraduate science to semi-technical hobbies. The job involves working closely with the Further Education, Published, copy-editing and proof-reading, as well as dealing with authors, freelance staff, and our own production staff. Accuracy, and the ability to rewrite where necessary are important. Training will be provided for a person with the required academic and work record.

We are based at Basingstoke, close to the M3. The right person will be offered a competitive salary, and will be entitled to 20 days' holiday per year. We also have a subsidised bar, canteen, social club, pension scheme, and free transport to and from the town centre.

Send your application in handwriting, along with a detailed curriculum vitae, to Sheldagh Browne, Personnel Manager, The Macmillan Press Ltd., 4 Little Essex Street, London WC2R 3LF. 333(A)



Experienced Research Chemist Semi-Synthetic Antibiotics

Europe-based

£15,000-£25,000

On behalf of clients based in Continental Europe, we seek a synthetic or process-development chemist with in-depth practical experience of semi-synthetic antibiotic synthesis.

He or she will take up an important new research appointment and, as well as playing a leading role in fundamental research, will be expected to contribute both ideas and direction to the company's development programme in this field.

The *ideal* candidate will probably be in the age range 28-35, and possess both wide knowledge and at least three years' practical experience, in industry, of semi-synthetic penicillin and/or

cephalosporin synthesis. This experience may have been gained either in fundamental research or in process development.

We recognise that there are few people with the specialist knowledge and experience we seek. Accordingly, our client aims to provide a salary and benefits package that should prove highly attractive to any suitably qualified candidate.

Please write in confidence for an application form and further information, or 'phone if you would like to discuss the appointment further before applying.

Talentmark
Leaders in Health Care Recruitment



Edward Cooper, Talentmark Limited,
King House, 5-11 Westbourne Grove,
London W2 4UA. 01-229 2266 (24 hrs).

317(A)

INVERTEBRATE NEUROBIOLOGY

Postdoctoral position available for two to three years for biochemist/pharmacologist interested in studying cyclic nucleotides/protein phosphorylation in identified molluscan neurons. C.V. and three letters of reference to: Dr I. Levitan, Friedrich Miescher-Institut, P.O. Box 273, CH-4002 Basel, Switzerland. W37(A)

TECHNICIAN required for Department of Microbiology to be responsible for the running of laboratories. Qualifications: O.N.C. or equivalent in Microbiology. Salary scale £2,688 to £3,060 per annum. Ref: 139/C/368. Application form from: Assistant Secretary, Personnel Office, University of Birmingham, P.O. Box 363, Birmingham B15 2TT. 334(A)

AGRICULTURAL RESEARCH COUNCIL INSTITUTE FOR RESEARCH ON ANIMAL DISEASES

Biochemist/Molecular Biologist

Required for studies on scrapie, ageing and the nature of virulence determinants in pathogenic bacteria. Among the main lines of research will be the physical characterisation of the small DNA species recently isolated from scrapie brain and the establishment of the relationship of this DNA to the genome of the scrapie agent. Also molecular approaches to the problem of ageing as applied to loss of production in farm animals.

Qualifications: A 1st or Upper 2nd class honours degree in Biochemistry or Biological Science or Chemistry.

Appointment as Scientific Officer (£2,839 to £4,415 or Higher Scientific Officer (£4,101 to £5,448) according to qualifications and experience. At least 2 years' relevant postgraduate experience is required for H.S.O. Non-contributory pension scheme.

Application Forms from: Secretary, Institute for Research on Animal Diseases, Compton, Nr. Newbury, Berks., quoting ref. 305. 325(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF EARTH SCIENCES Experimental Officer— Electron Probe Microanalysis

Applications are invited from Ph.D. graduates and other suitably qualified persons for a post of EXPERIMENTAL OFFICER concerned with operation and development in the electron probe microanalysis laboratory (JEOL JXA 50A). The post carries day-to-day responsibility for training and maintenance in the laboratory, and offers the opportunity to participate in major research projects involving the electron probe. It is hoped to make an appointment to the post as soon as possible.

Salary at an appropriate point on the IA scale for Other Related Staff (£3,883 to £6,555) (under review), according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 49/12/D. Closing date for applications May 8, 1979. 315(A)

UNIVERSITY OF ABERDEEN LECTURESHIP IN ZOOLOGY

Applications are invited for the above post.

Salary on scale £3,883 to £7,754 per annum, with appropriate placing. Superannuation and removal expenses.

Further particulars from the Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by April 27, 1979. 302(A)

UNIVERSITY OF RHODESIA

Applications are invited for the following posts:

LECTURESHIPS/ SENIOR LECTURESHIPS IN ENGINEERING

Applicants should have an engineering degree and appropriate postgraduate experience in at least one of the following specialist fields: **Civil Engineering**, Materials, Water Engineering, Geotechnical; **Electronic and Power Engineering**, Electric Power Systems, Electrical Machines, Electromagnetics, Electrical Networks, Control Systems; **Mechanical Engineering**, Dynamics, Thermodynamics, Heat Transfer, Mechanical Design, Fluid Mechanics; **Materials Science**, Metallurgy or Ceramics.

Salary Scales (Approx. Stg. equivs). Professor, £10,055 by 378 to £11,941; Senior Lecturer, £7,311 by 284 to £8,163 by 301 to £9,668; Lecture Grade I, £6,632 by 249 to £7,621; Lecture Grade II, £4,094 by 215 to £5,169 by 249 to £6,414. Subject to permission, earnings of up to approximately £3,400 per annum for consultancy may be retained in full.

Both Permanent Pensionable Term and Short-term Contracts are available. Unless born in Rhodesia, ex-patriate on contract are exempt from military service.

Further particulars and application procedure from the Association of Commonwealth Universities (Appts 36 Gordon Square, London WC1H 0PF).

Closing date for receipt of applications is **May 31, 1979.**

British subjects considering applying for posts in Rhodesia are urged to consult the Foreign and Commonwealth Office (Telephone 233 8727) or their nearest British Consular Office.

292(A)

UNIVERSITY OF
EDINBURGH
DEPARTMENT OF GENETICS
POSTDOCTORAL
RESEARCH FELLOW

Postdoctoral position, supported by the S.R.C., is available for two years to collaborate with Dr J. O. Bishop in the molecular cloning of nuclear protamine DNA sequences in the trout. Cloned cDNA sequences are already to hand. Starting date not later than June 30, 1979. Salary £4,382 per annum.

Applications with curriculum vitae and names of two referees should be sent to the Secretary to the University, Old College, South Bridge, Edinburgh EH8 9YL, not later than April 27, 1979. Please quote Reference 5016. 349(A)

UNIVERSITY OF NAIROBI
KENYA

Applications are invited for the post of

PROFESSOR
IN DEPARTMENT OF
PHYSICS

Applicants must have a Ph.D. degree in Physics. They should also have considerable years of University teaching and research experience as evidenced by publications in internationally reputable journals. Active areas of research in the department include Theoretical Physics, Ionospheric Physics, Solid State Physics, Applied Geophysics and Palaeomagnetism. Specialisation in one of the fields would be an added advantage. Salary scale KSh 4,632 to KSh 5,562 per annum (KSh equals £1 32 Sterling). The British Government may supplement salary by £6,552 per annum (Sterling) for married appointee or £4,644 per annum (Sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowance and holiday visit passages S.S.F. or P.S.U.; family passages, medical and scheme; housing allowance, various allowances. Detailed applications (two copies) including a curriculum vitae and naming three referees should be sent by airmail not later than May 10, 1979 to the Registrar (Recruitment and Training), University of Nairobi, P.O. Box 30197, Nairobi, Kenya. Applicants resident in U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address.

326(A)

UNIVERSITY OF DURHAM
DEPARTMENT OF
EXTRA-MURAL STUDIES
Applications are invited for the post of
STAFF TUTOR

in Biological Sciences (Zoology) to develop further a wide range of courses for both the general public and specialist groups. Experience in teaching adults is desirable but not essential.

Salary on the scale £3,883 to £7,754 per annum (under review) plus superannuation.

Applications (three copies) naming three referees should be sent by April 21, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. 281(A)

ST GEORGE'S HOSPITAL
MEDICAL SCHOOL
(University of London)
TECHNICIAN GRADE 4

urgently required in Structural Biology Department for a three-year Cancer Research Campaign project, to work on the analysis of cell surface molecules, using immunological methods in embryonic cells. Experience of tissue culture techniques and/or immunological methods desirable. Salary in the range £2,955, £3,042, £3,132 (under review) plus £275 London allowance. Application forms and further particulars available from the Establishment Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE.

342(A)

Exciting Opportunities in Reactor Physics Hinkley Point Power Station

Two interesting opportunities have arisen for experienced Reactor Physicists to take charge of the operation of the four reactors at Hinkley Point Power Station.

The posts are part of the Reactor Physics Department which consists of a team of technical staff responsible for the safe, effective and economic operation of the two Magnox Reactors in the 'A' Station, operational since 1965, and the two Advanced Gas Cooled Reactors in the 'B' Station, commissioned last year.

The more senior post will be assigned to duties on the 'A' Station. He or she will take part in the daily management of the reactors and in the development of medium and long term strategies. Duties also include safety analysis work which is a continuing and important part of the Department's work.

The second person will work at the 'B' Station and will be mainly concerned with the daily management of the reactors.

Applicants for both positions should possess a degree or equivalent qualification in Physics, Mathematics or Engineering. Experience in nuclear design or research would be relevant and an advantage.

Salary will be in the range £4110-£7060 plus a self-financing productivity payment. Terms and conditions are excellent and fringe benefits include relocation assistance where appropriate.

If you are interested, please write for an application form AF/1 from the Personnel Manager, Bedminster Down, Bridgwater Road, Bristol BS13 8AN. Please complete and return, quoting vacancy number 106/79/N.

357(A)

Central Electricity
Generating Board
South Western Region



PRESTON
POLYTECHNIC

Applications are invited for the following appointment.

SENIOR LECTURER
IN BIOLOGY

Preference will be given to applicants specialising in Ecology or Medical Laboratory Science.

Salary scale (under review) £6,051 to £7,065 to £7,572.

Application forms and further particulars may be obtained from the Chief Administrative Officer (Staffing), Preston Polytechnic, Corporation Street, Preston PR1 2TQ, to whom completed applications should be returned by April 23.

344(A)

MEDICAL RESEARCH
COUNCIL
CYCLOTRON UNIT
requires a
SCIENTIST

to investigate the effects of hyperthermia on mammalian cell membranes. The successful candidate will join a group which is investigating various aspects of the response of cells and tissues to elevated temperatures, with a view to using hyperthermia to treat cancer. It is essential that applicants already have research experience in membrane biochemistry. An appropriate biological degree is required together with a Ph.D. or equivalent research experience. The appointment will be for one year.

Salary, according to age and experience, in the range £5,081 to £6,530 (inclusive of London Weighting).

Please write, giving qualifications and curriculum vitae, to Miss A. Piles, Medical Research Council Cyclotron Unit, Hammersmith Hospital, Duane Road, London W12 0HS (Tel 01-743 4594, extn 103).

355(A)

THE EDINBURGH SCHOOL OF AGRICULTURE
DEMONSTRATOR—GRADE III OFFICER

AGRICULTURAL BIOCHEMISTRY DEPARTMENT (Ref 189N)

A demonstrator is required to assist in teaching degree and diploma students in agricultural biochemistry, animal nutrition and applied biology. An opportunity will also be available to carry out research in an approved area.

Candidates must possess a good honours degree in Agricultural Science, Biochemistry, Biology or related subject. Preference will be given to candidates with a postgraduate qualification and a knowledge of animal nutrition.

Appointment will be made on the scale of Grade III or Grade IV according to qualifications. Grade IV, £2,598 to £3,924, Grade III, £4,178 to £6,898. Non-contributory pension scheme.

For further particulars and application forms write to The Secretary, The East of Scotland College of Agriculture, West Mains Road, Edinburgh EH9 3JG. Closing date for return of completed applications April 30, 1979.

351(A)

POSTDOCTORAL position available to study protein mobility in cell membranes and model systems. Previous experience with optical spectroscopy would be advantageous. The position is funded by the Swiss National Science Foundation with a salary of approximately S Fr. 35,000 to S Fr. 40,000 per year, depending on age and experience. Please send applications with names of two referees to Dr R. J. Cherry, Laboratorium für Biochemie, Eidgenössische Technische Hochschule, ETH-Zentrum, CH 8092 Zurich, Switzerland.

W35(A)

UNIVERSITY OF OXFORD
DEPARTMENT OF THEORETICAL PHYSICS
RESEARCH ASSISTANT
IN THEORETICAL NUCLEAR PHYSICS

Applications are invited for the post of Research Assistant to the Nuclear Theory Group. The post is funded by the Science Research Council to support ongoing research in nuclear structure and heavy ion reactions. The appointment is temporary for one to three years from October 1, 1979.

The salary is on the Research Support Scale (A) currently £3,883 to £6,555 per annum with USS membership. The applicant should not exceed the age of 31 in the first year of the appointment.

Applications with the names of 2 referees to the Secretary, Department of Theoretical Physics, 1 Keble Road, Oxford, by April 27, 1979.

361(A)

**Directorate of Fisheries Research
Environment Protection Division,
Burnham on Crouch**

Sedimentologist

... to join a team of scientists assessing the effects of effluent discharges on the marine environment, and in particular the sea bed. The work will involve planning and participating in experiments, collecting samples from Ministry research vessels, organizing laboratory work, and analysing computer data to assess the chemical and physical effects of waste disposal on the marine environment. In addition, the successful candidate will represent the Division in specialist working groups.

Candidates, normally aged under 27, should have a good honours degree or equivalent in geology, geography, earth sciences or a closely related discipline. Experience of marine sedimentology is highly desirable. *Final Year Students may apply.*

Appointment as Scientific Officer (£2840 - £4410; under review) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 1st May 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote ref: SB/46/AD.*

**Ministry of Agriculture, Fisheries
and Food**

329(A)

**Science
Group**
CIVIL SERVICE

KING'S COLLEGE LONDON
University of London
APPOINTMENT OF PRINCIPAL

The Principal of King's College, Sir Richard Way, KCB, CBE, is due to retire on July 31, 1980. It is intended that a successor should be appointed from August 1, 1980.

The Principal is the chief academic and administrative officer of the College.

The salary is negotiable and living accommodation is provided.

Persons interested in being considered for this appointment, or wishing to suggest names, are invited to write in confidence not later than April 23, 1979, to the Chairman of the Delegacy, the Rt. Hon. Earl Jellicoe, DSO, MC, at King's College London, Strand, London WC2R 2LS, from whom further particulars are available.

308(A)

STATE UNIVERSITY OF UTRECHT
Biological Faculty
The Netherlands

Applications are invited for the position of
PROFESSOR
in Palaeobotany and Palynology.

Requirements: A Ph.D. degree in biology or geology with specialisation in palaeobotany, with emphasis on micropalaeobotany, and ability for productive research as well as leadership potential.

Persons who meet these qualifications and like to be considered as candidates or those wanting to mention likely candidates are requested to contact Dr H. O. Voorma, Department of Molecular Cell Biology, Padualaan 8, 3584 CH Utrecht, Netherlands. W29(A)

**PURDUE UNIVERSITY
RESEARCH ASSOCIATES
THEORETICAL
CHEMISTRY**

Two openings exist for theoretical chemists to study molecular liquids and solutions with applications in organic chemistry. A Ph.D. in chemistry or physics and background in computing and molecular quantum or statistical mechanics are required.

Start anytime in 1979. Salary is \$12,000/year. Appointments are renewable.

Send vitae and names of references to: Prof. Wm. L. Jorgensen, Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, U.S.A. An equal opportunity/affirmative action employer.

W19(A)

**UNIVERSITY OF
QUEENSLAND
Australia
TEMPORARY LECTURER
IN GEOGRAPHY**

Applicants should have a higher degree and tertiary teaching experience.

The appointee will take responsibility for teaching basic courses in Remote Sensing and Cartography and an advanced course in Geomorphology. (Quote Ref. No. 13079) April 30, 1979.

Salary: \$A15,786 to \$A20,737 per annum. Other Benefits: Single air fare for appointee only to Brisbane.

Additional information and application forms are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

322(A)

**UNIVERSITY OF
AUCKLAND
New Zealand**

Applications are invited for the following full-time teaching positions. Conditions of appointment and method of application are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications, in accordance with "Method of Application", will be accepted at any time up to **May 14, 1979** unless otherwise stated. Commencing salaries in accordance with qualifications and experience will be established within the appropriate scale. The scale for Lecturers is NZ\$11,529 per annum rising by annual increments to NZ\$14,250 per annum. In normal circumstances, a Lecturer whose services have proved satisfactory to the Council may expect in due course to be given the status of a Senior Lecturer, salary maximum NZ\$16,780 per annum. At present all salaries are supplemented by a General Wage Order of NZ\$365 per annum.

**Biochemistry
LECTURESHIP**

Applicants should have a first degree in biochemistry or chemistry and suitable postgraduate qualifications in biochemistry. Preference will be given to candidates who have completed a period of postdoctoral research.

**Numerical Analysis
LECTURESHIP**

Closing Date: July 15, 1979. For 1979 this position is within the Computational Mathematics Unit of the Department of Mathematics, but from 1980 it will be within the newly formed Department of Computer Science. Applications should have qualifications within any field of numerical analysis.

347(A)

**KENYATTA UNIVERSITY
COLLEGE—KENYA
(Constituent College of
University of Nairobi)**

Applicants are invited for the post of
**ASSOCIATE PROFESSOR
IN THE BOTANY
DEPARTMENT**

Applicants must have a Ph.D. degree in Botany and considerable experience at University level, at least to Senior Lecturer level. Further qualifications in Education or experience in the training of graduate teachers would be advantageous. They should also provide evidence of successful research activity through publications in reputed journals. The appointee will be expected to teach in the B.Ed. and M.Sc. programmes and to carry out and supervise research in his/her area of specialisation. He/she will also be expected to be capable of providing academic and administrative leadership in the department. Salary scale: K£3,864 to K£4,488 per annum, (K£1 Sterling equals £1.32). The British Government may supplement salary by £5,784 per annum (sterling) for married appointee or £3,876 per annum (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages: subsidised housing; S.S.F. or F.S.S.U.; non-contributory medical aid scheme. Detailed applications (two copies) with curriculum vitae and naming three referees should be sent by airmail to the Registrar, Kenyatta University College, P.O. Box 43844, Nairobi, Kenya by May 8, 1979. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

328(A)

**UNIVERSITY COLLEGE
OF SWAZILAND**

Applications are invited for the post of

**LECTURER IN
AGRICULTURAL
ENGINEERING**

in the Department of Land Use and Mechanisation. Candidates should possess a Degree or Diploma in Agricultural Engineering. Formal training in teaching Agricultural Engineering at College level would be an advantage. The appointee will be expected to provide lectures on Farm Machinery and Crop Mechanisation to Degree and Diploma students and practical demonstrations relevant to these courses. Salary scale: L4,140 to L7,860 per annum (£1 sterling equals L1.71). The British Government may supplement salary in range £1,650 to £1,986 per annum (sterling) for married appointee or £408 to £780 per annum (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. If appointment is for a limited period, a 25 per cent gratuity is paid in lieu of superannuation for the first two years of service; 27½ per cent for the second two years. A 10 per cent inducement allowance is payable to expatriates not qualifying for supplementation from other source. Education allowance; medical aid scheme; reasonable rented accommodation; family passages; biennial leave. Detailed applications (two copies) with curriculum vitae and naming three referees to be sent by May 9, 1979 to the Registrar, University College of Swaziland, Private Bag, Kwaluseni, Swaziland. Applicants resident in the U.K. should send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

327(A)

Guy's Hospital Medical School. Experienced **SECRETARY** required from June to work for Professor and Unit of Gastroenterology. Applicants must be competent shorthand-typists. Salary according to experience and qualifications. Apply in writing, stating age and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. G.R.1.

320(A)

UNIVERSITY OF STIRLING

DEPARTMENT OF BIOLOGY POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the post of postdoctoral research assistant to work on an N.E.R.C.-funded project to study the productivity of oligochaete worms in the Forth estuary.

The appointment will be for 2½ years, on the Research and Analogous Staff Scale 1A, £3,883 to £4,382 (subject to review), with the starting date to be arranged.

Further details are available from the Secretary, University of Stirling, Stirling FK9 4LA to whom applications should be submitted, together with the names of two referees, by April 17, 1979. 312(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BOTANY

SENIOR

RESEARCH ASSISTANT

Applications are invited for the post of Senior Research Assistant to work on the production of a new Check List of British Ascomycetes (including lichen-forming species) under the joint direction of Dr G. N. Greenhalgh (Liverpool University) and Dr D. L. Hawksworth (Commonwealth Mycological Institute, Kew). The work involves both a critical assessment of existing records and revisionary work, and the person appointed will divide their time between Liverpool University and the Commonwealth Mycological Institute. Candidates should have some experience in the field of taxonomy and nomenclature, preferably, though not necessarily, with fungi and lichens and should have, or expect to obtain, their PhD before taking up the appointment.

The project will be financed by the SRC for three years. The initial salary will be within the range £3,883 to £4,382 per annum (under review).

Applications (three copies), together with the names of two referees, should be received not later than April 30, 1979, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX from whom further particulars may be obtained. Quote ref RV/344/N 297(A)

THE WEST OF SCOTLAND AGRICULTURAL COLLEGE ADVISORY AND DEVELOPMENT SERVICE

Post of

SENIOR

EXPERIMENTAL OFFICER (Grade II)

Glasshouse Investigational Unit for Scotland

Applications are invited from suitably qualified persons for the post of Senior Experimental Officer, Grade II. Preference will be given to Honours graduates in horticulture or a closely related discipline. Candidates will be expected to have appreciable post-graduate experience of glasshouse experimental and development work and preferably some knowledge of commercial glasshouse production. Duties will include the initiation and implementation of experimental programmes and responsibility for the day-to-day supervision of work at the Unit.

Salary, Grade II, £6,447 rising to £7,651. Application forms and conditions of appointment may be obtained from the Secretary, The West of Scotland Agricultural College, Auchincruive, Avr 2A6 5HW, with whom applications should be lodged not later than May 4, 1979. 301(A)

Laboratory Technicians Pharmaceutical Development

Hoechst is one of the world's leading chemical and pharmaceutical manufacturers and a policy of continuous development has created the following opportunities in our research and development laboratories at Milton Keynes.

We are currently seeking experienced technicians for the following areas:—

To provide technical support in the development of animal feed products. The successful candidate will develop and improve analytical methodology and perform quality control assays, process investigations and stability evaluation for a range of medicated animal feed products. Candidates should have experience of working in an analytical laboratory and preferably be qualified to HNC level in Chemistry.

To assist with analytical aspects of the development of pharmaceutical products. The successful candidate will be primarily involved with the testing and stability evaluation of experimental pharmaceutical dosage forms using a wide variety of physical and chemical testing methods. Candidates should have some laboratory experience and preferably be qualified to GCE 'A' level in chemistry.

The posts carry good salaries, a free private health scheme, subsidised restaurant meals, at least 4 weeks' holiday a year and good sports facilities.

Please write or phone for an application form to:—

Mrs. Sheila Matthews, Personnel Dept., Hoechst UK Ltd., Walton Manor, Walton, Milton Keynes MK7 7AJ. Tel: Pineham 5068.

Hoechst



363(A)

UNIVERSITY OF JUBA SUDAN

Applications are invited for the following posts:

1. PROFESSOR/
ASSOCIATE PROFESSOR/
LECTURER IN ANATOMY.
2. PROFESSOR/
ASSOCIATE PROFESSOR/
LECTURER IN PHYSIOLOGY.
3. PROFESSOR/
ASSOCIATE PROFESSOR/
LECTURER IN
COMMUNITY MEDICINE

Salary scales (excluding cost-of-living allowance). Professor £55,460 per annum, Associate Professor £54,030 to £54,550 per annum, Lecturer £51,500 to £53,500 per annum (£51=£1.23 sterling). The British Government may supplement salaries in range £3,894 to £4,542 per annum (sterling) for married appointees or £1,830 to £2,310 per annum (sterling) for single appointees (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages, various allowances, superannuation scheme, annual overseas leave.

Detailed applications (2 copies) including a curriculum vitae and naming 3 referees, should be sent by air mail to the Vice-Chancellor, University of Juba, P.O. Box 82, Juba, Sudan, by May 8, 1979.

Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. 282(A)

AN X-RAY CRYSTALLOGRAPHER

OR ELECTRON MICROSCOPIST

is sought to take part in a structural study of crystals of SV-40 virus. This postdoctoral position begins May 1, 1979. For a crystallographer, experience with proteins is desirable, but not essential. For an electron microscopist, interest in 3-dimensional reconstruction or in 2-dimensional arrays is preferred. Send a resume and two letters of recommendation to: Eaton Lattman, Department of Biophysics, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA. (301) 955-8715. Johns Hopkins is an equal opportunity/affirmative action employer. W23(A)

EAST MALLING

RESEARCH STATION

PHYSIOLOGY DEPARTMENT

A BIOCHEMIST to join the Hormone Unit for work in support of a programme of biochemical research on plant hormones controlling the growth and cropping of fruit trees.

Applications are invited from those with honours degree in Chemistry or Biochemistry or an ordinary degree, or H.N.C., with relevant experience.

Appointment in Scientific Officer grade (£2,839 to £4,415) starting salary depending on qualifications and experience.

Details and application forms from Assistant to the Secretary, East Malling Research Station, East Malling, Maidstone, Kent. 324(A)

UNIVERSITY OF ZAMBIA

Applications are invited for the post of

SENIOR LECTURER/

LECTURER IN THE

DEPARTMENT OF BIOLOGY

Applicants must have a Ph.D. or equivalent. The areas of research interest given preference are comparative plant and animal metabolism, enzymology or morphogenetic biochemistry. Preference will be given to those with experience in University lecturing and enzymological studies. The appointee will be expected to take up duties as soon as possible and be responsible for the teaching of general Physiology and Biochemistry in component courses as well as conducting an advanced biochemistry course in the final year of the B.Sc. programme.

Salary scales. Senior Lecturer K7,638 to K8,016 per annum; Lecturer, K5,504 to K7,608 per annum (£1 sterling=K1.61). The British Government may supplement salary in range £2,922 to £4,350 per annum (sterling) for married appointee or £930 to £1,968 per annum (sterling) for a single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages, baggage allowance; superannuation; medical aid scheme; regular overseas leave.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees should be sent by air mail to Registrar, University of Zambia, P.O. Box 2379, Lusaka, Zambia, by May 7, 1979.

Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 287(A)

ROYAL DENTAL HOSPITAL OF LONDON

School of Dental Surgery
(University of London)

LECTURESHIP (RESTRICTED TENURE) IN ANATOMY IN RELATION TO DENTISTRY

Applications are invited for the post of Lecturer in Anatomy tenable for up to three years commencing on September 1, 1979.

The Department of Anatomy in relation to Dentistry is responsible for teaching human anatomy, histology and dental anatomy to dental students. This appointment will be at a junior level and the duties will include teaching part or all of these subjects. Previous experience in human anatomy would be a considerable advantage. Adequate research facilities are available, including histology, histochemistry, tissue culture, electron microscopy, etc.

The Department is situated in a modern building shared with St George's Hospital Medical School in Tooting, London, S.W.17.

Salary will be at the appropriate point on the scale £3,906 per annum (currently under review), plus London Allowance of £502 per annum, with superannuation under U.S.S.

Further details may be obtained from Dr A. F. Hayward, Department of Anatomy in Relation to Dentistry, Royal Dental School, Jenner Wing, St George's Hospital Medical School, Cranmer Terrace, London, S.W.17, telephone 01-672 1255 ext. 4633, to whom applications with the names of three referees should be submitted before May 5, 1979. 371(A)

CHEMICAL TOXICOLOGIST

TENURE-TRACK ASSISTANT
OR ASSOCIATE PROFESSOR

Available: August 1979

Ph.D. required

Previous training and research experience in toxicology is essential. Applicant should have a strong background in chemistry and biochemistry. The successful applicant will be expected to develop a strong research program in toxicology which would attract continued outside funding in support of our M.S. chemistry and Ph.D. biomedical sciences degree programs. Responsibilities include undergraduate teaching in the individual's specialty as well as graduate courses in toxicology.

Send resume, research project proposals, and three letters of recommendation to Dr Frank E. Scully, Jr., Chairman, Search Committee, Department of Chemical Sciences, Old Dominion University, Norfolk, VA 23508.

Applications must be received no later than April 30, 1979. Applications from all qualified individuals are invited. An Affirmative Action/Equal Opportunity Employer. W40(A)

UNIVERSITY OF WARWICK LECTURESHIP IN MICROBIOLOGY

Applications are invited for a new post tenable from October 1, 1979 in the Department of Biological Sciences. Teaching will be within the degree courses taught in the Department, the precise area depending upon the interests and experience of the candidate and requirements of the Department. The successful applicant will be appointed to the Microbiology Research Group led by Professor R. Whittenbury; and will be expected to undertake research which complements one or more of the existing projects. Applications from candidates with experience in or a particular interest in, the morphogenesis and differentiation of prokaryotes and who have skills in molecular biological, genetic and/or genetic manipulation techniques, will be particularly welcome. Initial salary will be within the first four points of the Lecturer scale: £3,883 to £4,631 to £7,754 p.a. (under review). Further details and application forms from the Academic Registrar, University of Warwick, Coventry CV4 7AL quoting Ref. No. 35/3A/79. Closing date for receipt of applications May 4, 1979. 367(A)

UNIVERSITY OF GLASGOW CHEMISTRY DEPARTMENT (GC-MS UNIT)

An immediate vacancy exists for a

POSTDOCTORAL RESEARCH APPOINTMENT

tenable for one year, for a project on the analytical and structural characterisation of hydroxysterols by GC-MS and ancillary techniques. Candidates should be steroid chemists, or others familiar with modern liquid-phase and gas-phase methods suitable for the study of microgram amounts of compounds in biological samples of complex composition. Experience of open-tubular glass columns in GLC or GC-MS would be especially valuable. There is a possibility of an overseas visit during tenure of the post. Equipment includes LKB9000 and DuPont 21-490F (CI/EI) instruments, either of which can be used on-line to VG20 Data System. Starting salary in the range £3,634 to £4,133 of the salary scales for Research and Analogous Staff (1A), with U.S.S. benefits.

Applications, naming two referees, should be sent, preferably by April 17, 1979, to Professor C. J. W. Brooks, Chemistry Department, University of Glasgow, Glasgow G12 8QQ, from whom further details can be obtained.

In reply please quote Ref. No. 4413M. 286(A)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London)

POSTDOCTORAL RESEARCH ASSISTANT

required in the Biochemistry Department for a three-year project to establish fusion hybrids of plasma cells producing antibodies against disease causing protozoa. Previous experience in cell culture and biochemistry or immunology desirable. Salary in the range £3,883 to £4,882 (under review) plus £502 per annum London allowance. Application forms and further particulars available from the Establishment Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. Closing date April 30. 343(A)

DIRECTOR, NATIONAL MUSEUM OF NATURAL HISTORY Smithsonian Institution

Applications are being accepted until June 1, 1979 for the position of Director, National Museum of Natural History, Smithsonian Institution, Washington, D.C. Salary is to be \$47,500.

For further information, including copy of the official position announcement, contact: Smithsonian Institution; The Search Committee, Office of Personnel Administration, Box 2500; 900 Jefferson Drive, S.W.; Washington, D.C. 20560.

Applicants should submit Standard Form 171 to the above address.

The Smithsonian Institution is an equal opportunity employer. W39(A)

EAST MALLING RESEARCH STATION POMOLOGY DEPARTMENT

A CROP PHYSIOLOGIST to join, in a support capacity, a team working on root physiology and on irrigation and other methods of reducing tree water stress.

Minimum qualifications needed are an ordinary degree, H.N.C. or equivalent in Botany/Agricultural Science/Horticultural Science.

Appointment in Scientific Officer grade £2,839 to £4,415 starting salary according to qualifications and experience. Non-contributory superannuation.

Details and application form from Assistant to the Secretary, East Malling Research Station, East Malling, Maidstone, Kent. 323(A)

UNIVERSITY OF ASTON IN BIRMINGHAM DEPARTMENT OF PHARMACY SENIOR LECTURESHIP IN PHARMACEUTICS

Applications are invited for the above post. The person appointed will be expected to undertake administrative responsibility for teaching and research in Pharmaceutics. Personal academic interests should be compatible with the existing work of the Pharmaceutics Section, which includes biopharmaceutics, pharmacokinetics, stability and technology. Research expertise relevant to the pharmaceutical industry would be an advantage.

SALARY SCALE: £7,506 to £9,263 per annum (under review). Appointments are normally made to the first point of the scale.

Application forms and further details may be obtained from the Staff Officer (quoting REF. NO. 352/6), University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET (Tel. 021-359 3611, Ext. 201) and applications should be returned not later than April 30, 1979. 314(A)

UNIVERSITY OF NEWCASTLE UPON TYNE POSTDOCTORAL RESEARCH ASSOCIATE IN BIOCHEMISTRY

A postdoctoral research associate is required to join a group working on the isolation and characterisation of monocyte-specific antigens, with techniques including monoclonal antibodies. The project is funded for three years by the Wellcome Trust and involves collaboration between the departments of Pathology and Biochemistry. Experience in membrane biochemistry and/or immunology would be desirable.

Enquiries and applications are invited from persons possessing or expecting to possess by summer 1979 a Ph.D. and should be addressed to Professor R. H. Pain, Department of Biochemistry, Ridley Building, The University, Newcastle upon Tyne, NE1 7RU. Tel. 0632-28511. 318(A)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF ORGANIC CHEMISTRY

Applications are invited for a number of posts of Demonstrator/Senior Demonstrator in the Department of Organic Chemistry. Successful candidates will be expected to supervise practical classes, undertake tutorial work, and carry out research in collaboration with a research group in the Department.

Initial salary within the range £3,384 to £4,882 per annum.

Applications, together with the names of three referees, should be received not later than June 30, 1979 by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/546/N. 365(A)

NEW ZEALAND UNIVERSITY OF CANTERBURY Christchurch

LECTURER IN GEOLOGY (ENGINEERING GEOLOGY)

Applications are invited for the above-mentioned position. Applicants should be capable of teaching courses to both Civil Engineering and Geology undergraduate students, to give assistance in postgraduate work and to undertake research.

Applications close on June 30, 1979. The salary for Lecturers is on a scale from NZ\$11,894 to NZ\$14,615 per annum.

Further particulars and Conditions of Appointment may be obtained from the Association of Commonwealth Universities (Apts.), 36 Gordon Square, London WC1H 0PF. 348(A)

QUEEN ELIZABETH COLLEGE Kensington (University of London) LECTURESHIP

IN MANAGEMENT STUDIES

Applications are invited for the post of Lecturer in Management Studies to join a developing group within the Department of Food Science. Applicants will be expected to have an appropriate academic background with an original discipline in areas such as Economic Geography, Operational Research, Applied Statistics or Applied Economics. The successful applicant will be required to teach courses in the general curriculum of management studies to students following inter-disciplinary degrees in the natural sciences and management. Outside contacts will be encouraged. Industrial research and other relevant experience would be an advantage, and placing on the salary scale will reflect both qualifications and experience; the current scale (under review) is £3,905 to £7,754 per annum plus £502 per annum London Allowance.

Application forms and further particulars are available from the College Secretary, Queen Elizabeth College, Campden Hill Road, London W8 7AH. Tel: 01-937 5411, ext. 209. Closing date: May 4, 1979. 307(A)

UNIVERSITY OF LONDON KING'S COLLEGE

LECTURESHIP IN GEOLOGY

Applications are invited for a LECTURESHIP in Geology. Applicants should have a good honours degree and experience in structural geology; an interest in engineering geology desirable. Field teaching ability is essential.

Current salary scale £3,883 to £7,754 plus £450 per annum London Allowance (both under review). The starting salary will be at an appropriate point on the scale. Universities Superannuation Scheme contributions would be payable.

Further information about the post and Department and application form may be obtained from the Assistant Registrar, King's College London Strand, London WC2R 2LS, to whom applications, in triplicate, should be sent, together with the names of two referees, to reach him by April 26, 1979, quoting reference No. 191669/N 309(A)

UNIVERSITY OF BRISTOL SCHOOL OF CHEMISTRY DEPARTMENT OF PHYSICAL CHEMISTRY

Applications are invited for a

LECTURESHIP IN PHYSICAL CHEMISTRY

tenable from October 1, 1979. Initial salary in the range £3,883 to £6,311 per annum on a scale rising to £7,754 (under review). The successful candidate will be expected to take a full part in laboratory supervision lecturing and tutoring; to engage in research and to participate in the administration of the Department.

Suitably qualified candidates should submit applications, together with the names of three referees, by April 30, 1979, to the Registrar, University of Bristol, Senate House, Bristol BS1 1TH, from whom further particulars may be obtained. Please quote reference EB. 295(A)

UNIVERSITY OF DURHAM DEPARTMENT OF BOTANY

Applications are invited from candidates with interests in molecular and microbial genetics for the post of

LECTURER IN GENETICS

tenable from October 1, 1979. Experience in recombinant DNA technology preferred.

Salary of the scale £3,883 to £7,754 (under review) plus superannuation.

Applications (3 copies) naming three referees should be sent by May 1, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 1LE (from whom further particulars may be obtained). 218(A)

**UNIVERSITY OF ZURICH
SWITZERLAND
DEPARTMENT OF ZOOLOGY
POSTDOCTORAL
OR SENIOR
RESEARCH ASSISTANT**

Applications are invited for a position to work on a project concerned with the development of muscle in *Drosophila*. Good training in electron microscopy would be preferred.

The appointment is for a period of two years, with the possibility of extension, and could commence as soon as possible. The salary would range from Sfr.27,000 to Sfr.47,000 according to experience.

Applications, in the form of a curriculum vitae, list of publications, names and addresses of two referees, should be sent to Prof. I. Deak, Zoologisches Institut der Universität Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. W33(A)

**BLOOD GROUP REFERENCE
LABORATORY
SCIENTIFIC OFFICER**

A graduate is required for research and development related to the production of antiglobulin reagents for use in blood transfusion laboratories. Some experience in immunochimistry, protein purification or radioimmunoassay desirable, but not essential.

Applications in writing with details of experience and the names of two referees to Miss K. Lomas, Blood Group Reference Laboratory, Gatliff Road, off Ebury Bridge Road, London SW1W 8QJ. 354(A)

**HOUGHTON POULTRY
RESEARCH STATION
LIAISON OFFICER**

Applications are invited for the post of Liaison Officer, the duties of which include liaison with, and an information service for, the poultry industry, veterinary profession, Agricultural Research Council and Ministry, the Press, journals and educational organisations, and the preparation of publications and exhibits.

Applicants should possess a degree, HNC or equivalent in a science subject, with at least 5 years' relevant experience. Experience in public relations and editorial work would be an advantage.

Salary in Higher Scientific Officer grade (£4,101 by 8 to £5,448).

Further particulars from the Secretary, Houghton Poultry Research Station, Houghton, Huntingdon, Cambs PE17 2DA. Closing date for applications: May 11, 1979. 311(A)

**ST GEORGE'S HOSPITAL
MEDICAL SCHOOL
(University of London)
LECTURER IN
IMMUNOLOGY**

The post, which is in an expanding Immunology Unit, is suitable for candidates with a background in immunochimistry or membrane chemistry and perhaps cell culture. The person appointed would be expected to devote a substantial amount of time to research in the first three years and would be well provided with research expenses. Salary in the range £3,975 to £8,250 (under review) plus £502 per annum London allowance. Further particulars available from the Establishment Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, to whom applications, with the names of two referees, should be sent to arrive by April 30, 1979. 340(A)

**UNIVERSITY OF
MARYLAND
Baltimore County (U.M.B.C.)
POSTDOCTORAL,
BIOCHEMISTRY**

Available summer, 1979. Research in isolation and characterisation of nucleic acid helix-destabilising (unwinding) proteins; chemical modification, physical, genetic, and immunological studies on these proteins, cationic metal and dye interactions with nucleic acids and polysaccharides. Metro. Baltimore-Washington, D.C. location. Send resumé and three letters of recommendation to Dr Richard L. Karpel, Department of Chemistry, University of Maryland Baltimore County, Catonsville, MD 21228. Equal opportunity/affirmative action employer. W38(A)

**UNIVERSITY OF
LEICESTER
DEPARTMENT OF GENETICS
Applications are invited for a
POSTDOCTORAL
RESEARCH ASSOCIATE**

to work on an M.R.C.-supported project on the mechanism of action of penicillin and the use of this antibiotic to the processes of cell division and shape determination in *Escherichia coli*.

The position is tenable until October 1981 on the range for Research Associates, £3,883 to £6,555. Applications, including the names of two referees, should be sent to Dr Brian G. Spratt at the Department of Genetics, University of Leicester, Leicester LE1 7RH. 296(A)

**MEDICAL RESEARCH
COUNCIL
DEVELOPMENTAL
NEUROBIOLOGY UNIT
INSTITUTE OF NEUROLOGY**

Applications are invited from young postdoctoral morphologists to participate in a programme to study effects of metabolic factors on brain development. Research experience in quantitative morphological method, light and electronmicroscopic autoradiographic, and immunohistochemical techniques will be of advantage.

The appointment will be for one year in the first instance with the possibility of extension for a further two years. Salary will be at the appropriate point on the M.R.C. scale for non-clinical scientific staff.

Applicants should send a Curriculum Vitae giving full details of qualifications and experience, and the names of two referees to Dr R. Balazs, M.R.C. Developmental Neurobiology Unit, Institute of Neurology, 33 John's Mews, London WC1N 2NS. 336(A)

FELLOWSHIPS

**UNIVERSITY OF
CAMBRIDGE
DEPARTMENT OF
ENGINEERING
THE PYE FOUNDATION
RESEARCH FELLOWSHIP IN
TELECOMMUNICATIONS**

Applications for the Fellowship are invited from established postdoctoral research workers to study techniques for the bandwidth compression of speech signals. The Fellowship is tenable from June 1, 1979 for three years, at a salary in the range £4,631 to £6,080 (under review) plus superannuation.

Further particulars may be obtained from the Secretary, Faculty of Engineering, Trumpington Street, Cambridge CB2 1PZ, to whom applications, including a brief curriculum vitae, and the names of two referees, should be sent by April 27, 1979. 360(E)

**THE UNIVERSITY
OF LEEDS
PROCTER DEPARTMENT
OF FOOD SCIENCE
PROCTER
RESEARCH FELLOWSHIP**

Applications are invited for a temporary post of POSTDOCTORAL RESEARCH FELLOW

to study the physics of gelation of water-soluble macromolecules such as gelatin. The project will involve experimental techniques of laser light-scattering and rheological measurement. Applicants should have (or expect to have by October 1979) a Ph.D. in physical chemistry, chemical physics or polymer physics. Previous light-scattering experience would be an advantage. The successful applicant will work in association with Dr. E. Dickinson and Dr. G. Stainby of the Procter Department of Food Science. The appointment will be made for a fixed term of one year. At the end of that period a further fixed term contract for a period of two years may be available. Salary in the range £3,883 to £4,382 on the 1A scale for Research and Analogous Staff (£3,883 to £6,555) (under review), according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 72/4/D. Closing date for applications May 21, 1979. 300(E)

**UNIVERSITY OF
EDINBURGH
DEPARTMENT OF
BIOCHEMISTRY
POSTDOCTORAL
RESEARCH
FELLOWSHIP**

Applications are invited for this post to work on the mode of action and uptake of the drug Suramin in *Trypanosoma brucei* and the cause of resistance to that drug.

Salary range £3,883 to £4,382 (plus superannuation). The post, financed by the World Health Organisation's Special Programme for Research and Training in Tropical Diseases, will be for one year initially with possible extension for a further two years.

Applications with curriculum vitae and the names of two referees should be sent to Dr I. B. R. Bowman, Department of Biochemistry, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, by April 23, 1979. Please quote Reference 5017. 350(E)

**UNIVERSITY OF
BIRMINGHAM
DEPARTMENT OF
PLANT BIOLOGY
POSTDOCTORAL
FELLOWSHIP**

Applications are invited for an Agricultural Research Council Postdoctoral Research Fellowship, tenable for three years, for work with Dr G. G. Henshaw on plant regeneration from potato tissue cultures. Salary in the range £3,883 to £6,555 (under review), plus superannuation. Maximum starting salary will be £4,382.

Applications including a curriculum vitae and the names and addresses of three referees should be sent to Assistant Registrar, Science and Engineering Registry, P.O. Box 363, Birmingham B15 2TT by Friday, April 20, 1979. Please quote ref: NK7. 337(E)

**HOWE
SENIOR
RESEARCH FELLOWSHIP**

Applications are invited by the Council of the Royal Society for the Howe Senior Research Fellowship, established from the Mr and Mrs R. L. Howe Bequest for the promotion and encouragement of scientific research. On this occasion applications are restricted to those working in the fields of fundamental or applied biological sciences. The appointment will be tenable in any appropriate university department or research institution in the United Kingdom approved by the Council of the Royal Society. Candidates, who must be citizens of the United Kingdom or the Commonwealth, must supply the usual personal details and give two referees. Testimonials will not be considered. The subject of the proposed research, and where it would be done, together with the name of the head of the department, whose consent should first be obtained, must be stated.

The appointment, which will be subject to the Society's general regulations governing research appointments, will be tenable for five years from October 1, 1979 (or another date to be arranged) and may be renewable for successive five-year periods until the end of the academic year in which the holder becomes 65. Applicants should preferably be under 45 on October 1, 1979. The stipend scale will be £8,031 x £252 to £8,878 x £249 to £9,036 per annum, and the point of entry will be determined by Council. Superannuation benefits will be provided. Some provision for research expenses will be available.

Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, and returned by April 30, 1979. 283(E)

**UNIVERSITY OF
BIRMINGHAM
DEPARTMENT OF
PLANT BIOLOGY
POSTDOCTORAL
FELLOWSHIP**

Applications are invited for a Postdoctoral Research Fellowship supported by the Ministry of Overseas Development which is tenable until December 31, 1979 to work on the storage of cocoa seeds. Salary in the range £3,883 to £6,555 per annum plus superannuation. Starting salary will not exceed £4,382.

Further particulars are available from Assistant Registrar, Science and Engineering, P.O. Box 363, Birmingham B15 2TT to whom applications (three copies) including a curriculum vitae and names and addresses of three referees should be sent by Friday, April 20, 1979. Please quote ref: NK6. 285(E)

ASSISTANTSHIPS

**THE UNIVERSITY OF
SHEFFIELD
DEPARTMENT OF
BOTANY**

Applications are invited from men and women for an

**S.R.C. POSTDOCTORAL
RESEARCH ASSISTANTSHIP**

to study physiological and biochemical aspects of seed dormancy in collaboration with Dr B. C. Jarvis. Tenable for two years from September 1, 1979. Initial salary up to £4,382 a year. Applications, together with the names of two referees, to the Registrar and Secretary, The University, Sheffield S10 2TN, by April 20, 1979. Particulars available on application. Quote ref: R 271/G. 331(F)

ASSISTANTSHIPS—cont.

UNIVERSITY OF DURHAM
DEPARTMENT OF CHEMISTRY

Applications are invited for chemists or chemical physicists for an

S.R.C. POSTDOCTORAL
SENIOR RESEARCH
ASSISTANTSHIP

or sixteen months from as soon as possible, to work in collaboration with Dr J. Yarwood on the interaction and dynamics of hydrogen bonded systems in the liquid phase. These studies which include infrared, far-infrared, Raman and neutron scattering studies are also supported by a N.A.T.O. research grant and would be carried out at Durham, the Technical University, Braunschweig and at A.E.R.E. Harwell. Some experience in one branch of molecular spectroscopy is essential and an interest in the chemical physics of molecular motions and interactions in the liquid phase would be an advantage.

Initial salary £3,883 or £4,133 per annum on National Research Range A plus superannuation.

Applications (three copies) naming three academic referees should be sent by April 18, 1979, to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars can be obtained. 278(F)

UNIVERSITY OF DURHAM
DEPARTMENT OF PHYSICS

Applications are invited for a

POSTDOCTORAL
SENIOR

RESEARCH ASSISTANTSHIP

enable for three years from 1st October, 1979. The successful applicant will participate in a new research project devoted to the theoretical study of low energy molecular collisions.

Initial salary in the range £3,883 to 4,882 (plus superannuation).

Applications (three copies) naming three referees should be sent by April 30, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. 280(P)

GRADUATE ASSISTANTSHIPS leading to M.Sc. and Ph.D. in Chemical Engineering. Stipend up to \$7,200 plus tuition for qualified candidates. Active and stimulating research program. Write Dr John A. Roth, Department of Chemical Engineering, Vanderbilt University, Nashville, Tennessee 37235. Equal Opportunity Employer. W31(P)

STUDENTSHIPS

AGRICULTURAL
RESEARCH COUNCIL
STUDENTSHIP

Applications are invited for an ARC postgraduate studentship tenable at the Meat Research Institute for research into the viscoelastic properties of semi-solid foodstuffs.

The work will involve a study of the transmission of mechanical pulses through foods and computer analysis of transient stresses generated with an Instron materials tester.

The project is expected to form the basis for a Ph.D. thesis in the University of Bristol and the appointment will commence in October 1979.

Candidates should possess a First or Upper Second Class degree in Physics or a related subject.

Further particulars of the award and allowances together with Application Forms may be obtained from the Personnel Officer, Agricultural Research Council, Meat Research Institute, Langford, Bristol BS18 7DY. 310(F)

UNIVERSITY OF
ST ANDREWS
POSTGRADUATE
STUDENTSHIP IN
BIOCHEMISTRY

Applications are invited for a postgraduate studentship financed by the National Fund for Research into Crippling Diseases. The project will involve biochemical research in relation to a particular inborn error of metabolism. Techniques employed in the project will include tissue culture, protein purification, methods of physico-chemical separation such as isoelectrofocusing, immuno- and electro-phoretic methods, enzymology.

The three-year studentship will commence from October 1979. Candidates should hold, or expect to obtain in 1979, a first or upper second class Honours Degree in Biochemistry.

Applications (preferably type-written) together with the names and addresses of three referees should be sent to Dr R. Griffiths, Department of Biochemistry and Microbiology, University of St Andrews, North Street, St Andrews, Fife, Scotland. 352(F)

UNIVERSITY OF BATH
BIOCHEMISTRY GROUP
SCHOOL OF
BIOLOGICAL SCIENCES
S.R.C. RESEARCH
STUDENTSHIP

Applications are invited for an S.R.C., C.A.S.E. Research Studentship, granted to the above department in collaboration with The Chester Beatty Institute for Cancer Research. It is proposed to investigate the potential anti-tumour activity of a range of newly-synthesised deuterio-analogues of glucose and the successful applicant will have the opportunity to gain experience in a range of cell-culture and also enzymological techniques. Further details may be obtained from Dr R. Harrison at the above address. 335(F)

UNIVERSITY OF
ABERDEEN
DEPARTMENT OF
PHYSIOLOGY

A N.E.R.C. Research Studentship is available to study the tolerance of fish red cells to temperature and pressure (Dr A. G. Macdonald) or the hormonal regulation of phosphate metabolism in fish (Dr C. G. Dacke: C.A.S.E. Studentship with N.E.R.C. Institute of Marine Biochemistry). Further information may be obtained from the Head of the Department of Physiology, University of Aberdeen, Marischal College, Broad Street, Aberdeen AB9 1AS. 346(F)

UNIVERSITY OF
ABERDEEN
DEPARTMENT OF GENETICS
Applications are invited for an
S.R.C.

RESEARCH STUDENTSHIP to support study for a Ph.D. degree. A variety of research topics are available in such areas as mutagenesis, comparative genetics of drug resistance in fungi, DNA replication patterns in related species, genetics of herbicide resistance in plants, human cytogenetics in relation to cancer, genetics of colour perception in man, ecological genetics of animal or plant populations.

Details of academic record and names of two referees should be sent to the Secretary, Department of Genetics, University of Aberdeen, 2 Tillydrone Avenue, Aberdeen, as soon as possible and not later than April 4, 1979. 303(F)

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SCIENCES (BIDSTON)STUDENTSHIP ON TILT
IN THE SOLID EARTH

Applications are invited for a Studentship in the Earth Tide Section of the Institute of Oceanographic Sciences at Bidston Observatory.

The project is concerned with Earth tidal tilt measurements and the effects of crustal structure, ocean tides and shelf tides on the observed tidal signal and will involve some fieldwork in the Lake District.

Candidates should possess a First or Upper Second Class degree in an appropriate scientific discipline.

The successful applicant will be registered for the degree of Ph.D. of the University of Liverpool of which this Institute is an Affiliated Body.

Further particulars of the project and the award may be obtained from Dr R. J. Edge, Institute of Oceanographic Sciences, Bidston Observatory, Birkenhead, Wirral L69 7RA. 294(F)

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Applications are invited from graduates, or those expecting to graduate this summer, for a N.E.R.C. Research Studentship. The Studentship is available for one only of the following research projects:

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(Supervisor: Professor H. Smith)

(2) Available genetic resources of turf grasses—C.A.S.E. studentship with the Sports Turf Research Institute.

(Supervisor: Dr C. A. Stace)

The first project will be of interest to a plant physiologist/ecologist whereas the second project would be more suited to a plant taxonomist. All promising candidates will be invited to the Department for informal discussions.

Requests for further information and applications should be addressed to Professor H. Smith, Department of Botany, University of Leicester, Leicester LE1 7RH. 313(F)

COURSES

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This course will be offered for the first time in October 1979; it is of one year's duration and is designed to instruct students in the theoretical and practical aspects of toxicology. The full M.Sc. course consists of five modules, each of about five weeks and includes a research project (12 weeks). Applicants should have an honours degree in Biochemistry, Chemistry, Physiology or Zoology or hold a medical or veterinary qualification of equivalent standing. Students having a degree in Chemistry should have taken a course in Biology.

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Application forms and further details of the course may be obtained from the Assistant Registrar (S), University of Birmingham, P.O. Box 363, Birmingham B15 2TT, quoting reference T2. Applications should be received as soon as possible and in any case not later than June 15, 1979. 304(D)

SYMPOSIUM

FEBS SYMPOSIUM
ON DNA

Liblice, near Prague,

Czechoslovakia

24-29 September 1979

Topics: Recombinant molecules of DNA; DNA-protein interactions; DNA recombinations *in vivo*; DNA repair.

Information from: Drs S. Zadrzil and J. Sponar, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Flemingovo nám.2, 166 10 Prague 6, Czechoslovakia.

The closing date for applications is May 15, 1979. 250(M)

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CONFERENCES

THE NEW YORK
ACADEMY OF SCIENCES
ANNOUNCEMENT OF
CONFERENCECONFERENCE ON THE
STRUCTURE OF
LIPOPROTEINS
New York City

September 10-12, 1979

The New York Academy of Sciences will sponsor a three-day conference on the STRUCTURE OF LIPOPROTEINS. The conference is co-chaired by Angelo Scarni (University of Chicago) and Frank Landsberger (Rockefeller University). Research on plasma lipoproteins is advancing at a very rapid pace. This conference will focus directly on analyses of the structure of these molecular complexes. Recent observations have shown that plasma lipoproteins have a regulatory role in intracellular lipid metabolism, and that they may also be implicated in the genesis of neurological disorders.

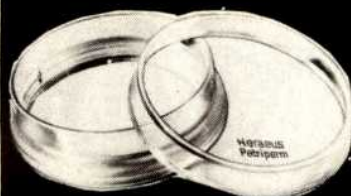
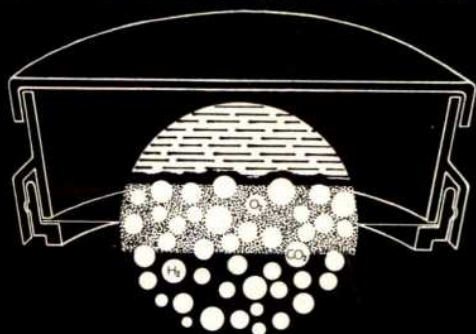
The conference will permit a critical analysis of the progress to date in the field of plasma lipoprotein structure and will alert multi-disciplinary investigators to the advances made in the general area of protein-lipid interactions and other relevant biological systems.

To supplement invited lectures, the organising committee will accept a limited number of suitable poster session presentations. The deadline for the submission of abstracts to be considered for poster presentation is on or about June 1, 1979. The abstract should include the names of the author(s), institution and 100 to 200 word summary of work to be presented in the poster. Send abstracts for poster session and inquiries about details of the programme and registration to:

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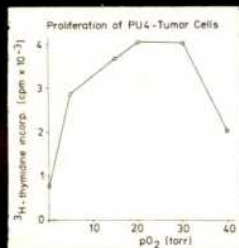
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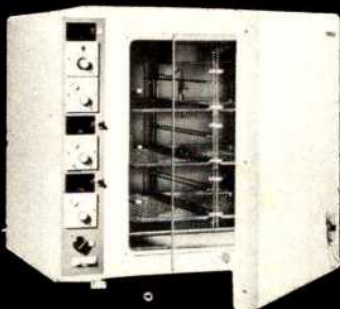
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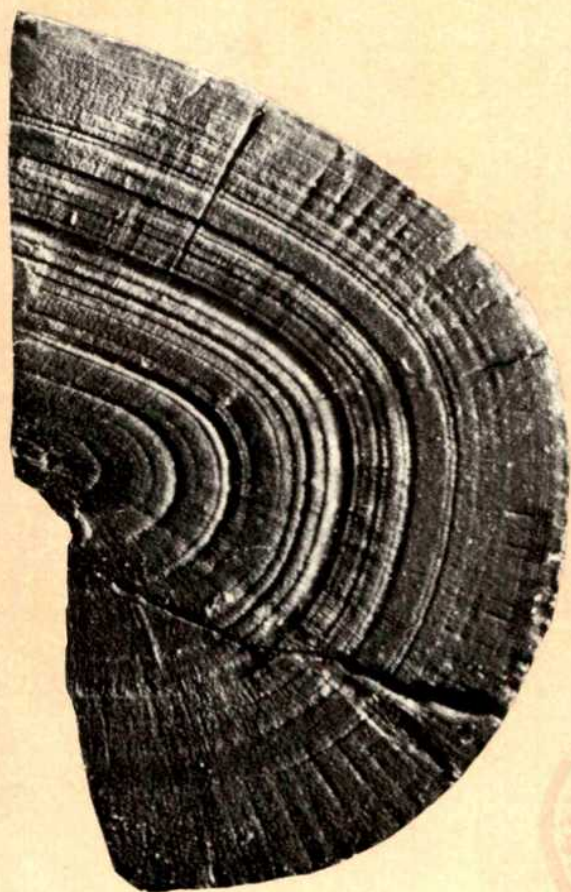
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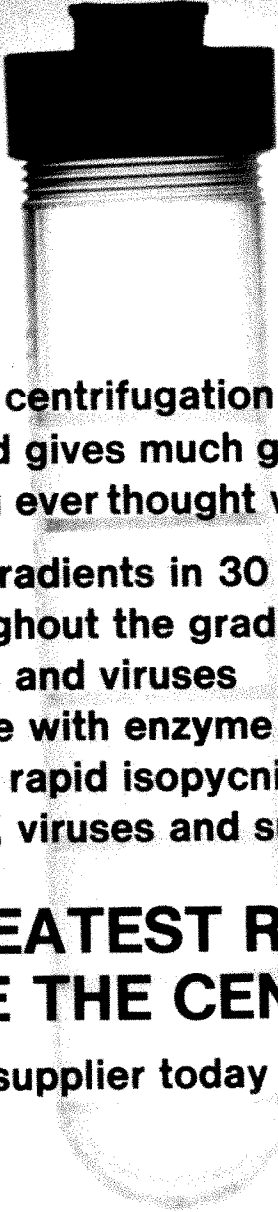
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Cover picture

Teeth of a dinosaur recovered from Late Cretaceous formations of Alberta, Canada. The concentric rings provide evidence of the seasonal growth characteristic of cold-blooded animals, and do not support the hypothesis that dinosaurs were warm-blooded. See page 635.

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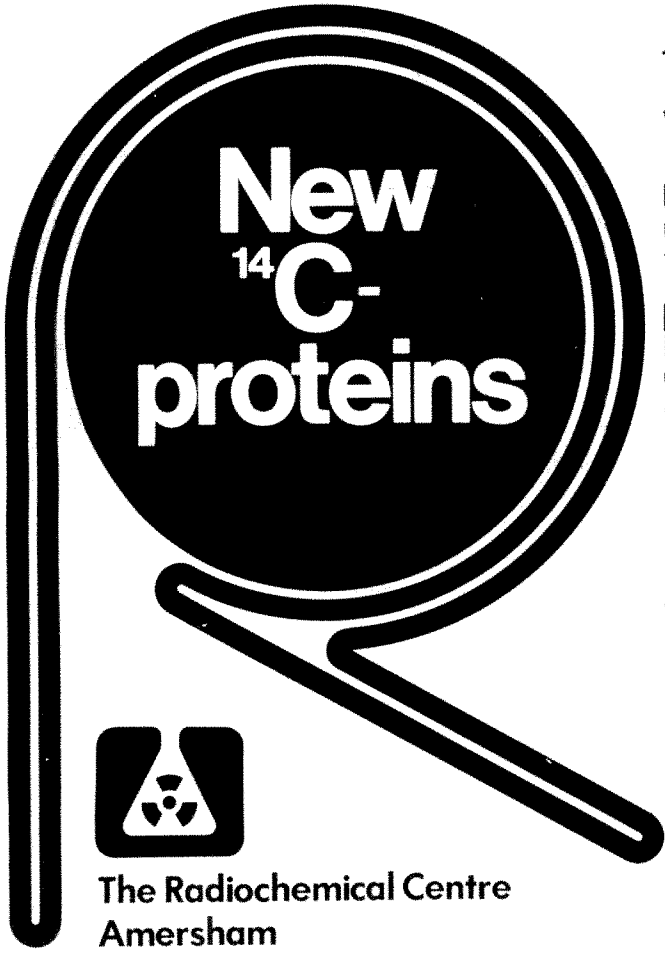
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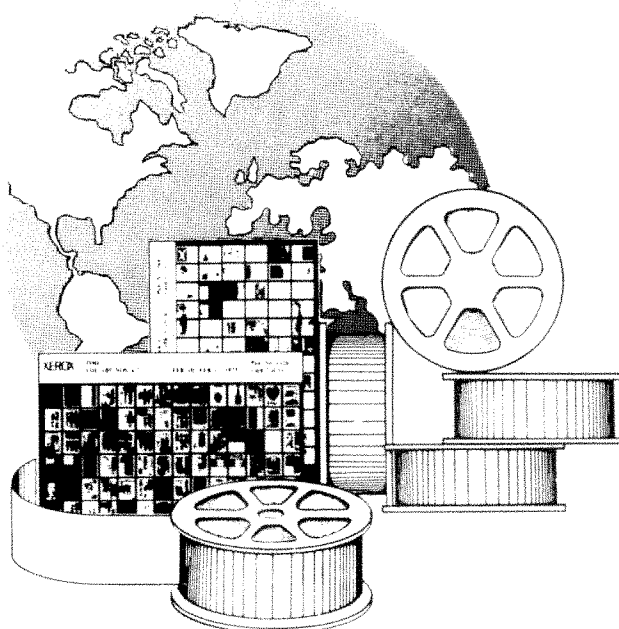
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Molecular biology: suffering from shock

THE recent meeting of molecular biologists convened by COGENE, the Committee on Genetic Experimentation of the International Council of Scientific Unions, was an instructive affair—but not in the way the organisers intended. The lesson came in their treatment of the press. First the meeting was open; then it was closed to journalists. Then it was open to three journalists picked by the Association of British Science Writers, but the proceedings were to be 'off the record'. Then at the last moment all restrictions were lifted.

To be fair, the organisers of the meeting—COGENE and the Royal Society—admitted they had made a mistake; they were under pressure, it seemed, from biologists who had had bad experiences with the US press. And the press is certainly no angel. But the meeting's attitude to it was symptomatic of a deeper malaise: that despite the promise of the 'Berg letter' (*Genesis*, someone called it) which launched molecular biology into politics, biologists have failed to grasp the nettle of their relationship to the public. The press, after all, represents all the fears and frustrations of its readers: it is the public writ in 48 point bold caps.

However the molecular biologists—if the COGENE meeting was a guide—now feel that their subject has no right to be in the public arena at all. The Berg letter called for a pause to assess the risks of the recombinant DNA technique, we have had the pause, and the risks are found to be negligible. So why don't the NIH, and GMAG, and the other regulatory agencies just go away? That was the strong mood of the meeting. "We want to achieve re-entry," someone said. But as Sir Frederick Warner, one of the assessors at the Windscale inquiry into nuclear reprocessing, told the meeting, the three great fears have been raised in the public mind: carcinogenesis, mutagenesis, and teratogenesis. The public won't go away. Re-entry into the womb is no longer possible, even if it seems rational.

Paul Berg's two revolutionary impacts on molecular biology—the recombinant DNA technique itself, and (with the other 10) the Berg letter—have left molecular biologists in a state of shock. Within six years they have found an immensely promising research technique; they have entered biotechnology, with undreamt-of profits to be made; and they have entered politics with their perfectly rational whistle-blowing over conjectural risks. All this happened at just the time when a new breed of radical scientists was searching for something to cut its teeth on, and when the regulation of science and technology was becoming fashionable among governments. So no wonder the biologists are reeling.

No doubt the organisers of the COGENE meeting intended that it should come to terms with this new climate.

But it only reacted to it. Four major issues facing molecular biology were either not addressed or left unresolved. First, what really are the arguments that the risks are negligible? Although there were interesting talks—such as that of Walter Bodmer on species barriers—which were relevant to the question, there was no scientific *debate* of the matter. Where were the critics—and there are scientific critics, with genuine arguments—to bring matters to a head? To the outside observer the view that risks are negligible went through on the nod. If the biologists are to face the public they must do better than communicate a mood; they must construct arguments that are for more than internal consumption. To do that they must embrace their heretics, not spit at them.

Second, there was not a minute of discussion of the new procedure for risk assessment devised by Sidney Brenner and recently adopted by Britain's Genetic Manipulation Advisory Group. Confounding its critics who complain that biological knowledge is to patery for it to work, this method is already in use in GMAG, and has resulted in the down-grading of a number of experiments. If it is false, it should be exposed; if it is correct, being simple and logical it could be the basis of communicating the biologists' new mood to the public.

Third, if there was no scientific debate, neither was there political debate, despite the fact that it is clear that the biologists' re-entry must be a political one. The most moderate voices, such as that of Mark Richmond, showing sensitivity to politics and public opinion, sounded radical against the growls of the rednecks, and were submerged.

Fourth and last, the role of molecular biology in technology, where recombinant DNA is the microprocessor chip of the pharmaceutical industry, was dealt with only at the level of bioengineering and gee-whizzery. And yet here is a technique which has already generated a new social world for the biologist, who is becoming suspicious of his colleague's motives (does he want my enzyme for profit or for science?) and hungry for a new kind of success. Is that not a significant development? Why was it not a subject for debate? Further, what impact will this technique have on medicine and agriculture? Should it be left to the few secretive new firms that are engaged on it, or do we have a chance to create a humane and democratic technology? These questions were ignored.

Molecular biologists are firmly in politics now, and it is totally irrational to ignore the fact. Nuclear physicists should envy them: for the physicists generated their technology first, became committed to it, and burnt their political fingers later. The biologists have the opportunity to learn their politics first, and grow with it. They should not miss the opportunity. □

California calls for nuclear shutdown

David Dickson reports from California on reactions to the accident at the Harrisburg nuclear power plant

THREE years ago, the citizens of California voted by a two to one majority against any curb on the development of nuclear power. Last week, following the accident at the Three Mile Island nuclear power plant in Harrisburg, Pennsylvania, which took over a week to bring under control, and resulted in the discharge of radioactive isotopes into the local environment, an informal survey by a San Francisco newspaper based on telephone calls from readers revealed a 56% majority in favour of shutting down all nuclear power plants.

No one pretends that the California figures are a true reflection of attitudes across the US. California's maverick tradition on "lifestyle" issues, together with a high incidence of sunshine that makes it relatively well placed with respect to alternative sources of energy, both tend to inflate public opposition to nuclear power.

However, the shift in attitude, to judge from press reports, does reflect a public mood. Governor Jerry Brown, his presidential ambitions not far below the surface, has argued with both the Nuclear Regulatory Commission and the local utility company for a temporary shutdown of the Rancho Seco power plant in California (which like the Harrisburg plant was designed by the engineering firm Babcock and Wilcox). And even some of those who had previously been staunch supporters of the nuclear industry are voicing concern that perhaps the Harrisburg incident indicates that not all is as it should—or had been made out—to be.

Defenders of the industry are using the fact that the amount of radiation released to the atmosphere has been minimal, and well below levels claimed to have a harmful effect on humans, to argue that the industry had shown that its safety precautions worked. "Reactors are designed to survive the failure of instruments, which is why they have back up systems," said Dr Edward Teller, so-called "father" of the hydrogen bomb, and now with Stanford University's Hoover Institution. "The worst-case estimate is that some people were exposed to about 50 millirems. Airline hostesses get that in about 50 hours of flight, which they can probably accumulate in about three weeks," Dr Teller said.

But Professor Joseph Rotblat, Emeritus Professor of Physics at St. Bartholomew's Hospital, London University, points out that if the official figure of 25 millirem per hour within a five-mile radius was sustained for the first week of the accident, the 24,000

inhabitants within the five-mile radius could have received a total of 5 rems. This exceeds the maximum allowable dosage for radiation workers of 3 rems in any 13-week period and is ten times the permissible value for the population at large. Official estimates attribute one cancer case per 10,000 population per rem, but, says Rotblat, "this estimate is perhaps a factor of ten too small", which would imply an additional 120 cancer cases because of the accident.

If the incident did little to shake the support of firm supporters of nuclear power, neither did it come as much surprised to nuclear critics. "This is just the type of thing that we have been warning people would happen," said Mr Bob Judd, director of California's Office of Appropriate Technology in Sacramento.



"But we've put a million dollars into Meltdown at Rancho Seco!"

Where there has been a perceptible shift, however, is in those who have yet to come down firmly on one side or another. In the California state legislature, a number of politicians who still support the development of nuclear energy voiced concern at the implications of Harrisburg—for example, at the fact that the formation of the hydrogen bubble which formed at the top of the containment vessel, and gave the greatest source of concern, had never entered anyone's calculations as a potential event—and supported Governor Brown's call for a temporary closure of Rancho Seco.

Similarly on university campuses some of those previously sympathetic to the anti-nuclear movement, but sceptical of its apparent "anti-technology" stance, admitted that the Harrisburg incident had shifted their perspective. "I am beginning to believe that there is something inherently unstable about nuclear power; that unlike other energy sources, the more you develop it, the more you have to develop systems to keep it under control," says Professor Charles Schwarz, professor of physics at the University of California at Berkeley.

The incident hung like a black cloud

over the proceedings of a San Francisco conference on "science, technology and the human prospect" which had been organised jointly by the Electric Power Research Institute and the Thomas Alva Edison Foundation as part of a year-long commemoration of the invention of the electric light one hundred years ago.

The opening day of the conference coincided with Governor Brown's call for the closing of Rancho Seco. Dr Chauncey Starr, Vice-President of EPRI, admitted at a press conference that he would have liked to discuss some of the broader issues being raised by the conference, billed as "a unique opportunity to explore the impact of science and technology on society's development". But all the questions, inevitably, turned on Harrisburg.

"Give us the facts. We want facts, not opinions," demanded one journalist, as Dr Starr explained that at present the precise sequence of events within the reactor that had led to the accident was unknown, but assured people that the levels of radiation to which the local community had been exposed were "so low that they were below any physiological danger to anyone, including pregnant women."

The accident did not change the long-term picture, he said. The initiating circumstances—the apparent failure of a pump circulating the cooling water—was not uncommon, and the statistical probability of the accident fell within the range that had been predicted for the nuclear power programme over 400 reactor years of operation. "Risk analysis is not going to be changed in any particular way by this," Dr Starr said.

"Although something happened in this plant that we do not yet fully understand, no one has been hurt, and there is no public danger that any of us can see. I do not think that shutting down nuclear power plants is a way to increase public confidence. We will continue to do our best to get the public to understand that there are sufficient assets to nuclear power compared to other energy sources, and that the health risks remain very much less. Remember that even steam boilers blow up."

Back in the less charged atmosphere of the conference, speakers talked in general terms about the need to maintain confidence, that society could learn to control the technology that it had developed, and claimed that the Harrisburg incident should reinforce—rather than dent—this confidence (though most were reluctant to get into a detailed discussion of the incident itself). □



President Carter is briefed in the control room at Three Mile Island by Harold Denton of the NRC (left).

Harrisburg: counting the cost

At Harrisburg, the accident still poses enormous clean-up problems. The radiation level inside the reactor containment dome reached 30,000 rems per hour, and officials face the task of cleaning up 250,000 gallons of radioactive water from inside the reactor vessel itself. "We are looking at plans to bring the reactor to a cold shutdown without an increased leakage from the plant" said Harold Denton, Director of Reactor Regulation of the Nuclear Regulatory Commission (NRC). But Senator Morris Udall said it would be months before any clean-up could begin, "if, indeed, a clean-up is possible".

In the meantime, there have been press reports blaming the Metropolitan Edison Company for violating Federal regulations for reactor operation. The *International Herald Tribune* cites detailed NRC reports that valves controlling the emergency water supply to the reactor's cooling system had been closed for two weeks for routine maintenance. Mr Darrell Eisenhut, an engineer for the NRC, told a press conference that in addition the analyses showed that the main cooling system had been turned off at the wrong time and that four auxiliary pumps were disengaged in violation of Commission regulations. These faults combined to cause 60,000 gallons of radioactive water to flood the reactor chamber to a depth of eight feet. The zirconium in the reactor fuel cladding then combined with the water to release hydrogen gas which created a large 1,000 cubic feet bubble at the top of the vessel, causing the fuel rods to overheat and rupture releasing fission products into the water.

In a letter to *The Guardian* in London, Sir Martin Ryle of the Cavendish Laboratory in Cambridge said that the deficiencies of the emergency core cooling system of

the Harrisburg family of pressurised water reactors (PWRs) had "been known publicly for five years and by the US Atomic Energy Commission for considerably longer". According to Ryle, the system consists of four separate methods of cooling and relies on correct working of monitored temperature, pressure and waterlevel and the correct sequencing of the various pumps and valves. "It is an excessively complicated system that has never been tested except for 'tests' by computer simulation", said Ryle. He added that the highly dangerous hydrogen bubble should have been predicted as a matter of "A-level textbook knowledge" or failing that as the result of an "afternoon's experiment".

In the financial world, the accident touched off heavy sales of nuclear power related stocks. The US stock market as a whole suffered a broad decline which was attributed to investors worrying over the implications of the accident for the US energy situation. However, stock in Columbia Pictures, owners of *The China Syndrome*, a film about a nuclear meltdown "somewhere in Pennsylvania", rose as its box office takings reached a new high. An anti-nuclear group in South Carolina reported that their phones had been busy 16 hours a day. "People call up and say I can't believe it. I saw the movie and then came home and saw the same thing on the 11 o'clock news."

Daniel Ford, executive director of the Union of Concerned Scientists, called on President Carter "to seek immediate removal" of Dr Joseph Hendrie as chairman of the NRC. Ford was reported as saying that Hendrie had participated in a "far reaching cover-up of critical nuclear safety difficulties".

Joe Schwartz

Joe Schwartz monitors world wide reactions

'We all live in Pennsylvania..'

IN THE UK, the Secretary of State for Energy, Tony Benn, said that before the accident he had faced continuous pressure to "go American" and replace Britain's gas cooled reactors with American PWRs similar to the Harrisburg design. Pressure came from advisers, civil servants, and the nuclear industry itself, but he rejected the plan. Benn said the Harrisburg accident proved that "energy decisions must be kept under democratic control of Parliament". Prime Minister James Callaghan told Parliament: "We have been very wise in concentrating on a safer type of reactor." One of the three firms competing for the order of Britain's first PWR is Babcock and Wilcox; the others are the West German company Kraft Werke Union and a consortium which includes Rolls Royce and Northern Engineering.

France and West Germany, the two most committed nuclear nations in Europe, sent scientific teams to Harrisburg to inspect the accident. Two West German experts reported that the reactor was closer to a core meltdown than was publicly admitted.

The French Government announced its intention to proceed as usual with nuclear development, but the Opposition and the trade unions have called for a re-examination. Francois Mitterand, leader of the Socialist Party, accused the government of secrecy and high handedness in pushing through its power programme. The Socialist minority in parliament called for a halt to France's plan to build nine additional PWRs similar to the Harrisburg reactor in the next five years.

Prime Minister Raymond Barre told the European Press Club: "We must multiply our security measures but France cannot renounce nuclear energy". Minister of Industry Andre Giraud said: "An accident of this type has been taken into account in the design of French nuclear plants."

Le Monde reported numerous criticisms of the Government's stance. The Committee of Interregional Ecological Movements (CIME) demanded an immediate halt to the French nuclear programme because of the danger to urban centres. A Harrisburg-type accident, CIME said, would contaminate 300,000 people around the Fessenheim reactor (Mulhouse), 100,000 around Flamanville (Cherbourg) and 500,000 around Pellerin (Nantes).

The most dramatic action in France

was at the Mediterranean naval base near Toulon with the bombing of several million dollars worth of reactor equipment intended for export to Iraq, West Germany and Belgium. Responsibility was claimed by a group of militant ecologists in a call to *Le Monde*. The caller said "We have succeeded in neutralising machines dangerous to human life." However, French officials are reported as believing that the bombing may have been an Israeli action directed primarily against the Iraqi reactor programme.

In West Germany, 35,000 demonstrators at the Gorleben hearing into nuclear reprocessing plans in Lower Saxony chanted, "we all live in Pennsylvania." Inside the hearings, a French scientist, Yves Lenoir of the mining academy of Fontainebleau, walked out of the meeting of 60 international experts saying that the hearings were a sham and had no influence over the decision. Lenoir said that any discussion of safety that did not take into

account the Harrisburg events was academic.

But the Ministry of the Interior in Bonn said that German safety standards were high compared with international standards but they would be "toughened up" drastically. "Utilities and the nuclear industry will have no chance to ease safety precautions," a spokesman said.

In Sweden the Opposition party demanded that the Ringhals Two plant near Goteberg—a PWR similar to Harrisburg—should be closed for inspection; and Premier Ola Ullsten announced a national referendum on the country's nuclear programme. Danish politicians urged greater debate before their country took a decision to build nuclear reactors, and in Belgium the mayor of the town of Huy, 40 miles south-east of Brussels, ordered the closing of the 870 MW reactor, Tihange 1, saying that the Harrisburg accident had shown the town's emergency preparations to be inadequate.

In Japan, Premier Masayoshi Ohira said there would be no change in Japan's programme which currently has 19 reactors producing 11% of the country's energy. But 100 demonstrators staged a sit-in at the Ministry of International Trade and Industry calling for a thorough inspection of all Japan's nuclear power plants. The ministry itself was holding an emergency meeting with officials from 15 areas to discuss nuclear safety measures.

In Eastern Europe, comment on Harrisburg was limited. Soviet television devoted 15 minutes to the event on 2 April saying, "the accident has provoked a profound anxiety and continues to alarm the American people. A particular indignation has been aroused by the fact that the energy monopolies, in searching for profits, do not take the necessary measures in order to assure the safe functioning of nuclear power stations." □

Guidelines should go, DNA meeting concludes

Eleanor Lawrence reports on a meeting where biologists scoured themselves for going public on conjectural risks

LAST week in the village of Wye, deep in the Kent countryside, an audience predominantly composed of molecular biologists overwhelmingly reiterated the now widely-held view that recombinant DNA research poses no special risks.

The meeting was convened by the Royal Society and COGENE, the Committee on Genetic Experimentation of the International Council of Scientific Unions, to discuss the status of recombinant DNA work and the guidelines controlling it.

Essentially most scientists working in the field now believe that the original fears were based on bad scientific judgment, and that recombinant DNA experiments at the very worst can pose no more hazard than that of working with the most dangerous organism involved in the experiment. Therefore, they argue, regulations for recombinant DNA research are unwarranted and should be abolished. The inconsistency inevitable in guidelines designed to guard against conjectural hazards and the bureaucracy involved in their implementation pose a threat to the freedom of scientific enquiry.

The scientific basis for the change of heart appears to rest first on advice from experts in infectious diseases that it is virtually impossible to convert the laboratory strain of the common gut organism *E. coli* into an epidemic pathogen by the random insertion of a block of foreign genes.

The fear that the insertion of animal virus genes into *E. coli* would result

in a new route for the virus to bypass normal host defences is now also held to be groundless. The current conventional wisdom is that work with cloned viral DNA poses, if anything, even less risk than work with the virus itself, and that cloned viral DNA fragments offer the safest way to study the molecular biology of the most lethal viruses such as Lassa or smallpox.

Joe Sambrook of Cold Spring Harbor Laboratory observed that had Professor Bedson been working in Birmingham with cloned smallpox sequences rather than the complete virus, both he and Janet Parker would be alive today. (The World Health Organisation is indeed considering the possibility that cloned fragments of smallpox DNA might be the safest way of conserving the smallpox genome for posterity.) Although little direct evidence addressing this question was available in 1977—when the Ascot Workshop's recommendation of such views to the NIH was influential in gaining considerable relaxation of guidelines for work with animal viruses—experimental support in the case of certain DNA viruses, at least, has recently been obtained from the NIH 'worst case' polyoma virus experiment (see box).

Participants in the debate also marshal evolutionary arguments, such as the growing appreciation that genetic exchange occurs across wide species barriers in microorganisms, as general ammunition. But it is not clear that

these arguments necessarily address the particular point that still worries those who see the need for guidelines. They ask to be assured that the specific product of any given recombinant DNA experiment is not going to be hazardous to those who may be exposed to it, either in the laboratory, or in the general environment.

Among those who call for the end of regulation a more subjective attitude is that expressed most forcibly by J. D. Watson of Cold Spring Harbor. Watson now attributes the call for the moratorium as mixture of fears over research with tumour viruses themselves and an attack of mild liberal guilt, and considers that he and this fellow signatories displayed a complete lack of scientific judgment. "We were jackasses," he told the conference. "It was a decision I regret; one that I am intellectually ashamed of." Watson adopted this position soon after the original 'Berg letter' was written.

Another signatory, Stanley Cohen of Stanford University, also felt the group's original action was irresponsible on scientific grounds, as well as politically naive. It was based simply on a "lack of certainty there was no risk" and was therefore an "irresponsible scientific argument."

The third signatory present, Norton Zinder, holds a somewhat different view. Although he now thinks their original fears to be groundless, in the circumstances as they saw them at the time there was no other action that could be taken.

Given that they now largely have the support of their scientific colleagues on

guideline committees, the problem now facing those who wish to get rid of public guidelines was neatly summed up by Mark Richmond, a member of the UK Genetic Manipulation Advisory Group. Publication of the Berg letter, he pointed out, was in effect a political act even though there had been no political intention. But through this political act the subject has lost its innocence, and it is now going to be hard for the scientists involved to regain a position in which their views can be regarded as objective.

"How are you going to reassure the public that you're not arguing from self-interest", said Richmond, "but that you are now arguing objectively—particularly when, looked at from the outside, you seem to make enormous quantum jumps between what you require for conditions at one time and what you required for the same experiments a couple of years ago".

R. Pritchard, Professor of Biochemistry at Leicester and a well-known opponent of the UK GMAG reiterated his opinion that the effect of the Berg letter was largely due to the eminent names appended to it, and that its message could only be countermanded by the authors themselves. They should state publicly and unequivocally what they now believe, he said, to the loudest round of applause of the meeting.

Not all the participants, however, judged the display of public recantation before the Inquisition to be warranted. In the perception of a historian of science, Charles Weiner of MIT, the recombinant DNA debate has not been the wasteful and illborn episode it seems to most of those concerned.

Weiner has followed the tortuous course of the debate from the beginning and has collected in his *Recombinant*

'Worst case' experiments show low risk

THE original fears which led to the call for a moratorium were largely prompted by the prospect that cloning animal viruses, and especially tumour viruses, would provide them with a new or more effective way of overcoming host defences. The first results, reported recently, from the NIH 'polyoma experiment' designed to test this proposition were reassuring. Tests of the infectivity of complete copies of polyoma DNA cloned in plasmids or phage lambda showed it to be non-infectious, or much less infectious (by a factor of 10^6) than polyoma virus itself.

One of the NIH experimenters, Dr M. Martin of the National Institute of Allergy and Infectious Diseases, told the Wye conference of results they now have on the tumorigenicity of cloned polyoma DNA. *E. coli* carrying plasmids containing one copy of polyoma DNA produced no tumours when introduced into newborn hamsters; polyoma virus introduced in comparable conditions produces tumours in all animals infected.

The circular recombinant plasmid DNA itself produced a few tumours when given in quantities equivalent to 10^6 times those at which polyoma virus is invariably tumorigenic. As expected from the previous infectivity experiments, bacteriophage lambda-containing dimeric polyoma DNA also caused tumours in a few cases.

The NIH experiment was delayed first by the regulations against the cloning of animal viruses and then by a private legal suit brought against NIH which sought to prevent the experiment. A group of European scientists sponsored by EMBO to undertake similar polyoma infectivity experiments completed initial *in vitro* infectivity tests (which came to essentially the same conclusions as the NIH study) last year under the then less restrictive UK regulations, but have been unable to carry out animal infectivity or tumorigenicity tests because of the lack of any approved facility for such animal experiments in Europe.

In contrast to the US team they were able to obtain dimeric polyoma DNA cloned in plasmids (the NIH scientists could only obtain dimeric polyoma inserts in lambda) which will now be tested for animal infectivity and tumorigenicity at Fort Detrick.

DNA Archive at MIT much of the formal documentation, supplemented by the informal accounts of those involved. Although molecular biologists found the experience of coming face to face with the apprehensions of society a traumatic one, they have, in Weiner's opinion, come through with their science and honour relatively unscathed.

Despite the trauma and despite the

bureaucracy said Weiner, recombinant DNA research flourishes. Has the whole debate and its attendant publicity, he wondered, stimulated rather than repressed the research? But he was concerned at the attitude he now sees amongst younger scientists, to whom the message of the recombinant DNA debate now seems to be "shut up or be shut down".

US regulators countenance abolishment of the guidelines

INDICATIONS that the administrators most closely concerned are sympathetic to the efforts of the US scientific community to disentangle itself from the coils of recombinant DNA guidelines were provided by the statement at the conference from the Director of NIH, Donald Fredrickson.

Fredrickson's decision to go public on the proposed guidelines early in 1976 exposed the scientists to the full force of a public debate. But Fredrickson maintains that this was the only way to release the rising tension and "to prepare to defend whatever actions would be taken, against certain criticism".

The first NIH guidelines were released in June 1976, and greatly relaxed guidelines came into force early this year. In his Wye address, Fredrickson described the latest guide-

lines as a "new set of rules painfully formulated during this unprecedented curtailment of experimentation in biology".

In his view one of the important achievements of the revision was to provide for continuous and orderly evolution of the rules—"even to their eventual elimination when the need passes". Further revisions are due to come into force within the next few weeks, notably the approval of strains of *Bacillus subtilis* and *Saccharomyces cerevisiae* host-vector systems for various levels of containment, a provision hitherto lacking in the NIH guidelines.

H. DeWitt Stetten, former Chairman of the Director's Recombinant DNA Advisory Committee, used his talk to describe his personal route to disenchantment. After battling with the

myriad inconsistencies embodied in the first guidelines he eventually came to the conclusion that they had "got off the track badly" and were doing things that were very wrong—given that the mission of NIH was to support and encourage research.

He decided that the hazards had been overstated as no one could give him any evidence that there was any hazard. He concluded that "we had erected a bastion against a phantom", and that the barriers built to exclude ghosts were composed of scientists' time, effort and money. He has proposed that the guidelines be reduced to one simple rule: "the appropriate conditions of containment for recombinant DNA experiments should be those of the most virulent micro-organism entering into the experiment".

Lower Saxony stalls for time in wake of Harrisburg:

The six-day international hearing on the planned nuclear reprocessing facility at Gorleben in West Germany was very much influenced by the events of Harrisburg. Most of the proponents or "neutral participants" were obviously interested in gaining time. The organisers of the hearings—the state government of Lower Saxony—and the increasingly involved public (the largest anti-nuclear demonstration in Germany with over 100,000 participants took place during the week) were presented with two entrenched and irreconcilable camps. The pro-nuclear lobby insisted that the gigantic project could be realised, and proposed 1994 as a new start-up date for the reprocessing plant. The anti-nuclear lobby maintained that the project was more complicated, dangerous and expensive than simply storing nuclear waste underground. The Lower Saxony government is expected to take its time in assessing the meeting and may defer a decision until after elections for the European Parliament and the state government of neighbouring Schleswig-Holstein. Surprisingly the government granted permission for further trial drilling to a depth of 250m but only outside the area of the salt domes where final storage is planned. The main aim of the drilling is to find out about the surrounding hydrological structure.

From Klaus Höpfner in Hanover

US tries to stop proliferation in Pakistan: The State Department has announced that the US is cutting off development aid to Pakistan amounting to £20m this year and £23m next year in an effort to prevent Pakistan building a uranium enrichment plant. The action was required by a Congressional ruling to deny aid to countries rejecting nuclear inspection or safeguards. US officials became suspicious because Pakistan does not have reactors that can use enriched uranium. According to *The Guardian*, a spokesman from the Pakistani Foreign Ministry in Islamabad "angrily rejected" claims that Pakistan was planning to build an atomic bomb. "Somehow it gives rise to more concern if an Islamic country carries out research into some areas of nuclear technology than if other countries in the world do so" said the spokesman.

US intelligence sources claim that Pakistan has been surreptitiously buying equipment to make a gas centrifuge enrichment facility. The centrifuge was purchased in Holland and high frequency electric power converters were purchased from Emerson Electric in the UK. The UK government stopped the export after it was told about its potential military use.

ESA and NASA agree on joint solar-polar mission: The European Space Agency and the US National Aeronautics and Space Administration have signed a memorandum of understanding for a joint International Solar Polar Mission (ISPM) to be launched in 1983. ESA and NASA will each provide a spacecraft, and ESA will supply software and support personnel to manage the ESA flight operations and data processing at the mission control and computing facility which will be provided by NASA. The two-spacecraft mission is designed to observe the Sun from above and below its polar regions. The spacecraft will be launched simultaneously by the Space Shuttle and will travel in the ecliptic plane out to Jupiter where they will swing around under the influence of Jupiter's gravity to head back towards the Sun on orbits out of the ecliptic plane, one heading towards the Sun's north pole, the other towards the Sun's south pole. The spacecraft will circle the Sun on this polar orbit and then head back to Jupiter. The period from

launch to the second polar passage will be about five years. The mission will also investigate the interplanetary medium during the initial Earth to Jupiter run and the Jovian magnetosphere during the Jupiter fly by.

Comprehensive schools maintain standards in science: Pupils at inner London's non-selective (comprehensive) schools obtained almost the same number of O level and CSE grade 1 passes in biology, chemistry and physics as those from selected intake schools in 1977, and the comprehensive schools achieved these results despite having inferior science facilities and spending less time on the subject. These are the conclusions of a report issued by the Inner London Education Authority science inspectorate. The report expressed concern about the imbalance in facilities, and emphasised that the excellent examination results achieved by dedicated comprehensive school staff should "in no way be taken as an excuse for doing nothing to improve the resources in these schools".

The poorest science facilities were in non-selective mixed and girls schools. According to the report, the main reason for the differences was probably the greater range of subjects taught in comprehensive schools. They had proportionately less resources to spend on science subjects than selective schools since they had to teach subjects such as home economics, design and technology. Selective schools tend to offer only traditional subjects. However improvements are in progress.

Dr John Price, ILEA senior science inspector, said the authority's schools subcommittee had recommended setting up a central task force of 30 science teachers by 1980-81 who will help out in any school where there is a temporary shortage of science staff. The schools subcommittee has also pressed for the urgent review of the staffing and pay points system which causes comprehensive schools to have fewer experienced teachers. Finally, all schools will be given minimum laboratory facilities which will be improved in later years when more money is available.

India prepares for monsoon measurements: India is making major preparations for the forthcoming Monsoon Experiment (MONEX-79) which will enhance understanding of monsoon circulation around the three seas bordering India: the Arabian Sea, the equatorial Indian Ocean, and the Bay of Bengal. Four ships and one plane from India will be supplemented by five Soviet ships and three US planes with further information to be gathered by meteorological balloons and cyclone warning radar stations situated along the Indian coast. Marine weather reports from merchant ships and upper air reports from commercial aircraft will also be analysed.

Three of the Indian ships are expected to be equipped with Navaid sounding systems obtained through the World Meteorological Organisation and the fourth with an Indian-made Omega system to enable them, for the first time, to make systematic recordings of upper winds over the seas around India. Indigenous 3-D scanners will be attached to the 10cm radars at Calcutta and Madras to enhance observational capability, while wind and temperature observations of the stratosphere will be measured by rockets fired from Thumba, Sriharikota and Balasore.

MONEX-79 is a sub-programme of the First Global Garp Experiment (FGGE) of the GARP (Global Atmospheric Research Programme). It will coincide with the launching of FGGE on 1 May and will end in August when the monsoon abates over India. Rs 250 million will be spent on the experiment.

From Dilip M. Salwi in New Delhi

How safe is the fast reactor?

Fast breeder reactors are an essential part of a nuclear future — but in the light of the incident at Three Mile Island would they be safe? **Professor F. R. Farmer**, Safety Adviser to the UK Atomic Energy Authority, discusses how to assess their dangers

FAST breeder reactor safety is currently an emotive topic, but it should be placed in context: absolute safety can never be achieved, and most industrial activities carry some risk of causing harm to people and the environment. One aim of design and operation must be to keep the risk sufficiently low, and there will be argument as to what this implies.

The increased recognition of risk has rendered obsolete the concept of maximum credible accident, and currently leads more to a discussion of residual uncertainties in accident and consequence modelling, rather than a prolonged description of the devices installed to minimise the chance of accidents and a demonstration of their reliability. In following this trend, it should be understood that some uncertainties will always remain, and it will only be possible to establish an increased degree of confidence that the system and its behaviour are well understood. This is important when considering the early versions of new systems—reactors or aircraft; it is not possible to establish the same degree of confidence in the first as may be developed by the fifth or the tenth, and to accelerate this learning process there should be extensive exchange of safety information and collaborative programmes on safety issues on an international scale. To date collaboration has been good, and it is to be hoped that commercial consideration will not prevent this exchange.

Since the fast reactor core is much more compact than thermal reactors, the heat production per unit length or volume is greater. The cooling system has to cope with this, and fast reactors use liquid sodium, a very effective heat transfer medium operating at near atmospheric pressure.

The control of power in a fast reactor is easier than most thermal reactors. Both depend on the small fraction of delayed neutrons, but the more compact core of the fast reactor simplifies the procedures for controlling power because the effectiveness of any control rod extends virtually throughout the reactor and is only slightly affected by the position of other rods. Elaborate systems of sector control, as in large thermal reactors, are thus unnecessary. It is easy to provide redundancy in the control and shut off sys-

tems, since in a large reactor with 30 rods, the insertion of any five would shut it down. The aim in current designs is to achieve sufficient diversity.

The removal of heat from the fuel in a fast reactor is in some ways easier and in others more difficult than in water or gas cooled reactors, as indicated by the following examples.

As sodium reacts violently with water—and additionally, hydrogen as a neutron moderator must be kept away from the core—all designs have intermediate sodium-to-sodium heat exchangers, and the final sodium-to-water exchangers are placed some distance from the reactor. On the other hand, the normal operating temperature of the sodium is several hundred degrees below its boiling point so that there is a wide margin to accommodate temporary disturbances of power or flow. In reactors of the tank design, the whole primary circuit is immersed in several thousand tons of sodium; as a result the fission product decay heat can be absorbed for several hours, sodium flow being by natural convective circulation. It is yet to be shown that the transition from the flow conditions under power to convective flow of a shut down reactor can take place safely—this is still being investigated.

There are several misunderstandings about the dangers of fast reactors; for example it has been said that a fast

reactor may behave like an atom bomb. The fuel of current fast reactors contains plutonium plus a higher percentage of ^{235}U , which is a neutron absorber, however, and this fuel cannot explode.

Another fear, that fast neutrons make the fast reactor more difficult to control, is likewise without foundation. The micro-second lifetime of neutrons in a fast reactor, as compared with milli-seconds in a thermal reactor, has no bearing on normal operation in which control operates through the delayed neutron fraction. Under most accident conditions the response is not dominated by the neutron lifetime, but by the doppler coefficient of the U/Pu fuel which cancels most changes in reactivity (resulting from some accident conditions) by a corresponding rapid change in the fuel temperature. The reactor becomes less reactive as the fuel temperature increases.

It has frequently been said that the particular feature of a fast reactor is that the core is not in its most reactive configuration. This is true, but it is an oversimplification in a comparison with thermal reactors. The light water reactors in their normal operation are not in their most reactive state although for different reasons. The light water reactors can be rendered prompt critical by changes in their coolant; the pressurised water reactors by a rapid temperature drop of the water; the BWR by collapse of the steam voidage in the reactor core. These conditions are now deemed to be reduced to an improbable event by design measures. The same degree of confidence has not yet been established for the fast reactor, perhaps because designs are viewed more critically. There are two types of fault conditions peculiar to a fast reactor which increase reactivity, one being the rapid expulsion of sodium from the mid-core regions, and the other a collapse or compression of the core into a slightly smaller volume.

As stated earlier, it is not, and never will be, possible to show that accidents cannot happen—it is only possible to show that the accidents are unlikely and their consequences reasonably understood and tolerable in relation to their estimated frequency.

How might this be done? It is likely that in newly developed systems such as the fast reactor, two lines will be followed in parallel. First, effective measures must be provided to prevent the escalation of minor faults into major accidents; hence all major accidents will have a very low probability of developing. Second, even if the preventative measures fail, the ways in which accidents develop and are limited and contained, are sufficiently well understood through an adequate (even if not perfect) appreciation of the

What Harrisburg means for the Rasmussen report

"I have heard recent statements that the accident at Harrisburg further devalues the Rasmussen report. I do not see any evidence for these statements; the sequence of events may or may not be consistent with the report. An accident may reveal new features and it is important to find out whether any new, unperceived sequence or new phenomena have occurred. However, the estimate of accident frequency is less important than the detailed assessment of each reactor and support systems. The chief merit of Professor Rasmussen's report lies in the detailed studies and in the control that is then exercised to ensure that his assumptions are met." **Professor F. R. Farmer.**

phenomena involved.

How far can these lines be followed at present? The normal classification of accidents into errors of control, heat removal, and so on, appear to be well covered with redundant and diverse protective equipment. The adequacy of these provisions may be subject to some discussion, mainly to improve confidence in the lower levels of accident frequency. It seems that two troublesome areas remain—a degree of uncertainty in the structural integrity of the internal reactor structures, when requiring confidence at low frequency levels, and some uncertainty in the various possible modes of failure propagation within fuel sub-assemblies and their subsequent development. Currently, many methods of early identification of onset of failure are possible, but the application of all sensors could lead to over-instrumentation to such an extent that the reactor could never operate effectively.

These uncertainties are likely to be resolved, although not solved, with sufficient confidence to enable the next stage to proceed. The greater stumbling block, ideologically, lies in the second parallel line of argument—that if preventative measures fail, the accident can still be contained. The difficulty in this approach is the continued escalation of the assumptions defining the accident. There is seldom an upper limit, and there is no maximum credible accident as conveniently postulated for the development of the light water reactors. Rather than question too closely the initiating assumptions, it may be more instructive to study two important phenomena, the mode of fuel behaviour and core dispersion in

a 'whole core accident', and the conditions of violent or passive interaction between hot fuel and sodium, known as FCI (Fuel Coolant Interactions).

The 'whole core accident' has been under study in USA, UK, France, Germany, Japan and USSR for up to 20 years. The research and theoretical programmes have been, and continue to be, extremely large and complex. The object has been to find out how fuel behaves when heated rapidly in the range 2,000 to over 4,000°C in timescales from a few milliseconds to a second or so.

Various core dispersive mechanisms exist. For a start the core must disperse if the fuel is vaporised: this mechanism can be violent and is studied to find the dynamic and static loadings in the core region and its surroundings. For some accidents, molten fuel may be swept out of the core by the coolant, and the loss of a small fraction of fuel would terminate the reactivity excursion. A third dispersive mechanism exists through internal gas pressures generated in the fuel by fission products. Theoretically, this could be very effective and could prevent fuel vaporisation, or reduce its extent. This mechanism has only been studied in the last few years, but experiments are continuing.

Fuel-coolant interactions have also been studied for well over 10 years, using various materials, including tin, aluminium, uranium oxide, water, sodium, and freon. Some theoretical correlations have been suggested in recent years, but there is not yet a generally agreed model, and more work is required particularly with UO_2 and sodium.

The question now arises of when there will be sufficient understanding of 'whole core accidents' and 'FCI', and what constitutes 'sufficient understanding'. Returning to the proposition of a two-pronged approach to safety, the first step is to show that accidents can be identified at an early stage and safely terminated—this is likely to be developed with considerable confidence. The other approach is to study accident conditions assuming protective measures fail. This route is open-ended, for at no time will it be shown that any accident developing in any way with no safeguards can be tolerated by any one design. However, these studies should lead to some optimisation in core materials, core geometry and surrounding containment, so as to tolerate a wide range of possible accidents even if these are of low probability.

This present stage in safety analysis is described by Hafele (Fusion and Fast Breeder Reactors, IAEA, A-2361 Laxenburg, Austria RR-7-8 November 1976 (Revised July 1977)) as the third stage in the safety debate. It is instructive to follow his argument. The first phase (1944–1959) gave much attention to the short neutron lifetime, the relatively small fraction of delayed neutrons, the speed of control devices, and a positive power coefficient, basically because the reactors were small and used metallic fuel. The second phase, up to 1970, considered larger reactors with mixed oxide fuel. The debate was then around the sign and size of the doppler coefficient, then around superheat, the sodium void coefficient and FCI.

The overriding feature of the third phase is a widespread and multilevel approach of proving the elements of the design concept that arose from the second phase. In this connection, one has to realise that the nature of what is considered a proof has evolved very substantially in the years since 1970. Engineering judgement is no longer considered sufficient. The probability approach to reactor safety has been introduced and related to the concept of residual risks, extremely small in probability but very large in the perceived consequences. The focus of the scientific and technical attention of the third phase is therefore the depth and credibility of the experimental and theoretical proof of the design concept. In many countries there is now a growing interest in the attempt to quantify confidence and the degree of uncertainty. This is likely to continue. □

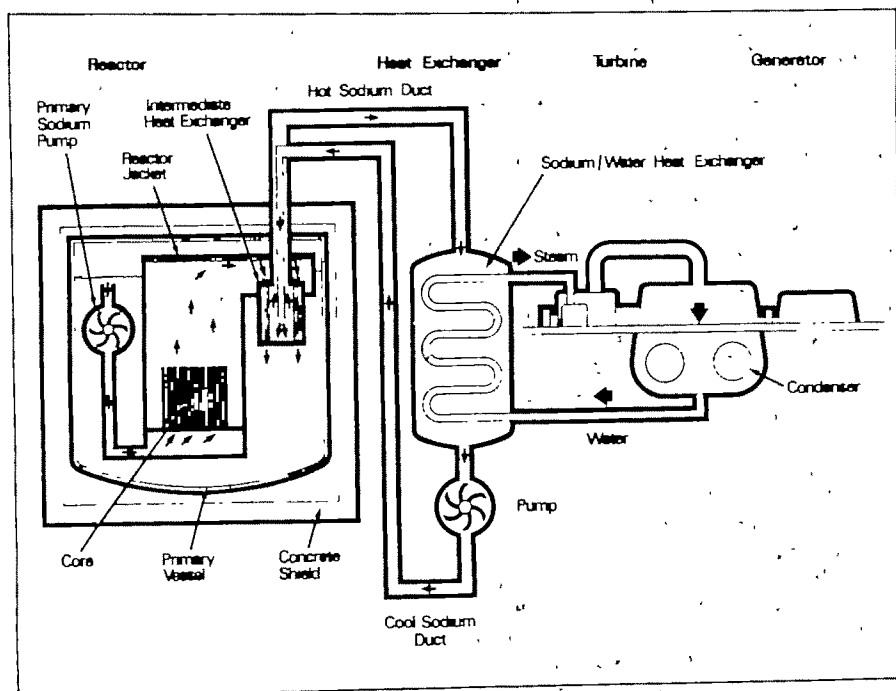


Diagram of the core and cooling system of the UKAEA's prototype fast reactor.

This article is based on a talk given by Professor Farmer at a conference on the politics of fast breeders at the end of last year. The conference proceedings are to be published by Macmillan.

How the West was scientifically explored

The US Geological Survey celebrates its centenary this year.

Harold L. Burstyn, the agency's official historian, surveys the past 100 years

At the close of the Civil War in 1865, only three and a half million people lived in the western half of the United States. A generation later, when the Superintendent of the Census of 1890 declared that he could no longer find on the map a continuous frontier line of settlement, more than eight and a half million Americans lived to the west of the first tier of states beyond the Mississippi River. In one of history's great migrations, Americans settled 430 m acres from 1870 to 1900.

With such a headlong rush westward of people seeking new opportunity on the frontier, exploration barely kept pace with settlement. From 1867 to the end of the 1870s, four separate groups, known collectively as the Territorial Surveys, mapped the west to uncover its mineral resources and assess the uses for its land. They were independent of the General Land Office, whose locally-hired surveyors divided the land into the rectangular plots acquired by settlers.

At a much higher level of precision, the Coast Survey, founded by Jefferson in 1807 as the federal government's first scientific agency, mapped both coasts by primary and secondary triangulation. The need to connect the Pacific to the Atlantic network led to a triangulation across the country and the change of the agency's name in 1878 to the Coast and Geodetic Survey. This change took place at a time of hope for political reformers anxious to restore the good name of the United States and the Republican party after the corruption of the Grant administration. President Rutherford B. Hayes and Secretary of the Interior Carl Schurz hoped to achieve their twin aims of reform and retrenchment by consolidating the Territorial Surveys into a single organisation and placing the parcelling of land in the hands of scientists.

On Joseph Henry's death in 1878 his place as leader of the National Academy of Sciences went to O. C. Marsh, the paleontologist. Under Marsh's direction, the academy prepared at the request of the forty-fifth Congress a plan for surveying and mapping the Territories. It recommended that the work be in a single agency, the Coast and Interior Survey, which would be the old Coast and Geodetic Survey re-



Survey director Charles Walcott (left) and John Wesley Powell, with Sir Archibald Geikie, their British counterpart, at Harper's Ferry, 1897.

named and transferred from the Treasury to the Interior Department. The geological work of the separate territorial surveys, three of them still in the field, would be combined into another agency, the Geological Survey. Finally, the academy report called for a federal commission, among whose members would be the heads of both surveys, to recommend changes in the laws concerning the public lands.

The final session of the forty-fifth Congress to which Marsh and his colleagues reported met after the election of 1878. At that time bills approved in the rush to adjourn each session often contained the only substantive legislation of the entire two years of the Congress, and appropriations bills (which had to pass if the federal government was to function) were the favourite vehicles for new departures. Thus the Sundry Appropriations Bill, the last to be passed by the forty-fifth Congress, attempted to reform the disposal of the public lands by placing their measurement and appraisal in the hands of scientists. But only two of the academy's proposals were in the Bill that President Hayes signed into law just before the Congress expired at midnight on 4 March 1879: the founding of the United States Geological Survey in the Department of the Interior for "the classification of the public lands, and examination of the Geological Structure, mineral resources, and products of the national domain"; and the formation of a commission to codify and propose revisions to the land laws. Both the Democratic-controlled House and the Republican-controlled Senate rejected the attempt to change the system by which the public lands were parcelled out to settlers. And when the commission on

the land laws made its recommendations two years later, they, too, were rejected by Congress.

To direct the new Geological Survey, Hayes and Schurz chose Clarence King, leader of the Geological Exploration of the Fortieth Parallel. From 1867 to 1873, King and his civilian colleagues had reconnoitered the natural resources along the route of the first transcontinental railway and had begun a series of monographs that set the highest scientific standards for the geology of the American west. King, "the best and brightest man of his generation . . . with talents immeasurably beyond any of his contemporaries", organised the new agency into districts, headquartered in Denver, Salt Lake City, and San Francisco. His topographers and geologists spread out to determine the character of the public lands, beginning with the major mining districts: Leadville in Colorado, Eureka and the Comstock Lode in Nevada, and the gold fields of California. King hoped that a decade of concentration on the principal ore bodies would lead to a rigorous theory of their formation. A sound geological understanding of the way metals were found in the earth might also show notorious wastefulness, while the work of the geologists could help investors in American mines sort out the favourable from the unfavourable prospects.

King wanted to extend the survey over the whole country, but the Interior Department was uncertain of the survey's mandate from the Congress to study those parts of the country that did not belong to the federal government, and it confined the survey to the public lands. Frustrated by petty politics and anxious to seek his fortune in mining, King resigned in 1881.



From the Survey's archives: earthquake damage in Charleston, 1886; geologist C. Willard Hayes on the limb of an exposed syncline in Maryland, 1897.

President Garfield replaced King with John Wesley Powell. Powell and his colleagues of the Geographical and Geological Survey of the Rocky Mountain Region had found the principles of a new approach to the earth, emphasising the role of erosion and deposition in forming the landscape. As an explorer he paid as much attention to the human as he did to the natural environment. He concluded that much of the interior lacked the rainfall to support traditional family farms. Dividing the public lands into uniform rectangles would invite monopoly by whoever controlled the banks of the streams. He called for a new system of land parcelling based on topography, for the setting aside of potential reservoir sites for public ownership, and for larger homestead grants in the dry areas. Powell's ideas lay behind the projected land reform of 1879 that the Congress had rejected.

Powell abolished the district offices, and based the survey's organisation on subjects such as glacial, volcanic, and other branches of geology, rather than territory. Determined to build a national agency, he used the Congressional authorisation, "To continue the preparation of a geologic map of the United States" to expand the survey to cover the whole country. Powell countered the "states' rights" argument against his federal agency by beginning in 1884 a cooperative programme of topographic mapping with Massachusetts, which, as one of the original states, had never contained any public lands. From Massachusetts, cooperation extended into other states and into geology and hydrology until it covered virtually the whole country and all its resources. After a broad investigation of economy in the federal government's scientific agencies, the Congress in 1886 renewed the survey's mandate for earth science.

Powell's chance to press for his programme of reform came with the droughts that began in 1886. By 1888,

after drought and blizzards had destroyed the open-range cattle industry and three consecutive dry seasons had brought despair to farmers in the central Great Plains, the Congress authorised Powell to conduct an irrigation survey. He immediately began an ambitious programme, including topographic mapping of a vast area, measuring the flow of its streams, and designating the sites for irrigation works and the lands they would irrigate. The Congress closed the remaining public lands to entry until Powell and his men were finished, but this provision generated such opposition to the irrigation survey that Congress abolished all of it except the designation of sites for reservoirs. At the same time the survey suffered the first of a series of budget cuts that finally drove Powell to resign in 1894.

Though the most drastic reductions owed more to both party politics and the Panic of 1893 than they did to opposition to Powell, his colleagues and successors in the Geological Survey never forgot the lesson of the 1890s. In the United States the primary purpose of federal civilian agencies for the physical sciences (until the National Science Foundation was created in 1950) has been to regulate. As a result, in times of constraint the regulatory function can usurp the resources devoted to research, however meagre they may be. From the failure of Powell's Irrigation Survey the Geological Survey's leaders learned that science could be saved only if administrative power was eschewed. For the next 30 years, they ensured that all possible regulatory roles were transferred to other agencies. As a consequence, its leaders believe, "the government's most productive research agency during the nineteenth century" has maintained its vitality in scientific research into the final quarter of the twentieth.

The trees of the United States passed rapidly into private hands until 1891

when the lame-duck fifty-first Congress empowered the President to create forest reserves by removing timbered lands from the market. Under Powell's successor, Charles D. Walcott, the Geological Survey mapped these national forests. Walcott also continued the Survey's inventory of the nation's water supplies.

As the vast fields of coal began to power the rapidly growing US industry, the Geological Survey began to test coals for their heating value and rocks for their use as structural materials. In 1908 the Congress asked the survey to look into mining safety, but since it was still averse to a regulatory role, these tasks were both transferred by the Congress to the Bureau of Standards and the newly-created Bureau of Mines.

Only in 1927, towards the end of the longest term of any of the survey's ten directors, George Otis Smith, did the survey abandon its opposition to becoming a regulatory agency. In 1925 Secretary of Commerce Herbert Hoover had wrested the Bureau of Mines out of the Interior Department into his own. The responsibility of the Bureau of Mines for supervising mining on public land was moved to the Survey's Land Classification Branch which became the Conservation Branch. With the growth of oil production on the outer continental shelf, the Geological Survey's Conservation Division is now its fastest-growing part.

The present Geological Survey continues to map the topography and the geology of the far-flung United States, to test its waters for quality and quantity, and to investigate the processes which formed the earth and the planets. The research ideal that has animated the US Geological Survey has borne fruit for a century. Yet the goal for which the Survey was founded—to make science the basis for the use of the land and its products—has not yet been reached. It remains the goal for the Survey's second century. □

correspondence

Dismantling DNA regulations

SIR,—I am delighted that John Maddox has taken up the debate on the problems related to regulations governing genetic manipulation (1 March, page 10). His analysis of the situation is perceptive when he states that the choice, if GMAG's procedures don't change soon, will be only whether to work with NIH guidelines here or elsewhere. In this regard John Maddox and I probably disagree only on time scale. He clearly feels that all may be resolved by the end of 1980 and asks if this is not enough, is it "a chance worth taking"?

If we are going to gamble we must know the odds and the stakes. At stake is the future of most genetic manipulation work in this country. The odds are heavily against anyone trying to continue in this highly competitive field if work has to be conducted under regulations so much more restrictive than elsewhere.

It is particularly bitter irony to find myself grouped with those scientists who invited constraints four years ago. That group was dominated by US scientists who are now working under relaxed NIH guidelines, while their colleagues here languish in the aftermath of their public outburst. From the outset, I have thought that those who invited constraints were doing both science and the public a huge disservice; I see no evidence to change my mind. A bureaucracy has been established and will be hard, if not impossible, to dismantle rationally.

In attacking the NIH guidelines, John Maddox subscribes to currently fashionable scare stories, those of "conjectured immunological hazards" and other problems "of expression". In fact when we talk of known proteins we can estimate possible dangers because we handle the proteins in their regular environment. We can therefore assess how to take precautions in handling the same products in genetic manipulation experiments. Such decisions, at least at the research laboratory level, are a matter of day-to-day care and not a matter for committee rulings and elaborate bureaucratic procedures. If there were risks which could be quantified and if there were a need for regulations which which could be formulated rationally, then we might start to build from scratch. Unfortunately, we are not in a position to build, but rather in the position of dismantling excessively restrictive, empirically conceived, regulations.

If we were to accept the idea of dismantling the regulations by rational means we could fall into an expensive trap. Any conjectured risk would need to be quantified and the limit to conjecture and to expense involved in collecting the necessary data cannot be foreseen. What I asked for previously was an acceptance of the spirit of the NIH guidelines. Let us recognise the need for care when there is a known hazard, but let us not get lost in a morass of conjectured risks.

Yours faithfully,

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Mediterranean scientists stymied by bureaucrats

SIR,—Rather than focus on who attacked UN research programmes on the Mediterranean at the recent Geneva Conference of the Mediterranean States (15 February, page 506), and who succeeded in cutting three scientific research projects of major importance, one should analyse who failed (and why) to support the scientific component of the Mediterranean Action Plan (MAP). Incidentally, this component has been fully in line with the proclaimed "target areas" of the Group of 77 and of the UN system, among which the "reinforcement of scientific and technological capacities of the developing countries" is foremost.

That research results, produced by the 83-odd laboratories of the region, are still inferior in quality compared to those from (for example) Liverpool, Woods Hole, or Villefranche-sur-Mer, would hardly surprise anybody. Obviously, in recent years something different from competing with the centres of excellence has been attempted. Recently it became clear that this implicit aim has met with considerable success. At the November 1978 joint ICSEM-UNEP Review Meeting on the Mediterranean Pollution Research Pilot Projects, held at Antalya, Turkey, a large number of interesting contributions were reported. These came from many laboratories never before known to produce anything of international significance. UNEP's contribution to the execution of these programmes was in the form of equipment, training fellowships, and travel and subsistence support for such meetings. It never amounted to more than some 10% of the total on-the-spot expenditure for the participating laboratories' projects. However, its importance should be judged by the fact that it mobilised sizeable national resources for research in marine sciences in many developing countries; but, unfortunately, not in all. The latter, instead of asking for additional help in creating national research centres, have joined the ranks of critics of the programmes, although for different reasons.

The UNEP-sponsored and -catalysed multilateralism in scientific training, technical cooperation and data exchange, has indicated, for better or worse, the eclipse of the old northern paternalism. Even the well produced 'show-biz' contribution to the Antalya meeting by one of the most developed countries of the region, has not, and probably would not reverse this trend. It has become apparent that all around the Mediterranean there is emerging a broadly based international community of marine scientists, which is procuring the necessary atmosphere for appraisal of scientific data, for discussion of results, and for mutual understanding irrespective of present day political or economic disagreements. The bureaucrats, a large majority in the ranks of the 60, or so official governmental representatives at the Geneva Conference, have seen the old, more-or-less comfortable, order vanish,

and see themselves increasingly made superfluous by the internationally oriented groups of scientists. The latter, as well known from experience, are difficult to control: they will soon be asking for more money, and will generally become a pain in the neck. Thus the hatchet was used, not surprisingly, on those who have produced the only tangible result in the four years of this unique exercise called MAP. Conversely, and in line with this thinking, a boost has been given to appealing, but vaguely defined, future plans. Among these, as Dr Walgate has correctly indicated, the development of alternative energy sources by the less developed countries has been accepted as one of the short term priorities.

The Geneva Conference was, in a way, a scaled down rehearsal for the UNCSTD. The message is clear and fully supports Moravcsik's admonition: do not let scientific and technological issues be decided by bureaucrats alone.

Yours faithfully,

VELIMIR PRAVDIC

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Cooperation in German health programme

SIR,—In his article about research in Germany (8 February, page 423) Robert Walgate refers to the federal government's programme on promoting research and development in the service of health 1978-1981 and to some worries of officials of the German research society (DFG). I am afraid there has been a misunderstanding, since there is no conflict between DFG and the federal ministries responsible for the health research programme.

When setting up the government programme, DFG and the federal government agreed that a close cooperation should take place. This is routine by now, DFG officials taking part in assessment meetings and quite often making valuable suggestions. In addition to this we have asked DFG to inform the government whenever they have the feeling that a project in health research is being funded by the federal government although the scientific quality does not seem to be sufficient. No example has been reported so far.

It is obvious that only research of good scientific quality should have an impact on government policy: poor research would do more harm than good. However, the federal ministries reserve the right not to fund a research project if it is likely that possible results would not have any bearing on the work of the ministries. In these cases, which happen quite often, the applicants are advised to submit their proposals to DFG or other institutions which do not have the same restrictions as we have, and whose financial means are not changed by the arrival of the new programme.

Yours faithfully,

HANS P. LORENZEN

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Technology, Bonn, West Germany.

Plant protection

SIR,—Your correspondent writing on 'European plant health' (4 January, page 16), draws attention to and criticises certain aspects of the policy of the European and Mediterranean Plant Protection Organisation (EPPO) in listing organisms of plant quarantine significance for the EPPO region and describing them in its data sheets.

It is the function of EPPO to advise member governments on the technical, administrative and legislative measures necessary to prevent the introduction and spread of pests and diseases of plants and plant products. Thus it does through the agency of its working party on phytosanitary regulations, made up of specialists from nine of the 35 EPPO countries. The working party is, with the full approval of Council, developing procedures in three distinct areas. The first of these is the formulation of lists of non-European (A1) and European (A2) pests, based on extensive research data and the common experience of the plant health services of 35 member countries. The purpose of these lists is to draw the attention of countries to the organisms considered of greatest quarantine importance to the EPPO region so that appropriate precautions are taken in the form of stringent regulations. The second stage is the preparation, for each listed organism, of 'specific quarantine requirements', which EPPO countries are recommended to use as a basis for their phytosanitary regulations. These requirements are addressed specifically to the exporting country and specify conditions which have to be met for the importation of particular plants and plant products, based on the most detailed knowledge available on ease of detection of quarantine organisms in consignments and in the field, on persistence in soil, and so on. It is the formulation and acceptance of these recommendations as a basis for phytosanitary regulations which is the backbone of EPPO's international strategy to ensure that correct and adequate precautions are taken to prevent as far as possible the introduction of new pests without bringing the international exchange of commodities to a standstill. The third element is the preparation of data sheets on the A1 and A2 organisms. These sheets are addressed to a wider readership, including on the one hand the plant inspectors of importing and exporting countries and on the other, the general scientific public, which should be better informed on these organisms, many of which are poorly documented at present. Further details on the activities of EPPO and its working parties will appear in the proceedings of a conference (Plant Health: the Scientific Basis for Administrative Control of Plant Parasites, ed D. L. Ebbels & J. E. King) to be published by Blackwells for the Federation of British Plant Pathologists.

At all stages in the formulation of the A1 and A2 lists and in the preparation of specific quarantine requirements and data sheets, extensive consultations have been made with research specialists in the EPPO region and throughout the world. The exact status of some potentially dangerous diseases of virus, or virus-like, origin is less certain than that of most of the organisms on the EPPO lists. These uncertainties, which will doubtless be resolved by further research, are clearly stated in the EPPO data sheets and will similarly be reflected in the appropriate

specific quarantine requirements. The problem of new strains of quarantine organisms is a very difficult one, which has been thoroughly considered by the working party and can only be resolved at present by applying requirements to all those entities which are indistinguishable in inspection practice from the dangerous ones, or, in extreme cases, by prohibiting the importation of certain plants. It is impossible to foresee the appearance elsewhere in the world of a new aggressive strain of a pathogen already present in a country. Phytosanitary regulations can only be formulated in practice in terms of what is known to exist, not what might exist or appear. This also applies to pesticide-resistant strains, which may cause considerable trouble when introduced into new areas.

We believe that EPPO's phytosanitary strategy, which has been formulated by and has the support of the plant health services of its member countries in consultation with their own specialists, is a sound basis from which to tackle the practical problem of excluding dangerous pests from the region at a time when increasing quantities of exotic products from many parts of the world are being shipped to Europe in fast-moving freight transport. The A1 and A2 lists are under constant review and will be extended as necessary to include other quarantine pests as information on their importance becomes available. If the formulation of the lists and the publication of the data sheets encourages research workers to try to resolve some of the uncertainties about these pests, so much the better.

Yours faithfully,

A. H. STRICKLAND
G. MATHYS
P. JOURNET
JAC FJELDDALEN

EPPO, Paris, France.

Safety in the news

SIR,—Perhaps some other readers noticed a remarkable coincidence of news items in the 8 March issue of *Nature*.

First there is the ban, after a ten-year struggle, of the 2,4,5-T herbicide in the US, because of the presence of dioxin. Then there is the recent release of the file on past nuclear reactor incidents, with the official comment "the absence of more serious effects is largely the result of luck", and the occurrence of accidents with a Rasmussen probability of 10^{-12} per year. Also, there is the new official concern in the US with the potential dangers of low levels of radiation previously treated as safe.

Finally, J. D. Watson calls for the deregulation of all DNA research, not merely that on a laboratory scale of the past few years, but also the industrial-scale research of the future.

Yours faithfully,

J. R. RAVETZ

University of Leeds, UK.

Detecting frozen lunar gases

SIR,—The possibility of sending a lunar satellite into a polar orbit round the moon is being discussed. This will allow the observation of the interiors of craters near the poles, the bottoms of which may never have been reached by the sun since their time of formation. I have suggested earlier (*Phys. Bull.* 24, 312 and 453; 1973)

that in such cold crater bottoms one might find a continuous record of the evolution from the moon's interior of gases such as water, carbon dioxide, and many other materials volatile at lunar day-time temperatures.

In the absence of a significant permanent atmosphere on the moon, individual molecules will travel over a large part of the lunar surface; any which are not swept away by the solar wind or by becoming ionised will eventually find their way to a cold crater and freeze out there. The deposited strata of condensed gases cannot be studied without the landing of appropriate instruments, but the orbiter may make it possible to identify likely places for this.

Over the whole lunar surface, one could expect the top metre of regolith to contain something like fifty million curies of uranium. A large part of the radon-222 derived from the decay of this should be able to escape and with a $3\frac{1}{2}$ day half-life has a good chance of reaching the polar region before it decays. An atom of radon would be held in a sunless area and such an area will therefore concentrate the radon itself and its daughters from a much larger area. An observable amount of alpha-activity might therefore be concentrated although good spatial resolution would be needed to observe it. Detection would be made easier by the fact that the emission would be effectively from an infinitely thin source since the cold condensed layer would be thick enough to stop all alpha-particles from the underlying long-lived alpha-emitters. It might, however, be worth looking carefully at the energy distribution of the alpha-particles of polonium-210 which, together with its parent lead-210, could be expected to have remained in situ for some decades, during which there might have been some deposition of a detectable thickness of water if there had been any significant emissions of this during the period.

Over most of the lunar surface one would of course expect a thick-source spectrum derived from alpha-emitters still within the regolith with only a small thin-source contribution from the radon in the "gas phase" above the surface. A limit to the relative size of this may be estimated. The mean thermal velocity of ^{222}Rn at 0°C is about 160 m s^{-1} . Leaving the moon's surface normally with this velocity and without collision it would fall back to the surface again in 200 seconds. An average excursion would take about two minutes during which time it would travel horizontally about 20 km. In an average lifetime of five days it could thus make some 3,500 returns to the surface, and a cold crater bottom could expect to collect all the radon from 3,500 times its own area.

This figure must certainly be too high since it assumes an effective albedo of one, or zero resorption by the rest of the surface. This is quite unrealistic. The time of dwell on or in the surface after each return is not known but is clearly not zero. On the other hand, if the satellite could be made to travel well below the top of the radon atmosphere (half height nearly 10 km) the background of thin-source particles would be usefully reduced. All in all, it would certainly seem worthwhile to look for possible anomalies of alpha-emission near the poles.

Yours faithfully,

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news and views

Acetylcholine receptor clusters

from Eric Frank

THE acetylcholine receptor, a membrane protein that mediates transmission of information between nerve and muscle, has been extensively studied both physiologically and biochemically. Yet despite the wealth of available information concerning its distribution, pharmacological properties, synthesis, degradation and subunit structure, one still knows very little of how receptors come to be so precisely located at neuromuscular junctions. Small clusters of ACh receptors found on non-innervated muscle cells resemble the high density patches at synapses, and a knowledge of how these receptor clusters are formed may provide some insights into the development of the postsynaptic muscle membrane.

In mature muscles, the sensitivity to ACh falls 50 to 100 times when the point of ACh application is moved only 2 μm away from a nerve terminal, and within 100 μm the sensitivity is down by more than a factor of 1,000 (Kuffler & Yoshikami *J. Physiol. Lond.* **244**, 703, 1975). Anatomical studies using α -bungarotoxin as a specific, high affinity ligand for cholinergic receptors in vertebrate muscle have shown a similar degree of localisation of the ACh receptor to neuromuscular synapses (Barnard, Wieckowski & Chiu *Nature*, **234**, 207, 1971; Hartzell & Fambrough *J. gen. Physiol.* **60**, 248, 1972; Albuquerque, *et al.* *Proc. natn. Acad. Sci. U.S.A.* **71**, 1376, 1974; Fertuck & Salpeter, *J. Cell Biol.* **69**, 144, 1976). The distribution of ACh receptors on muscle fibres without a well established innervation is markedly different, however. Denervated, embryonic and neonatal muscles all have high densities of receptors over their entire surface (Katz & Miledi *J. Physiol. Lond.* **162**, 393, 1962; Diamond & Miledi **170**, 389, 1964). Until recently, extrasynaptic receptors in denervated and embryonic muscle

were thought to be uniformly distributed in the muscle membrane. With the idea of membrane as a fluid mosaic (Singer & Nicholson *Science*, **175**, 720, 1972), a reasonable model was that a motor nerve somehow kept the receptors organised in a cluster at synapses, but that in the absence of this neural influence, the receptors were randomly distributed throughout the membrane.

It came as some surprise, therefore, when ACh receptors on muscle cells cultured in the absence of nerve cells were found to be clustered (Vogel, Sytkowski & Nirenberg *Proc. natn. Acad. Sci. U.S.A.* **69**, 3180, 1972; Fischbach & Cohen *Devl Biol.* **31**, 147, 1973). The clusters were present even if special precautions were taken to ensure that the muscle cells had not been contacted by nerves *in ovo* before they were isolated and put into culture (Beckoff & Betz *Science*, **193**, 915, 1976). Since the discovery of receptor clusters on myotubes *in vitro*, they have been observed in extrasynaptic regions of denervated mammalian muscle *in vivo* as well (Ko *et al. Science*, **196**, 540, 1977).

Characteristics

Clusters of ACh receptors, often called hot spots because of their high sensitivity to ACh, are typically round or ovoid patches about 5–10 μm across. In culture, each myotube usually has at least one cluster, more commonly two or three, and two clusters can occur in close proximity. Patches occur both at the edges of cells and in the thicker, more central regions where they are frequently associated with a muscle nucleus (Fischbach & Cohen *Devl Biol.* **31**, 147, 1973; Anderson *et al. J. Physiol. Lond.* **268**, 731, 1977). They are found on both cell surfaces, either juxtaposed to the substrate or on the free surface. Most clusters have discrete boundaries, the receptor density falls to background levels within a couple of micrometres. Estimates of receptor density within a cluster made using radiolabelled α -bungarotoxin are

two-to-threefold lower than those at mature synapses (Sytkowski *et al. Proc. natn. Acad. Sci. U.S.A.* **70**, 270, 1974). Freeze-fractures of identified hot spots also show a particle density two or threefold less than at neuromuscular synapses (Yee *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 3007, 1978). In myocytes of *Xenopus*, receptors in hot spots are grouped into many tiny spots or fine lines (Anderson *et al. op. cit.*). Given this uneven distribution, the peak density within a hot spot may be as high as in mature postsynaptic membrane. One ultrastructural study failed to demonstrate any unique membrane or cytoplasmic specialisation at receptor clusters, but the immunoperoxidase method used may have obscured some fine details (Vogel *J. Cell Biol.* **69**, 501, 1976).

Hot spots increase in number throughout the first week in culture (Sytkowski *et al. op. cit.*). Once formed, they are stable structures. Only rarely does a cluster move or disappear on a morphologically stable muscle cell (Anderson *et al. op. cit.*; Frank & Fischbach *Cell and Tissue Interactions*, (eds Lash & Burger) 1977), although their total number does decrease in older cultures (Prives *Cell*, **7**, 543, 1976). After the initial stage of cluster formation, the appearance of a nerve fibre has not been observed on morphologically stable muscle cells. This positional stability is correlated with a lateral stability of individual receptor molecules in a cluster. By labelling receptors with fluorescent α -bungarotoxin, Podleski and his collaborators showed that receptors within a cluster are not free to migrate within the plane of the membrane, whereas receptors not associated with a cluster can diffuse quite readily (Axelrod *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 4594, 1976). Clustered receptors are not metabolically stable, however. Twenty-four hours after receptors are irreversibly blocked with toxin, hot spots reappear at their previous locations (Frank *et al. Neurobiology*, (eds Otsuka & Hall)

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1979). Thus individual receptors within a cluster are being constantly replaced even though the cluster as a whole is stable.

Receptor clusters share several characteristics with the subneural patches at nerve-muscle synapses in culture. Both types of cluster are positionally stable over time, although they can disappear if the muscle cell is innervated at another location. Individual receptor molecules are present at a similar density and are metabolised relatively rapidly, with a half-time in the membrane much shorter than at adult neuromuscular junctions but comparable to that of non-clustered receptors (Schuetze *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 520; 1978). The only consistent difference that has been noted is that in cultures of nerve and muscle from *Xenopus* larvae, neural patches tend to be long and narrow, similar in shape to the overlying neurite, while aneural patches are circular or oval.

Significance of receptor clusters

Almost as soon as receptor clusters were discovered, it was hypothesised that they might serve as recognition sites for cholinergic nerves innervating muscle (Fischbach & Cohen *Devl Biol.* **31**, 147; 1973; Vogel *et al. Proc. natn. Acad. Sci. U.S.A.* **70**, 270; 1973). Under a variety of conditions, neuromuscular synapse formation is correlated with a high density of ACh receptors, both during embryonic development and during the reinnervation of denervated muscle. Clusters of ACh receptors on cultured muscle cells therefore might act as preformed postsynaptic sites. A cholinergic nerve, encountering a receptor cluster would form a synapse there.

This idea, however, turned out to be wrong. Work from two different laboratories using different animals and different methods are in full agreement that most, if not all, nerve-muscle synapses do not form at pre-existing receptor clusters. M. Cohen and his collaborators used fluorescently labelled α -bungarotoxin to follow the distribution of receptors during synapse formation on myocytes cultured from *Xenopus* larvae (Anderson *et al. op. cit.*; Anderson & Cohen *J. Physiol. Lond.* **268**, 757; 1977). The fluorescent patches under nerve processes, presumably at synapses, were very different in shape from those on myocytes uncontacted by nerve cells. In a few cases, the distribution of receptors was followed on a single myocyte while it was contacted by a neurite. Receptor molecules already labelled with fluorescent toxin coalesced under the neurite, and adjacent pre-existing fluorescent patches often disappeared. Neural clusters were thus formed by re-

cruitment of surrounding unclustered receptors, and perhaps by receptors from dispersed pre-existing clusters as well. Similar results were obtained in G. D. Fischbach's laboratory where the ACh sensitivity profile of chick myotubes in culture was determined physiologically during innervation by explants of embryonic chick spinal cord (Frank & Fischbach *Neurobiology*, (eds Otsuka & Hall) 1979). New areas of high sensitivity appeared at newly formed synapses. In no case was a synapse observed to form at a pre-existing sensitive spot. These pre-existing clusters often disappeared when they were adjacent to synapses, just as observed in the cultures of amphibian muscle. Because of the method used to assay ACh receptor distribution, it could not be determined whether receptors were recruited from adjacent areas or represented the incorporation of new receptors into the postsynaptic membrane.

It should be stressed that these findings do not bear on the importance of ACh receptors during synapse formation. There are appreciable numbers of receptors everywhere on these cultured muscles, at a density comparable to that in extrasynaptic regions of denervated, supersensitive muscle. Nor should one conclude that there are no preferred sites of innervation. If such sites do exist, however, they are not characterised by clusters of ACh receptors.

Another possibility is that receptor clusters on non-innervated muscles do not serve any useful function but are produced by the same mechanisms that operate during synapse formation to form high receptor densities in the postsynaptic membrane. During synapse formation, a nerve could simply trigger off an innate mechanism in the muscle resulting in the creation of a subneural cluster. Under normal conditions *in vivo*, motor nerves are present at very early stages of myogenesis, so that receptor clusters might normally appear only at synapses. But in culture, where muscle can be grown in the absence of nerve cells, cluster formation might be inappropriately triggered by other factors. Consistent with this idea is that synaptic and aneural receptor clusters in culture appear to be very similar to each other.

How nerves induce receptor clusters is unknown. An old theory was that ACh itself acted as a trophic agent, keeping the receptors clustered at the synapse. More recently, it has been suggested that the mere physical presence of the nerve could produce a high receptor density, since a thread or dead nerve placed on the surface of a muscle can produce a local area of high sensitivity. However, recent experiments by M. Cohen show that

clusters do not form under sensory nerves (personal communication). The most promising current idea is that motor nerves release a chemical substance other than ACh that induces receptor accumulation and/or cluster formation. Three laboratories have reported that extracts of nervous tissue added to muscle cultures increase the number of ACh receptors (Podleski *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 2035; 1978; Christian *et al.* **75**, 4011; 1978; Jessel *et al. Neuro Abs.* **4**, 369, 1978) and at least one of these extracts increases the number of receptor clusters as well. The isolation of the relevant substance in these extracts may provide an important tool in the study of the molecular mechanisms of receptor clustering. □

Cell lineage in early development

from Jonathan Slack

THE days of descriptive biology are often thought to be over, but in the field of experimental embryology there is a type of pure observation which retains its importance and which continues to pose considerable technical difficulties. This is the construction of the 'fate map' for an animal embryo, which shows what will happen to each region of the embryo during normal development. An accurate fate map is essential for the correct interpretation of experiments which seek to establish the causal relations of development by interfering with the normal processes.

Only a limited amount of information can be obtained by the direct observation of embryos because of the complexity of cell numbers and morphogenetic movements, although it has been possible to trace the cell lineage of the nematode *Caenorhabditis elegans* up to 182 cells by Nomarski optics (Deppe *et al. Proc. Natn. Acad. Sci. U.S.A.* **75**, 376; 1978). So the technical problem is that of specifically labelling a certain region of the embryo in such a way that the progeny of the marked cells can subsequently be found and identified, and to do this without damaging the marked cells or otherwise perturbing normal development. The methods used in the past have included staining with vital dyes, application of extracellular carbon particles and insertion of homologous

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grafts whose cell nuclei are labelled with ^3H -thymidine or with a chromosomal marker. Much has been done with these methods, particularly on the amphibian and chick embryos, but each has its own limitations and disadvantages and in particular it is not often possible to mark single cells in the early embryo.

A technique has recently been introduced which seems to offer considerable scope for the construction of accurate fate maps for several types of embryo and which is particularly suitable for the marking of single cells. It is described in a paper by D. A. Weisblat, R. T. Sawyer and G. S. Stent (*Science* **202**, 1295; 1978). The marking is carried out by the injection of horseradish peroxidase (HRP) into one of the large cells arising from an early cleavage division (blastomeres). The embryo is then allowed to develop for a certain time during which the enzyme persists passively in the cytoplasm of the daughters of the injected blastomere. When the embryo has reached the desired stage it is fixed and the regions containing the peroxidase can be visualised by standard histochemical procedures, either in whole embryos, if they are small enough, or on frozen sections. In this paper the fates of various early blastomeres of the leech *Helobdella triserialis* are traced. The enzyme persists in the appropriate clones for several days in an active form and it does not seem to interfere with normal development. HRP has a molecular weight of 40,000 and would not therefore be expected to pass from cell to cell through gap junctions. By contrast it is shown that a low molecular weight fluorescent dye, lucifer yellow, does spread to the whole embryo after injection into a single blastomere.

The technique has been applied to the early amphibian embryo by M. Jacobson and G. Hirose (*Science* **202**, 637; 1978; *Zoon* **6**, 149; 1978). When one of the first two blastomeres of *Xenopus laevis* is injected the whole of the appropriate side of the embryo can subsequently be shown to be labelled. In only one organ is there any contribution of cells from the contralateral side and this is the ventral part of the retina which is found to consist on both sides of a mixture of labelled and unlabelled cells. By means of injections of single blastomeres at 4, 8 and 16-cell stages it is shown that only the dorsal blastomeres contribute to the neural plate and that within the neural plate the clones make up longitudinally arranged strips.

These authors also make use of mosaic embryos which are produced by subjecting newly fertilised eggs to a heat shock. This causes suppression of

the second polar body and in a small proportion of cases leads to a situation in which the first two blastomeres contain different numbers of chromosome sets. This difference of ploidy is visible in the progeny clones as a difference of cell size and nuclear staining intensity. The results obtained from this method with respect to the shapes of clones and the provenance of the retina are substantially the same as those obtained from the HRP procedure, but the introduction of mosaics for the fate mapping of amphibia focuses attention on a rather important aspect of the interpretation of these maps. This is highlighted by another technique known as gynandromorph mapping which has been used to produce fate maps for the blastoderm stage of *Drosophila simulans* and other insects (reviewed by Janning in *Genetic Mosaics and Cell Differentiation*, Springer-Verlag, 1978). This also depends on the production of a mosaic after the first nuclear division, but in contrast to *Xenopus* in which the first cleavage is usually in the median plane for all embryos, in the insect the first

nuclear division is randomly oriented. So the early stages have no definable fate map because different individual embryos are different, and the mapping is carried out with respect to the later, blastoderm, stage by statistical inference from the frequencies with which adult structures appear within the same clone.

This dramatic difference between two types of animal embryo: one with reproducibly oriented early divisions and the other without, should help clear up a misconception often found in the literature. It is often assumed that an account of a cell lineage which does not vary between individual embryos gives some information about the decisions made during development and the states of determination of the cells. But it does not. As fate mapping is a purely descriptive procedure, additional investigations must always be made to establish whether clonal boundaries separate regions of different intrinsic cellular characteristics or whether they are simply passive consequences of the orientation of early cleavages. □

Specificity of DNA uptake in bacterial transformation

from J. R. Saunders

THE genetic transformation of bacteria by purified DNA has been of great significance in the development of molecular biology and is an essential technique in recombinant DNA research. However, little is known of the overall importance of transformation as a means of genetic exchange between wild populations of bacteria. Some bacterial species such as *Streptococcus pneumoniae*, *Bacillus subtilis*, *Haemophilus influenzae* and *Neisseria gonorrhoeae* become naturally competent (able to bind and take up DNA into a DNase-resistant form) in particular growth conditions. This suggests that gene transfer by transformation may be a relatively frequent event in the biology of these organisms.

B. subtilis or *S. pneumoniae* can take up DNA from unrelated (hetero-specific) organisms as well as homo-specific DNA. On the other hand *H. influenzae* can discriminate against foreign DNA and will only take up *Haemophilus* DNA efficiently (Scocca *et al.* *J. Bact.* **118**, 369; 1974). Two elegant papers from Hamilton Smith's laboratory at Johns Hopkins have re-

cently provided some exciting insights into the mechanisms involved in the specificity of DNA transport during *Haemophilus* transformation. Sisco and Smith (*Proc. natn. Acad. Sci. U.S.A.* **76**, 972; 1979) have examined transformation specificity in *H. influenzae* using as a probe an 8.1 kilobase fragment of *H. parainfluenzae* DNA cloned in *Escherichia coli* on the plasmid pBR322. This fragment was adsorbed very well by competent *H. influenzae* cells whereas pBR322 DNA was not. Recognition of *Haemophilus* DNA could not possibly involve conventional restriction/modification systems because the probe DNA had been propagated in *E. coli* and would have lost the specific *Haemophilus* modification pattern. It seemed likely therefore that *Haemophilus* DNA contains a special base sequence which determines uptake. Sisco and Smith then located these putative sites of specificity on two smaller fragments (120 and 140 base pairs long) lying 3.8 kb apart on the restriction endonuclease cleavage map of the 8.1 kb fragment. Competition for binding to *H. influenzae* cells between labelled 8.1 kb fragment DNA and chromosomal DNA from *H. influenzae* or *H. parainfluenzae* indicated that the *Haemophilus* genome contains

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about 600 copies of this uptake sequence (approximately 1 site per 4.0 kb). This specificity site does occur in certain heterologous DNA species but only at a low frequency (about 1 site per 300 kb). This limits successful uptake to fragments of foreign DNA carrying the recognition sequence. The actual recognition site is estimated to be only 8–12 bases long but the exact sequence awaits elucidation.

The ability to recognise the uptake sequence apparently resides in a membrane protein which is capable of specific binding of *Haemophilus* DNA and which is found only in competent cells (Deich & Smith in *Transformation 1978* (eds. Glover & Butler) 343, Cotsword Press, Oxford; 1979). This protein could merely be a specific surface receptor for DNA but it might also act as a pilot protein guiding homologous DNA into the cells. It is worth noting that a surface-located endonuclease is required for transformation of *S. pneumoniae* where one strand of double-stranded transforming DNA is degraded whilst the complementary strand is drawn into the competent cell (Lacks in *Microbial Interactions. Receptors and Recognition. Series B.* 3, 179; 1977). However, the situation must be different in *Haemophilus* because DNA remains double-stranded for several minutes after uptake and there is no evidence as yet that the receptor protein has nuclease activity.

A paper from the laboratory of Alexander Tomasz at the Rockefeller University, New York shows that *N. gonorrhoeae* also has a system for discriminating against foreign DNA during transformation (Dougherty *et al.* *Biochem. biophys. Res. Commun.* 86, 97; 1979). Gonococci will adsorb large amounts of both homologous (*Neisseria*) and heterologous DNA but only the former is taken up into a DNase-resistant (presumably intracellular) form. Competition experiments suggest that gonococci have two kinds of receptor on the cell surface. One receptor binds all types of DNA and the other is specific for DNA from closely related species. DNA is transported into the cell solely from the latter receptor. Whether or not the molecular basis for the specificity observed in *N. gonorrhoeae* is similar to that found in *Haemophilus* remains to be seen.

The specificity of DNA uptake exhibited by *H. influenzae* and *N. gonorrhoeae* raises some interesting possibilities as to the role of transformation in natural populations of these bacteria. Both organisms are prone to autolysis, releasing DNA, and both are highly competent, suggesting that transformation may often occur in the wild. Efficient transformation, however, poses its own problems since indiscriminate uptake of heterologous DNA on a large

scale could swamp restriction systems and compromise the genetic integrity of the host. Surface-located systems for distinguishing homologous DNA may therefore have evolved as additional genetic isolating mechanisms. Even so other bacteria which are also readily transformed *in vivo* such as pneumococci have maintained their species identity without the benefit of a discriminatory uptake system for DNA. The origin and evolution of specificity sites in *Haemophilus* together with possible similarities with restriction endonuclease recognition sites should become more apparent when their full base sequence is known. The specificity of *Haemophilus* transformation is, however, evidently yet another example of the ability of proteins to recognise defined sequences in DNA. □

Myofibrillar connection

from Pauline Bennett

It has long been suspected that in the striated muscle fibre there is a cytoskeleton which holds the constituent myofibrils in place. The presence of structural elements parallel to the fibre axis is indicated by the elastic resistance of the muscle to stretch which is not attributable to the contractile apparatus itself. Further, when the bulk of myosin and actin are extracted from myofibrils there remain 'ghosts' whose integrity is not lost even though in the light microscope only the Z-disks can be seen. The presence of transverse elements is suggested by the tendency for the Z-disks of neighbouring myofibrils to be in axial register across the fibre giving rise to the cross-striated appearance of the muscle cell. The components of this framework have not until recently been identified.

There have been suggestions, usually based on electron micrographs of muscle from which the contractile proteins have been extracted that a fibrous element is involved. For example, from such observations and an investigation of the components of extracted muscle, Maruyama and his colleagues (*J. Biochem.* 82, 317; 1977) consider that there is present a framework of longitudinal elastic filaments around each myofibril and that these filaments are composed of a protein, connectin. Connectin is however insoluble and has proved difficult to characterise.

In micrographs of intact muscle such

filaments are not in general observed, only membranous material is seen lying between the myofibrils. These membranes are obvious candidates for a supportive role. The sarcoplasmic reticulum sheaths each myofibril and could contribute to the longitudinal element whereas the transverse (T) tubule system is invaginated across the muscle fibre and could form the transverse elements. Furthermore the T-tubules occur at very precise positions with respect to the sarcomere structure, this position depending on the source of the muscle. In avian and amphibian skeletal muscle the T-tubules circle the myofibril at every Z-disk level and this relationship is maintained during contraction of the muscle. The question therefore is, what structural links are there, if any, between the membrane system and the contractile apparatus? One component of the transverse links seems now to have been identified.

Lazarides and his colleagues in a recent series of papers have been investigating the role and distribution of a protein they call desmin (from the Greek *desmos*, a link). This protein of chain weight 55,000 they first found in chicken smooth muscle. They have shown by indirect immunofluorescence that its antibody labels structures in all avian muscle cell types including the Z-lines of skeletal muscle fibres. Now Granger and Lazarides (*Cell* 15, 1253; 1978) have studied in more detail the three-dimensional distribution of antibody binding in striated muscle. To do this they have been aided by the valuable discovery of a method for obtaining sheets of Z-disks. Whole muscle, when homogenised, tends to be sheared lengthwise to form myofibrils. However, if most of the myosin and actin are first extracted from the muscle with KCl and KI the axial strength is reduced and blending shears the fibre across its width giving honeycomb-like sheets of Z-disks. SDS gel electrophoresis shows that these sheets are enriched in desmin compared with the original muscle although they still contain substantial amounts of actin, some α -actinin and traces of myosin and tropomyosin. Indirect fluorescent antibody labelling of ghost myofibrils shows that desmin, α -actinin and actin are all present at the Z-disk level. Similar labelling of Z disk sheets reveals that α -actinin is located as expected in the Z-disks whereas desmin labels the areas between the Z-disks forming an interconnecting network across the fibre. Antibody to actin seems to label the whole Z-disk sheet.

Previous observations of Lazarides and Granger (*Proc. natn. Acad. Sci. U.S.A.* 75, 3683; 1978) using a fluorescent dye to mark the hydrophobic,

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and hence presumably the membrane positions in glycerol-extracted fibres showed substantial fluorescence at the Z-line level and around the Z-disks in Z-disk sheets. The location of desmin thus seems to coincide with that of the membrane, probably the T-tubule system.

Since the resolution of the light microscope does not allow the precise position of desmin to be determined, Lazarides and his colleagues suggest a number of possible interpretations of the data. First the desmin may be involved in mediating the adhesion of the T-tubule to neighbouring Z-disks. Alternatively, desmin might be associated with direct fibrous links between Z-disks which are independent of the T-tubules. The first possibility offers a good explanation for the precise location of the T-tubules in the muscle cell and finds support in the general observation of the authors that desmin is present where membranes and actin appear to be associated such as in the intercalating disk in heart muscle. A test of this hypothesis would be to study the distribution of desmin in mammalian skeletal muscle. Here the T-tubules are located at the junction of the A and I-bands, not at the Z-disk level as in avian muscle, which should lead to a different antibody staining pattern. Unfortunately Lazarides and Balzer (*Cell* 14, 429; 1978) have shown that their antibody is specific only for avian muscle, but they have some evidence from two-dimensional gel electrophoresis that a similar protein is present in mammalian muscle.

The alternative proposal that desmin forms direct links between Z-disks is based in addition on the observations of filaments lying between Z-disks in ghost fibres. However, these are considerably longer than the distance between myofibrils in intact muscle. The evidence that these filaments are composed of desmin is indirect. Lazarides and his colleagues consider that desmin is the same as the protein, skeletin, isolated by Small and Sobieszek (*J. Cell Sci.* 23, 243; 1977) from chicken smooth muscle where it is the major component of the 100 Å filaments. There is a possibility that these two proteins are not identical. The extraction procedures used by the two groups are different, as is, slightly, the molecular weight attributed to the proteins. Furthermore it is unusual to find a protein which does not have a similar morphology in very different cell types and no 100 Å filaments have been detected in skeletal muscle, although a few could easily go unnoticed. If these objections are not valid can I broadcast a plea for the groups involved to get together to decide on a common nomenclature. In

view of the, at present, confused state of the field of 100 Å filaments, this is an important point to clarify.

Whatever the state of desmin in the muscle it is clear that in this protein we now have a handle with which to tackle the problem of how the transverse order is maintained in the striated muscle cell and can further hope to discover how it is related to the putative longitudinal cytoskeleton.

Enzyme therapy in genetic diseases

from Gregory Gregoriadis and Michael F. Dean

THERE are numerous genetic disorders in which, because of a partial or total absence of a specific enzyme, substrates normally metabolised by the enzyme accumulate intracellularly or extracellularly. This leads to profound defects in the function of the patient's tissues and in many instances to severe incapacitation or early death. Treatment for most of these disorders has been mainly symptomatic and, in a few cases, dietary. However, as knowledge of the molecular mechanism of these diseases progresses, more sophisticated attempts at therapy can be undertaken. Since therapy by genetic manipulations is only a distant possibility, efforts have concentrated on more immediate solutions, namely reduction of the substrate load by restoration of enzyme activity or other methods.

Enzyme therapy has recently gained a new impetus as a result of the advances in methodology discussed by D. Robinson (Queen Elizabeth College, London) which have provided us with bulk quantities of enzymes from human tissues, especially placenta (for example α -galactosidase, C. C. Sweeley, Michigan State University, East Lansing; glucocerebrosidase, G. L. Dale, City of Hope Medical Center, Duarte) and in the possible use of bacteria for the synthesis of biologically active proteins (L. Villa-Komaroff, University of Massachusetts Medical School, Worcester; R. D. Schmickel, University of Michigan, Ann Arbor). Another reason is the discovery that many enzymes possess recognition markers which facilitate their very rapid specific uptake by cells (E. Neufeld, National Institutes of Health, Bethesda). There is, for instance, a

phosphomannosyl recognition marker on several lysosomal hydrolases mediating their uptake by fibroblasts (W. Sly, Washington University School of Medicine, St. Louis), a fucosyl marker on glycoproteins recognised by hepatocytes (R. L. Hill, Duke University Medical Center, Durham) and an as yet uncharacterised group on β -hexosaminidase A which seems to promote binding of the enzyme by brain synaptic plasma membranes (J. W. Kusiak, National Institutes of Health). In the case of human β -hexosaminidase of which hepatic uptake in cats is due to a receptor recognising N-acetylglucosaminyl and mannosyl residues on the enzyme, unwanted participation of the liver can be suppressed (in favour of other tissues) by substances which compete for similar binding sites. This allows the enzyme to circulate longer and by increasing blood brain barrier permeability after exposing animals to hyperbaric oxygen, enzyme can thus reach nervous tissue (M. C. Rattazzi, State University of New York at Buffalo). Accessibility of enzymes to brain is also achieved by hypertonic shock using arabinose or mannitol which shrink the cells lining the vascular endothelium (J. A. Barranger, National Institutes of Health).

Difficulties in controlling the fate and effect of enzymes *in vivo* could be overcome by the use of carriers (naturally occurring or synthetic) which may, in various ways, improve enzyme distribution and action. Disappointingly, in type I Gaucher's disease in which glucocerebroside storage results in hepatomegaly (and splenomegaly), multiple injections of human glucocerebrosidase: β -glucosidase entrapped in γ -globulin-coated erythrocytes (E. Beutler, Scripps Clinic and Research Foundation, La Jolla) or in liposomes (G. Gregoriadis, Clinical Research Centre, Harrow) failed to reduce appreciably the size of the liver even though this tissue is the principal site for localisation of the carrier-associated enzyme. Nonetheless, after 3 years of treatment with liposomal enzyme there was some clinical improvement of the patient. Fortunately, neither of the carriers affected blood clotting factors or α -macroglobulin (liposomes). Some clinical improvement was also seen in Gaucher's disease patients injected with the free enzyme but a substantial reduction of hepatic glucocerebrosidase (also shown in liver biopsies exposed to the enzyme *in vitro*) was not paralleled by a reduction in the organ's size (R. O. Brady, National Institutes of Health). It may be that long-term therapy is required for the reversal of the process of organ enlargement and, at any rate, the amount of enzyme given should be sufficiently high to at least prevent further glycolipid storage.

The 2nd International Symposium on Enzyme Therapy of Genetic Diseases was held at Hilton Head Island, South Carolina on 4-7 March and was organised by R. J. Denick under the sponsorship of the National Foundation-March of Dimes and the Mount Sinai School of Medicine.

Enzyme therapy by transplanting hepatic or bone marrow cells in immunosuppressed animals with model diseases, was partly successful (D Sutherland, University of Minnesota, Minneapolis) although, with the exception of acute hepatic failure (where only temporary action is required), rejection remains a formidable obstacle for use in man. There has been, however, some success with bone marrow transplants in immune deficiency disease. Furthermore, transplants of HLA-identical fibroblasts increased levels of the relevant enzymes in patients with Hurler's, Hunter's and Sanfilippo type A diseases for up to several years and there was no evidence of graft rejection (M. F. Dean, Kennedy Institute of Rheumatology, London; D. Gibbs, Clinical Research Centre, Harrow). A more hopeful alternative would, of course, be transplantation of cells after they have been corrected for the malfunctioning or missing gene by one of the available methods.

A subtle and perhaps more efficient method of restoring enzyme activity in a large group of disorders is the administration of massive doses of specific vitamins (see review by C. R. Scriver, in *Treatment of Inborn Errors of Metabolism* (eds Seakins, Saunders & Toothill) 127, Churchill Livingstone, Edinburgh and London, 1973) which through a variety of mechanisms (for example the coenzyme may form part of the active site of the enzyme, affect apoenzyme synthesis and degradation or influence the specific activity of the enzyme by altering its conformation), result in beneficial biochemical and clinical changes (G. E. Gaull, Mount Sinai School of Medicine, New York). Much of the understanding of vitamin action has come from work with vitamin B and aspartate- β -decarboxylase (A. Meister, Cornell University Medical College, New York). A similar (but limited to mannosidosis) approach may be ingestion of Zn which can activate the partially missing α -mannosidase (G. A. Grabowski, University of Minnesota, Minneapolis). The almost certain effects of Zn on the absorption and metabolism of other trace metals could, however, prove unacceptable. In yet another approach advantage is taken of the equilibrium thought to exist between the various pools of substrate in the body. For instance, plasmapheresis coupled with a phytanic acid-poor diet lowered serum phytanic acid considerably and slightly improved muscular strength in a patient with Refsum's disease. Plasmapheresis alone reduced tri-

hexosylceramide levels to normal for a several day period in a patient with Fabry's disease (H. W. Moser, The John F. Kennedy Institute, Baltimore). The latter finding was also observed after repeated injections of plasma α -galactosidase A isozyme in two brothers with the same disease (R. J. Desnick, Mount Sinai School of Medicine).

Although some of these attempts at therapy have already benefited patients and some may have saved lives, most of the clinical trials were not preceded by animal experimentation, because animal models of human genetic diseases are extremely rare. The setting-up of such models is essential if therapy is to become more effective. An interesting example of a mouse with multiple lysosomal enzyme

deficiencies was demonstrated by P. Pentchev (National Institutes of Health). Even so, direct clinical trials are often a necessity. For instance, work with animal models is of little use in determining the likely immunogenicity of enzymes or cells prepared from human sources or whether cells from seemingly healthy donors may be contaminated with viruses, infectious viral components or with malignant cells. Some of these contaminants are impossible to measure or even anticipate and recipients, especially if immunosuppressed, may therefore be at risk. Similarly, artificial carriers such as liposomes, although controllable in terms of infectious contaminants, may exaggerate antigenicity through an adjuvant action



A hundred years ago

In the interests of British science we have refrained now for some time from referring to the evil days which have fallen upon one of the most reputable of our learned societies. The time, however, has now come when silence is impossible. At the meeting of the Royal Astronomical Council yesterday, the Astronomer-Royal, in consequence of the recent action of the Council—an action inevitable when the present constitution of that body is considered—resigned his seat at the board. We cannot too much regret that this Society, the traditions of which are second to none in Europe, should have been utilised for some years past by an advertising clique who have everything to gain by their connection with a body of honourable students of science. The withdrawal of men long known for their astronomical work from the Council commenced some time since. It has now culminated in the resignation of the Astronomer-Royal, and we are informed that other resignations are to follow, indeed, a man of scientific repute risks somewhat in being found amongst the Councillors. Surely the Fellows of the Royal Astronomical Society of London are strong enough to remedy such a state of things as this.

At a recent meeting of the Medical Society of Berlin Prof. Virchow gave (by previous request) his views on the subject of the Plague of Astrakhan.

At the beginning of most pestilential epidemics a Committee of doctors has generally declared that it was not the plague. They pronounced it petechial typhus. This was the case immediately before the outbreak of the plague at Rescht, when the disease had been long confined in Kurdistan and Mesopotamia. M. Tholozan was the first to say it was plague, and that the case was that, not of a great epidemic, but of a latent disease, spreading slowly and attacking only a few. It is indubitable that we have there a true centre,

whence the disease gradually spread, and I do not see why we should go to India, where the disease has not prevailed for many years past. Proceeding logically, we shall accept this course. From Kurdistan and Mesopotamia to Persia and on to beyond the Caspian. Even if the present cases on the other side of the Caspian were accompanied by pneumorrhagia, I would not hesitate to say they belonged to the plague proper, and that the disease is the same as that in Mesopotamia. The symptoms are very different from those of petechial typhus, the disease which the Turkish doctors affirmed. If near Salonichi (Xanthi) there be really petechial typhus accompanied by *Metastasis bubonica*, I fear it is the plague. It remains to keep our eyes open and see what happens after the return of the Russian army from the infected country (an occurrence which may well rouse grave apprehensions).

What has been done for our protection is little apt to tranquillise us. A blockade comprising all the frontier as well as the coast, from the Baltic to the Black Sea, seems to me illusory.

One example of severe quarantine has occurred in this century in the case of the plague at Noja, in Bari (Kingdom of Naples), in 1815. Trenches were dug, and three cordons of sentinels were formed (the third round the entire province), with orders to kill whoever tried to break the blockade and did not stop at the first summons; and some individuals were actually killed. But I cannot think an entire country is able to protect itself thus. Examination of passports would be excellent if those who deliver passports and certificates of health were angels. But the Russian functionaries are men, and think like men. The impossibility of always getting true certificates of origin has been seen in the case of the cattle plague. I consider, however, that pressure should be exerted on Russia to form a blockade of the infected districts. And especially it should be seen to, that the returning Russian army does not bring any pestilential germs, with it. As to restrictions on communications by land, the greatest of these are ineffectual for the end desired.

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review article

The anthropic principle and the structure of the physical world

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The basic features of galaxies, stars, planets and the everyday world are essentially determined by a few microphysical constants and by the effects of gravitation. Many interrelations between different scales that at first sight seem surprising are straightforward consequences of simple physical arguments. But several aspects of our Universe—some of which seem to be prerequisites for the evolution of any form of life—depend rather delicately on apparent ‘coincidences’ among the physical constants.

THE structure of the physical world is manifested on many different scales, ranging from the Universe on the largest scale, down through galaxies, stars and planets, to living creatures, cells and atoms. Only objects such as quarks and leptons may be devoid of further substructure. Each level of structure requires for its description and explanation a different branch of physical theory, so it is not always appreciated how intimately they are related. We will show here that most natural scales are determined (to an order of magnitude) by just a few physical constants. In particular, the mass scale and length scale (in units of the proton mass m_p and the Bohr radius a_0) of all structures down to the atom can be expressed in terms of the electromagnetic fine structure constant, $\alpha = e^2/\hbar c$, the gravitational fine structure constant, $\alpha_G = Gm_p^2/\hbar c$ and the electron-to-proton mass ratio, m_e/m_p . The quantity m_e/m_p is related to α due to a coincidence in nuclear physics. These dependences are indicated explicitly in Fig. 1.

There are several amusing relationships between the different scales. For example, the size of a planet is the geometric mean of the size of the Universe and the size of an atom; the mass of man is the geometric mean of the mass of a planet and the mass of a proton. Such relationships, as well as the basic dependences on α and α_G from which they derive, might be regarded as coincidences if one did not appreciate that they can be deduced from known physical theory.

However, one of the scales in Fig. 1, that associated with the Universe, cannot be explained directly from known physics: it is apparently a coincidence that the present age of the Universe is of the order of α_G^{-1} times the electron timescale $\hbar/m_e c^2$. This led Dirac¹ to conjecture that G decreases with time as t^{-1} , so that the two timescales are always comparable. A more metaphysical explanation by Dicke² is that conditions are propitious for the existence of observers only when $t \approx \alpha_G^{-1} \hbar/m_e c^2$, so that this ‘coincidence’ should be of no surprise. This line of argument, which is discussed later, appeals to the ‘anthropic’ principle³. Later we shall also mention other features of the material world that seem sensitive to apparent coincidences among physical constants.

To describe structures on scales smaller than the atom one needs extra constants: in particular, the weak and strong inter-

action coupling constants g_w and f . It might be assumed that these are independent of α and α_G but there are some ‘anthropic’ interconnections between these numbers. For example, the condition that neutrinos can blow off the envelope of a star in its supernova phase will be shown to be, roughly speaking, $\alpha_G \sim \alpha_w^4$ where $\alpha_w = g_w^2 c/\hbar^3$ is the weak fine structure constant. Supernovae are essential if the heavy elements which are (presumably) necessary for life are to spread from their production sites throughout space. The same relationship between α_G and α_w explains why the cosmological helium production is $\sim 25\%$ by mass. If α_w were slightly smaller or larger, the helium production would be either 100% (in which case there would never be any water, perhaps another prerequisite for life) or 0% (in which case stellar evolution would be rather different). Finally there are coincidences between f and α and the elementary particle mass ratios which may be necessary for chemistry. There may be enough independent anthropic constraints to pin down the order of magnitude of α and α_G , and also that of α_w and f . Such considerations do not provide a real physical explanation, but they may indicate why these fundamental ratios are found to have their measured values.

One other important parameter in the Universe is the entropy per baryon or, equivalently, the photon-to-baryon ratio $\mathcal{S}$, which is of the order of 10^8 . The value of $\mathcal{S}$ has no explanation within the conventional hot big bang theory but it is also associated with a coincidence: if $\mathcal{S} \sim 10^8$, the matter and radiation densities are comparable at the time they thermally decouple. Although the anthropic principle does not seem to require a specific value for $\mathcal{S}$, it does require an upper limit on $\mathcal{S}$ of order $\alpha_G^{-1/4}$; otherwise, galaxies and stars would be unable to form through gravitational condensation. In fact, there are several schemes which suggest how an entropy parameter of the order of $\alpha_G^{-1/4}$ could be generated naturally.

Scales of structure in nature

We now present some simple arguments for the mass and length scales shown in Fig. 1. These are mostly straightforward consequences of simple physics. Many have been given before, but it is useful to bring them together. All our discussions will be based on order of magnitude arguments, so equations will be of the ‘ $\sim$ ’ rather than ‘ $=$ ’ kind, factors like π being neglected.

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Protons: the size of a proton is taken to be the Compton wavelength associated with its rest mass:

$$r_p \sim \frac{\hbar}{m_p c} \sim 10^{-13} \text{ cm} \quad (1)$$

This is roughly the range of the strong interaction. Associated with r_p is the timescale $t_p = r_p/c \sim 10^{-23}$ s, the typical lifetime of a strong interaction resonance. By analogy with equation (1), the size or localisability of an electron is sometimes referred to as

$$r_e \sim \frac{\hbar}{m_e c} \sim 10^{-10} \text{ cm} \quad (2)$$

and we can define a corresponding electron timescale $t_e = r_e/c \sim 10^{-20}$ s.

Atoms: for a hydrogen atom the radius of the lowest energy electron orbit is

$$a_0 \sim \frac{\hbar^2}{m_e e^2} \sim 10^{-8} \text{ cm} \sim 1 \text{ Bohr} \quad (3)$$

and the associated energy is

$$E_0 \sim \frac{e^4 m_e}{\hbar^2} \sim 10 \text{ eV} \sim 1 \text{ Rydberg} \quad (4)$$

As one considers atoms of greater atomic number Z , the binding energy increases roughly as Z^2 and the radius of the inner shell electron orbit decreases roughly as Z^{-1} . However, the radius of the outer shell electron orbit remains of the order of a_0 , so all atoms have about the same size. If one introduces the fine structure constant

$$\alpha = \frac{e^2}{\hbar c} \sim \frac{1}{137} \quad (5)$$

one can write a_0 and E_0 as

$$a_0 \sim \alpha^{-2} \left(\frac{e^2}{m_e c^2} \right) \quad E_0 \sim \alpha^2 m_e c^2 \quad (6)$$

The quantity $(e^2/m_e c^2)$ is the 'classical' radius of the electron $\sim 10^{-13}$ cm. Atomic masses span a range from 1 to roughly α^{-1} times m_p . The nuclei of atoms with atomic number exceeding α^{-1} have so much electrostatic binding energy that they are unstable to electron-positron pair production. In practice, the instability to fissioning comes in at a somewhat smaller atomic number.

The Planck scales: the only quantities of dimensions mass and length which can be constructed from G , $\hbar$ and c are the Planck scales:

$$M_{\text{Pl}} \sim \left(\frac{G}{\hbar c} \right)^{-1/2} \sim 10^{-5} \text{ g} \quad R_{\text{Pl}} \sim \left(\frac{G}{\hbar c^3} \right)^{1/2} \sim 10^{-33} \text{ cm} \quad (7)$$

By introducing the gravitational fine structure constant,

$$\alpha_G = \frac{G m_p^2}{\hbar c} \sim 5 \times 10^{-39} \quad (8)$$

these scales can be expressed as

$$M_{\text{Pl}} \sim \alpha_G^{-1/2} m_p \quad R_{\text{Pl}} \sim \alpha_G^{1/2} r_p \quad (9)$$

So M_{Pl} is much larger than m_p but R_{Pl} is much smaller than r_p . The Planck length is the scale on which quantum gravitational fluctuations in the metric become of the order of unity, so the concept of space breaks down at such small scales. M_{Pl} can be interpreted as the mass of a black hole of radius R_{Pl} . Space may be thought of as being filled with virtual black holes of this size. Such 'instantons' have an important role in quantum gravity theory.

Black holes: the radius of a spherically symmetrical black hole of mass M is

$$R = \frac{2GM}{c^2} \sim \alpha_G \left(\frac{M}{m_p} \right) r_p \quad (10)$$

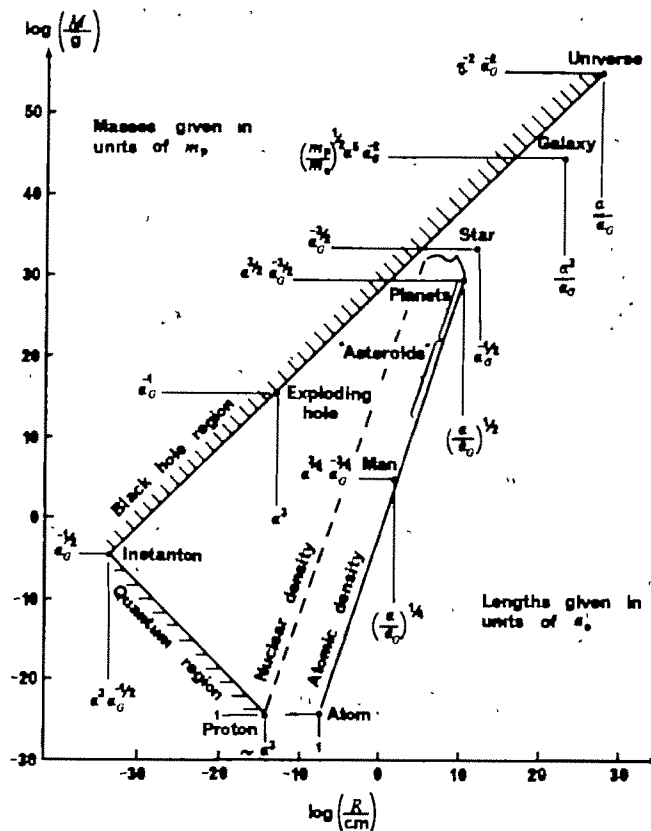


Fig. 1 The mass and length scales of various natural structures expressed in terms of the electromagnetic and gravitational fine structure constants, α and α_G . Some of these scales also depend on the electron-to-proton mass ratio, but this we have eliminated using $m_e/m_p \sim 10\alpha^2$. The asteroid scale also depends on the molecular weight A of rocky material. All these scales can be deduced directly from known physics except for the mass and length scale of the Universe, which depends on the age of the Universe being α_G^{-1} times the electron timescale $\hbar/m_e c^2$. Also shown are the atomic density line, the nuclear density line, the black hole density line and the 'quantum line' corresponding to the Compton wavelength. Most characteristic scales depend on simple powers of α_G ; the wide span of so many orders of magnitudes is a consequence of the huge numerical value of α_G^{-1} , which reflects the weakness of gravity on the microscopic scale.

This is the radius of the event horizon, the region from within which nothing can ever escape (at least, classically). Black holes larger than $1 M_\odot$ may form from the collapse of stars or dense star clusters. Smaller holes require much greater compression for their formation than could arise in the present epoch, but they might have been produced in the first instants after the big bang when the required compression could have occurred naturally. Such 'primordial' black holes could have any mass down to the Planck mass. In fact, Hawking⁴ has shown that small black holes are not black at all; because of quantum effects they emit particles like a black body of temperature given by

$$k\theta \sim \frac{\hbar c^3}{GM} \sim \alpha_G^{-1} \left(\frac{M}{m_p} \right)^{-1} m_p c^2 \quad (11)$$

This means that a hole of mass M will evaporate completely in a time

$$t_{\text{evap}} \sim \alpha_G^2 \left(\frac{M}{m_p} \right)^3 t_p (N(\theta))^{-1} \quad (12)$$

$N(\theta)$ is the number of species contributing to the thermal radiation; for $k\theta \ll m_e c^2$ these include only photons, neutrinos and gravitons; but at higher temperatures other species may contribute. The evaporation terminates in a violent explosion. For a solar mass hole, this quantum radiance is negligible: θ is

only 10^{-7} K and $t_{\text{evap}} \sim 10^{64}$ yr. But for small holes it is very important. A Planck mass hole has a temperature of 10^{32} K and only survives for a time $\sim R_{\text{Pl}}/c \sim 10^{-43}$ s. Those holes which are terminating their evaporation in the present epoch are particularly interesting. As the age of the Universe is $t_0 \sim \alpha_G^{-1} t_e$, the mass of such holes would be

$$M_h \sim \alpha_G^{-2/3} \left(\frac{t_0}{t_p} \right)^{1/3} m_p \sim \alpha_G^{-1} m_p \sim 10^{15} \text{ g} \quad (13)$$

and, from equation (10), their radius would be r_p . (The corresponding temperature is ~ 10 Mev: low enough to eliminate any uncertainty in $N(\theta)$ due to species of exotic heavy particles.)

Stars: Jordan⁵ first noted that the Sun has a mass of about $\alpha_G^{-3/2}$ times the proton mass; most stars have a mass in the range 0.1–10 times this. Jordan constructed an elaborate cosmological theory to explain this ‘coincidence’, but we will now see why stars are expected to lie in this mass range^{6,7}. The virial theorem implies that the gravitational binding energy of a star must be of the order of its internal energy. Its internal energy comprises the kinetic energy per particle (radiation pressure being assumed negligible for the moment) and the degeneracy energy per particle. The degeneracy energy will be associated primarily with the Fermi-momentum of the free electrons, $p \sim \hbar/d$, where d is their average separation. Provided the electrons are non-relativistic, the degeneracy energy is $p^2/2m_e$, so the virial theorem implies

$$kT + \frac{\hbar^2}{2m_e d^2} \sim \frac{GMm_p}{R} \sim \left(\frac{N}{N_0} \right)^{2/3} \frac{\hbar c}{d} \quad (14)$$

Here N is the number of protons in the star, $N_0 \equiv \alpha_G^{-3/2}$, and $R \sim N^{1/3} d$ is its radius. As a cloud collapses under gravity, equation (14) implies that, for large d , T increases as d^{-1} . For small d , however, T will reach a maximum

$$kT_{\text{max}} \sim \left(\frac{N}{N_0} \right)^{4/3} m_e c^2 \quad (15)$$

and then decrease, reaching zero when d is

$$d_{\text{min}} \sim \left(\frac{N}{N_0} \right)^{-2/3} r_e \quad (16)$$

A collapsing cloud becomes a star only if T_{max} is high enough for nuclear reactions to occur, that is $kT_{\text{max}} > qm_e c^2$ where q depends on the strong and electromagnetic interaction constants and is $\sim 10^{-2}$. From equation (15), one therefore needs $N > 0.1 N_0$. Once a star has ignited, further collapse will be postponed until it has burnt all its nuclear fuel. An upper limit to the mass of a star derives from the requirement that it should not be radiation-pressure dominated. Such a star would be unstable to pulsations which would probably result in its disruption. Using the virial theorem (that is, equation (14) with the degeneracy term assumed negligible) to relate a star's temperature T to its mass $M \sim Nm_p$ and radius R , the ratio of radiation pressure to matter pressure can be shown to be

$$\frac{P_{\text{rad}}}{P_{\text{mat}}} \sim \frac{aT^4 R^3}{NkT} \sim \left(\frac{N}{N_0} \right)^2 \quad (17)$$

so the upper limit to the mass of a star is also $\sim N_0 m_p$. A more careful calculation shows that there is an extra numerical factor of the order of 10, so one expects all main-sequence stars to lie in the range $0.1 \leq N/N_0 \leq 10$ observed.

In other words, only assemblages of 10^{56} – 10^{58} particles can turn into stable main sequence stars with H-burning cores. Less massive bodies held together by their own gravity can be supported by electron ‘exclusion principle’ forces at lower temperatures (they would not get hot enough to undergo nuclear fusion unless squeezed by an external pressure); heavier bodies are fragile and unstable owing to radiation pressure effects.

The M – R relationship for main-sequence stars is shown in Fig. 2, and can be deduced from the virial theorem and a knowledge of T , the central temperature. T adjusts itself so that

the nuclear energy generation rate balances the luminosity, the radiant energy content divided by the photon leakage time,

$$L \simeq acT^4 R^4 / \kappa M \quad (18)$$

where κ is the opacity. The appropriate κ decreases as M increases (electron scattering being the dominant opacity for upper main-sequence stars) but the energy generation increases so steeply with T that M/R depends only weakly on M .

White dwarfs and neutron stars: when a star has burnt all its nuclear fuel, it will continue to collapse according to equation (14) and, providing it is not too large, it will end up as a cold electron-degeneracy supported ‘white dwarf’ with the radius $R \propto M^{-1/3}$ indicated by equation (16). However, when M gets so large that $kT_{\text{max}} \sim m_e c^2$, the electrons will end up relativistic, with the degeneracy energy being pc rather than $p^2/2m_e$. Thus the degeneracy term in equation (14) acts as d^{-1} instead of d^{-2} , and consequently there is no $T=0$ equilibrium state. From equation (15) this happens if M exceeds the mass

$$M_c \sim \alpha_G^{-3/2} m_p \sim 1 M_\odot \quad (19)$$

which characterises stars in general. A more precise expression for this critical value of M (Chandrasekhar mass) is $5.6 \mu^2 M_\odot$ where μ is the number of free electrons per nucleon⁸. For a star which has burned all the way up to iron, $\mu \approx 1/2$ and the limiting Chandrasekhar mass, taking into account the onset of inverse β -decay when electrons get relativistic, is $\sim 1.25 M_\odot$. Note that the white dwarf line in Fig. 2 meets the main-sequence line on the nuclear ignition ($T \sim 10^7$ K) isotherm. Stars bigger than M_c will collapse beyond the white dwarf density but may manage to shed some of their mass in a supernova explosion. The remnant core will comprise mainly neutrons, the electrons having been squeezed onto the protons through $p + e^- \rightarrow n + \nu$, and this core,

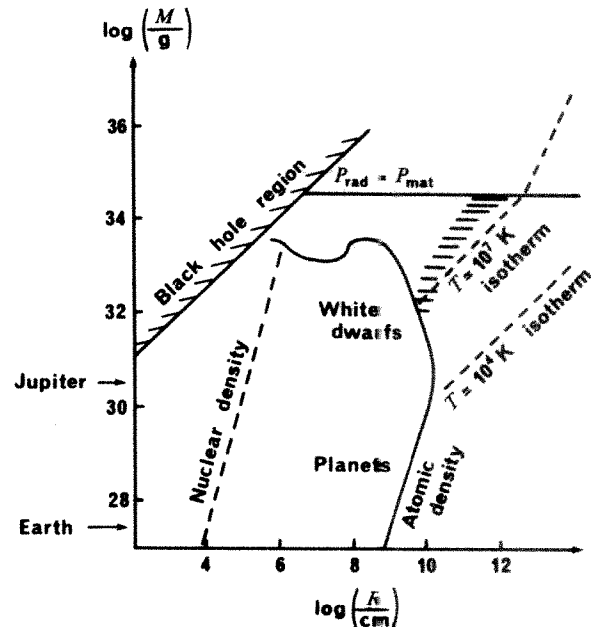


Fig. 2 An enlargement of part of Fig. 1. All solid planets lie on the atomic density line and all main sequence stars lie in the narrow mass range 0.1–10 times $\alpha_G^{-3/2} m_p$ (shaded). Regions with mass below this range would never get hot enough to ignite their nuclear fuel; regions with mass above this range would be radiation pressure dominated and consequently unstable. General relativistic instabilities are important for masses $\gg M_c$ (not shown). The precise form of the main-sequence band depends on the opacity and the nuclear reaction rate. All stars smaller than the Chandrasekhar mass ($\sim 1.4 M_\odot$) must eventually end up on the electron-degeneracy-supported white dwarf line. Stars bigger than this either shed some of their mass in a supernova explosion and end up as a neutron star or collapse to a black hole. Objects on the dotted line which bridges the white dwarf and nuclear density lines are unstable. Also shown are the ($T \sim 10^7$ K) nuclear ignition isotherm and the ($T \sim 10^4$ K) ionisation isotherm.

if small enough, may be supported by the neutron-degeneracy pressure. The limiting mass for a neutron star is more difficult to calculate than that for a white dwarf because of strong interaction effects and because, from equation (15), with $m_e \rightarrow m_p$, the particles which dominate the neutron star's mass are relativistic. The maximum mass is still of the order of $1 M_\odot$, however, and a neutron star bigger than this must collapse to a black hole. The maximum mass lies close to the intercept of the black hole line, given by equation (10), and the nuclear density line $\rho \sim m_p/r_p^3$. The intricacies of the line which bridges the white dwarf and neutron star regimes in Fig. 2 reflect the effects of gradual neutronisation and strong interactions. Stars on this bridge would be unstable and so are not of physical interest.

The above order-of-magnitude arguments show why the effects of radiation pressure, and relativistic degeneracy both become important for masses $\geq \alpha_G^{-3/2} m_p$. (Note also that general relativity is unimportant for white dwarfs because the binding energy per unit mass is only $\sim (m_e/m_p)$ of c^2 at the Chandrasekhar limit.)

Planets and asteroids: the above analysis applies only to objects where the thermal energy kT and/or the degeneracy energy per electron exceeds the ionisation energy $\sim \alpha^2 m_e c^2$. If T is too small, the object will be solid or liquid rather than gaseous, in which case its structure is determined by the balance of degeneracy and chemical bonds rather than degeneracy and gravity. Since the degeneracy and electrostatic binding energy per particle are $\hbar^2 n^{2/3} m_e^{-1}$ and $e^2 n^{1/3}$, the density of any solid or liquid is of the order of the atomic density

$$\rho_0 \sim e^6 m_e^3 m_p \hbar^{-6} \sim \frac{m_p}{a_0^3} \sim 1 \text{ g cm}^{-3} \quad (20)$$

From equation (16), the atomic density line meets the white dwarf line ($d_{\min} \sim a_0$) at a mass and radius

$$\begin{aligned} M &\sim \left(\frac{\alpha}{\alpha_G}\right)^{3/2} m_p \sim \alpha^{3/2} M_e \sim 10^{30} \text{ g} \\ R &\sim \left(\frac{\alpha}{\alpha_G}\right)^{1/2} a_0 \sim 10^{10} \text{ cm} \end{aligned} \quad (21)$$

This point in Fig. 2 also lies on the $kT \sim \alpha^2 m_e c^2$ isotherm. Equation (21) characterises the maximum size of a planet and is of the order of the size of Jupiter. The central pressure of a self-gravitating body more massive than this can be balanced by degeneracy pressure only if the electrons are crushed closer together than in an ordinary solid. A lower limit for the size of a (solid) planet can be specified if the planet is reasonably round—that it be bigger than its own mountains. The maximum size of a mountain can be found from the following argument⁷. A mountain of height h must not provide so much pressure on the planet's surface that it liquifies its base. The condition for this is easily shown to be

$$h < h_{\max} \sim \frac{E_{\text{liq}}}{A m_p g} \quad (22)$$

where A is the molecular weight of the planetary material, g is the surface gravity and E_{liq} is the liquefaction energy per molecule. The binding energy of a solid is about 0.1 Rydberg per molecule and E_{liq} is about a tenth of this (as only the directionality of the bonds is broken in liquefaction), so

$$E_{\text{liq}} \sim \beta \alpha^2 m_e c^2 \quad (\beta \sim 10^{-2}) \quad (23)$$

For the Earth, $g \sim 10^3 \text{ cm s}^{-2}$ and equation (22) implies a maximum height of the order of 10 km. In general one has

$$g \sim \frac{GM}{R^2} \sim G \left(\frac{M}{m_p}\right)^{1/3} A^{2/3} (y a_0)^{-2} m_p \quad (24)$$

where $y a_0$ is the molecular size of the planetary material. (A solid terrestrial-type planet is mostly SiO_2 and iron, for which $A \sim 60$ and $y \sim 4$.) Equations (22–24) thus imply

$$h_{\max} \sim (\beta y^2) \left(\frac{\alpha}{\alpha_G}\right) \left(\frac{M}{m_p}\right)^{-1/3} A^{-5/3} \quad (25)$$

This is less than the radius of the planet, $R \sim y a_0 (M/A m_p)^{1/3}$, provided M and R exceed

$$M \sim (y\beta)^{3/2} A^{-2} \left(\frac{\alpha}{\alpha_G}\right)^{3/2} m_p, \quad R \sim A^{-1} \left(\frac{\alpha}{\alpha_G}\right)^{1/2} a_0 (y^3 \beta)^{1/2} \quad (26)$$

which is thus the maximum size of an irregularly shaped 'asteroid'. The value of A is constrained by the requirement that solid planets must be made of material whose atomic number is sufficiently high that it is not vaporised by the high temperatures attained during the formation of the Solar System.

'Habitable' planets: the mass range of habitable planets can be narrowed down still further if an atmosphere and an appropriate surface temperature are required⁹. The optimum temperature for organisms is $T \sim \epsilon/k$, where $\epsilon \sim 10^{-3}$ Rydberg is the typical energy released in relevant reactions involving complex molecules: were T much larger, the molecules would be disrupted; were it much smaller, biological processes would require an exponentially long timescale. If an atmosphere composed of gas heavier than hydrogen were necessary for life, then the thermal velocity of hydrogen at this temperature should be slightly greater than the escape velocity from the planet, which implies

$$\frac{GMm_p}{R} \sim \epsilon \alpha^2 m_e c^2 \quad (27)$$

With the relationship $M/R^3 \sim A m_p / y^3 a_0^3 \sim m_p / a_0^3$, this implies that a life-supporting planet must have a mass

$$M \sim \epsilon^{3/2} \left(\frac{\alpha}{\alpha_G}\right)^{3/2} m_p \quad (28)$$

Only a small fraction of planets in the mass range permitted by equations (21) and (26) would satisfy this criterion.

Press⁹ has used this line of argument to set an upper limit to the size of living creatures on the basis that they must not 'break' when they fall: the energy released by a body of mass M_m in falling from height h_m on a planet whose surface gravity is given by equation (28) must be insufficient to break the molecular bonds on an area h_m^2 . This yields an upper limit

$$M_m h_m \left(\frac{GM}{R^2}\right) \leq \left(\frac{M_m}{m_p}\right)^{2/3} \epsilon \alpha^2 m_e c^2 \quad (29)$$

Substituting for M with equation (28) and assuming atomic density, this implies

$$h_m \sim \epsilon^{1/4} \left(\frac{\alpha}{\alpha_G}\right)^{1/4} a_0 \sim \left(\frac{\epsilon}{0.001}\right)^{1/4} \text{ cm} \quad (30)$$

with only a weak dependence on ϵ . If one assumes that the surface stresses are distributed along faults as wide as a polymer, the estimate for h_m is increased by a factor of 100. (In fact, equation (30) is more appropriate as the estimate for the size of a water droplet, which can be derived from a similar argument⁷.) Allowing for the factor of 100, the mass associated with h_m is

$$M_m \sim 10^3 \left(\frac{\alpha}{\alpha_G}\right)^{3/4} m_p \sim 10^5 \text{ g} \quad (31)$$

which is roughly the mass of a man. We plot this mass in Fig. 1, although its value is very uncertain (and is in any case irrelevant to swimming organisms); note its dependence on $\alpha_G^{-3/4}$.

The Universe: in the simplest Friedmann cosmological model, the age of the Universe t_0 is of the order of H_0^{-1} where H_0 is the Hubble parameter. (This relation fails only if the Universe is closed and near its maximum expansion.) Since $H_0 \sim 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$, this implies $t_0 \sim 10^{10} \text{ yr}$, a conclusion which is supported by several independent arguments. The associated horizon size (the distance travelled by light since the beginning of the Universe) satisfies the approximate relationship

$$ct_0 \sim \alpha_G^{-1} \left(\frac{\hbar}{m_e c}\right) \sim \left(\frac{\alpha}{\alpha_G}\right) a_0 \quad (32)$$

In other words the ratio of the size of the observable Universe to the size of an atom is comparable with the ratio of the electrical (or nuclear) and gravitational forces between elementary particles. There is no explanation for this well known coincidence within conventional physics, but Dirac¹ has conjectured that α_G is always given by

$$\alpha_G \sim \frac{\hbar}{m_e c^2 t} \sim \left(\frac{t}{t_0}\right)^{-1} \quad (33)$$

Assuming that $\hbar$, c , and m_e are constant in time, this requires that G decreases as t^{-1} , so Dirac invokes equation (32) as the basis for a new cosmology. Such a variation of G may be inconsistent with observation.

An alternative 'anthropic' explanation for equation (32) was first suggested by Dicke². As life presumably requires elements heavier than hydrogen and helium and such elements can only be produced and spread throughout the Universe by supernovae, Dicke argued that any 'cognisable' Universe would be one in which some stars have already completed their main-sequence evolution. The luminosity of a star whose opacity is dominated by electron scattering (as applies for large stars) is $\sim (P_{\text{rad}}/p)L_E$; (P_{rad}/p) is given in terms of M by equation (17) and L_E is the Eddington luminosity, $4\pi GMm_p c/\sigma_T$, where $\sigma_T = \alpha^2 r_e^2$ is the Thomson cross-section. A characteristic timescale¹⁰ is that over which an object of luminosity L_E would radiate away its entire rest mass:

$$t_* = \frac{c\sigma_T}{4\pi GMm_p} = \left(\frac{\alpha^2}{\alpha_G}\right) \left(\frac{m_p}{m_e}\right)^2 t_p \quad (34)$$

and this timescale involves the 'large number' α_G^{-1} explicitly. If $\eta \ll 10^{-2}$ is the fraction of a star's rest mass that can be released through nuclear burning, the main sequence lifetime is thus

$$t_{\text{MS}} \sim \eta \left(\frac{P_{\text{rad}}}{p}\right)^{-1} t_* \sim \left\{ \eta \alpha^2 \left(\frac{m_p}{m_e}\right)^2 \right\} \alpha_G^{-1} \left(\frac{M}{M_\odot}\right)^{-2} t_p \quad (35)$$

The quantity in braces is of the order of unity; as stars must have a mass of the order of $M_\odot$ living observers could exist only when

$$t_0 > t_{\text{MS}} \sim \alpha_G^{-1} t_p \quad (36)$$

However, t_0 cannot be much bigger than t_{MS} or else most of the Universe would have been processed into white dwarfs, neutron stars or black holes. Therefore observers are most likely to exist at an epoch $t_0 \sim t_{\text{MS}}$, and they will find equation (32) automatically fulfilled.

In fact, t_0 exceeds the value of t_{MS} in equation (36) by a factor $m_p/m_e \sim 10^3$. However, t_{MS} is sensitive to the value of M and equation (36) is really appropriate only for an upper main-sequence star. Dicke's argument would therefore be more convincing if one could show that the first stars would be of low mass. A possible reason might come from the general argument^{11,12} that the mass scale at which a collapsing cloud must stop fragmenting is

$$M_{\text{frag}} \sim x^{-1/2} \left(\frac{kT}{m_p c^2}\right)^{1/4} M_\odot \sim 10^{-2} x^{-1/2} M_\odot \quad (37)$$

where T is the cloud's temperature (expected to be $\leq 10^4$ K) and x is the ratio of its luminosity to that of a black body with this temperature. M_{frag} has only a weak dependence on T and is at least an order of magnitude smaller than $M_\odot$, thus increasing t_{MS} to a value more comparable to t_0 . Alternatively, one might argue that the first stars do have $t_{\text{MS}} \ll t_0$ but that some of the elements vital for life must be generated through the s-processes associated with later-forming less massive stars.

The total mass associated with the observable Universe (the mass within the horizon volume) is $\sim \rho_0 c^2 t_0^3$ where ρ_0 is the

present matter density, given by the Friedmann equation:

$$\rho_0 = \frac{3H_0^2}{8\pi G} + \frac{Kc^2}{16\pi G} \quad (38)$$

Here K is the scalar curvature of the Universe. Providing the K term is smaller than the others, we deduce that the mass of the Universe is

$$M_u \sim c^3 t_0^3 G^{-1} H_0^2 \sim \frac{c^3 t_0}{G} \sim \alpha_G^{-2} \left(\frac{m_p}{m_e}\right) m_p \quad (39)$$

The fact that the number of protons in the Universe is of the order of α_G^{-2} , another cosmological coincidence, is thus explained providing one can justify neglecting the K term in equation (38). It has been argued that K must always be zero by appealing to Mach's principle¹³ but, apart from this, there may be anthropic reasons for expecting that the K term is small. If K is negative, galaxies could not have condensed out from the general expansion unless $(-K)$ were less than $G\rho/c^2$ at their formation epoch. Otherwise, their gravitational binding energy would not have been large enough to halt their expansion. $(-K)$ may, in fact, exceed $G\rho/c^2$ now, but not by a large factor. If K is positive, it must be $< G\rho_0/c^2$, otherwise the whole Universe would have recollapsed before $t = t_{\text{MS}}$. The simple relationship between M_u and t_0 given by equation (39), with equation (36), explains why the Universe must be as big and diffuse as it is to last long enough to give rise to life. Relationships (32) and (39) also mean that the Universe has an optical depth of the order of unity to electron scattering.

Galaxies: it is not certain how galaxies form, so any estimate of their scale is very model dependent. The scale indicated in Fig. 1 derives from the following argument¹⁴⁻¹⁶. We assume that galaxies originate from overdense regions in the gaseous primordial material, and that they have a mass M and radius R , when they become bound. After binding, protogalaxies may virialise at a radius $\sim R/2$. Thereafter, they will deflate on a cooling timescale, with a virial temperature

$$T \sim \frac{GMm_p}{kR} \quad (40)$$

Providing kT exceeds one Rydberg the dominant cooling mechanism is bremsstrahlung and the associated cooling timescale is

$$t_{\text{cool}} \sim \frac{1}{n\alpha\sigma_T c} \left(\frac{kT}{m_e c^2}\right)^{1/2} \sim \frac{m_e^2 c^3}{ae^4 n} \left(\frac{kT}{m_e c^2}\right)^{1/2} \quad (41)$$

The free-fall timescale is

$$t_f \sim \left(\frac{GM}{R^3}\right)^{-1/2} \quad (42)$$

and this exceeds t_{cool} when R falls below a mass-independent value

$$R_* \sim \alpha^4 \alpha_G^{-1} \left(\frac{m_p}{m_e}\right)^{1/2} a_0 \quad (43)$$

which, from a more precise calculation, is 75 kpc. Until a massive cloud gets within this radius it will contract quasistatically and cannot fragment into stars.

This argument applies only if the mass is so high that $kT_{\text{virial}} \gg \alpha^2 m_e c^2$ at the 'magic radius' R_* ; that is,

$$M \gg M_* = \alpha^{-2} \alpha^5 \left(\frac{m_p}{m_e}\right)^{1/2} = 10^{12} M_\odot \quad (44)$$

Gas clouds of mass $< M_*$ cool more efficiently owing to recombination, and can never be pressure supported. Thus, M_* is a characteristic maximum galactic mass. Primordial clouds of mass $> M_*$ are inhibited from fragmentation and may remain as

hot pressure-supported clouds. This type of argument can be elaborated and made more realistic¹⁴⁻¹⁶, but one still obtains a mass $\sim M_*$ above which any fluctuations are likely to remain amorphous and gaseous, and which may thus relate to the mass-scale of galaxies. M_* and R_* may thus characterise the mass and radius of a galaxy. Of all the scale estimates in Fig. 1, this is the least certain—the properties of galaxies may be a consequence of irregularities imprinted in the Universe by processes at early epochs.

This completes the justification of Fig. 1 except that we may eliminate the dependences on m_*/m_p using the relationship

$$m_*/m_p \sim 10\alpha^2 \quad (45)$$

which results from a coincidence in the nuclear physics (see equation (58)). From Fig. 1 some amusing interconnections can be deduced. Regarding the length scales, for example; $\text{man} \sim (\text{planet} \times \text{atom})^{1/2}$; $\text{planet} \sim (\text{Universe} \times \text{atom})^{1/2}$. Regarding the mass-scales: $\text{Planck} \sim (\text{exploding hole} \times \text{proton})^{1/2}$; $\text{exploding black hole} \sim (\text{Universe} \times \text{proton})^{1/2}$; $\text{man} \sim (\text{planet} \times \text{proton})^{1/2}$. These relationships cannot of course be regarded as coincidences as they can be expected *a priori*.

Other cosmological coincidences

Another dimensionless constant which characterises our Universe is the photon-to-baryon ratio $\mathcal{S} \sim 10^8 \Omega^{-1} \text{ g l}$ (where Ω is the baryon density in units of the critical density). According to the hot big bang model the background radiation dominated the density of the Universe until a time

$$t_{\text{eq}} \sim \mathcal{S}^2 \alpha_G^{-1/2} t_p \sim 10^{11} \Omega^{-2} \text{ s} \quad (46)$$

and thermally decoupled from the matter when T fell below $\sim 0.1\alpha^2 m_e c^2/k$ at a later time

$$t_{\text{dec}} \sim \mathcal{S}^{1/2} \alpha_G^{-1/2} \alpha^{-3} \left(\frac{m_p}{m_e}\right)^{3/2} t_p \sim 10 \text{ g l}^3 \Omega^{-1/2} \text{ s} \quad (47)$$

(These equations derive from the fact that the radiation density and temperature fall like R^{-4} and R^{-1} respectively. Here R is the length scale of the Universe, which is $\propto t^{1/2}$ for $t < t_{\text{eq}}$ and $\propto t^{2/3}$ for $t > t_{\text{eq}}$.) The observation that t_{eq} and t_{dec} are within an order of magnitude of each other (for $\Omega \sim 0.1$) corresponds to the coincidence

$$\mathcal{S} \sim 10\alpha^{-2} \left(\frac{m_p}{m_e}\right) \sim \alpha^{-4} \quad (48)$$

Now, the formation of galaxies cannot occur until, first, T has fallen below the decoupling temperature, and second, the radiation density has fallen below the matter density. Thus, the anthropic principle requires that t_0 exceeds both t_{eq} and t_{dec} these conditions being satisfied if

$$\mathcal{S} < \alpha_G^{-1/4} \left(\frac{m_p}{m_e}\right)^{1/2} \sim \alpha_G^{-1/4} \alpha^{-1} \sim 10^{11} \quad (49)$$

A lower limit on $\mathcal{S}$ is obtained if one requires that the Jeans mass in the period t_{eq} to t_{dec}

$$M_J \sim G^{-3/2} \rho^{3/2} \rho^{-2} c^3 \sim G^{-1} t_{\text{eq}} c^3 \sim \alpha_G^{-3/2} \mathcal{S}^2 m_p \quad (50)$$

exceed the galactic mass indicated by equation (44). Such a condition would have to be satisfied if one identified M_J with the mass of a supercluster of galaxies¹² and it implies

$$\mathcal{S} > \alpha_G^{-1/4} \alpha^{9/2} \left(\frac{m_p}{m_e}\right)^{5/4} \sim \alpha_G^{-1/4} \alpha^2 \sim 10^6 \quad (51)$$

Equations (49) and (51) constrain $\mathcal{S}$ to lie fairly close to its observed value.

The rough equality between t_{eq} and t_{dec} has prompted suggestions that the background radiation may have been generated in some way during the early history of the Universe. For example, the radiation may have been produced by a first generation of pregalactic objects¹⁷. Such objects have a characteristic

lifetime $\sim t_{\text{eq}}$ given by equation (35), so after t_{eq} the radiation density would be expected to be ηF times the matter density, where F is the fraction of the Universe which goes into the stars and η is the fraction of each star's rest mass released through nuclear energy generation and supernova outbursts. The value of t_{eq} associated with the generated $\mathcal{S}$ is thus $(\eta F)^{3/2} t_{\text{eq}}$ and equations (35) and (46) imply, for objects with $L \sim L_E$, that

$$\mathcal{S} \sim \alpha_G^{-1/4} \left\{ \alpha \left(\frac{m_p}{m_e}\right) F^{3/4} \eta^{5/4} \right\} \quad (52)$$

If the term in braces is of the order of unity, one would expect $\mathcal{S} \sim \alpha_G^{-1/4}$ as observed. The same relationship between $\mathcal{S}$ and α_G would apply if the radiation were generated by black holes accreting at the Eddington limit¹⁸. Another possibility^{19,20} is that a photon-baryon ratio of the order of

$$\mathcal{S} \sim \left(\frac{G m_W^2}{\hbar c}\right)^{1/4} \quad (53)$$

where m_W is the W-boson mass, could be generated by CP violating processes in primordial black hole evaporation, or by the large fluctuations which might arise at the epoch of weak/electromagnetic interaction symmetry breaking¹⁸. This is just the relation $\mathcal{S} \sim \alpha_G^{-1/4}$ with m_p replaced by m_W . One could probably conceive of several cosmological scenarios in which a value of $\mathcal{S}$ of the order of $(G m^2/\hbar c)^{-1/4}$ would be generated, where m is the mass of some elementary particle. The important point is that the coincidences involving $\mathcal{S}$ can be explained naturally without recourse to anthropic considerations. (The processes of primordial nucleosynthesis, apart from deuterium production, are insensitive to $\mathcal{S}$ provided that $\mathcal{S} \gg 10^3$.)

Dicke's anthropic interpretation² of equation (32) was advanced before the microwave background was discovered. One could perhaps replace (or strengthen) the argument leading to equation (36) by the more general statement—independent of considerations of stellar physics—that observers require $kT \ll 0.1\alpha^2 m_e c^2$ and the possibility of thermodynamic disequilibrium, and therefore could survey the Universe only when $t_0 \gg t_{\text{dec}}$. From equation (46), if $\mathcal{S} \sim \alpha_G^{-1/4}$ the condition $t_0 > t_{\text{eq}}$ is the same as equation (36).

Dicke's argument helps us to understand why the 'coincidence' of equation (32) prevails—why a similar large number arises in two contexts that might at first sight seem unrelated. It does not tell us why α_G^{-1} has its particular immense value. The task of deriving such a large pure number from basic theory might seem a daunting one. However, Zeldovich²¹ points out that a complete quantum theory of gravity, where tunnelling and topology changes play a key role, is likely to involve exponentials of pure numbers, so that a factor $\sim 10^{40}$ may readily arise. Although no such number has been predicted, a relationship between α and α_G may be forthcoming from quantum field theory. It has been suggested^{22,23} that all space integrals in quantum electrodynamics should be cut off at the Planck length (given by equation (7)), thereby reducing otherwise divergent integrals to finite functions of the parameter $(\alpha \log \alpha_G)$. Various arguments suggest that a self-consistent electrodynamics is possible only if this parameter has some specific value of the order of unity, that is, one requires

$$\alpha^{-1} \sim \log \alpha_G^{-1} \quad (54)$$

This argument may not be convincing, but empirically relation (54) is satisfied. Figure 1 mainly shows that all objects where gravity is important have masses exceeding m_p by some simple power of α_G^{-1} . As the scaling is so simple, could one envisage a hypothetical universe in which all microphysical laws were unchanged, but G was (say) a million times stronger? Planetary and stellar masses ($\propto \alpha_G^{3/2}$) would then be lowered by 10^9 ; but hydrogen-burning main-sequence stars would still exist albeit with lifetimes ($t_{\text{eq}} \propto \alpha_G^{-1}$ according to equation (35)) of only ~ 100 yr rather than $\sim 10^8$ yr. Moreover, Dicke's argument would still apply: a hypothetical observer looking at the

Universe when $t_0 \approx t_{\text{max}}$ would still find equation (32) fulfilled, although the number of particles in his universe would be 10^{12} times lower than in ours. Are there anthropic arguments against the 'cognisability' of the kind of small-scale speeded-up universe that corresponds to a much smaller α_G^{-1} ?

One might argue that the gradual evolutionary emergence of complex organisms demands a large ratio between cosmic and microphysical timescales, and requires also that organised structures can become large (masses $\gg m_p$) before gravity overwhelms chemical forces. This favours a very large α_G^{-1} ; moreover, as $M_*/M_\odot \propto \alpha_G^{-1}$, a universe where α_G^{-1} is large contains more independent sites where evolution might occur. But there is no basis here for being at all quantitative. There are, however, some more specific (though somewhat contrived) anthropic arguments that pin down α_G .

One connection between α and α_G , given by Carter^{3,24}, is related to the existence of convective stars. If radiation transport is unable to maintain a star's surface temperature T_s above the ionisation temperature $\sim 0.1\alpha^2 m_e c^2/k$, a convective outer layer develops and this supplements the heat transport so that it does. The value of T_s which can be maintained by radiative transport, assuming (from the Coulomb penetration condition) a central temperature $T_C \sim 10^{-2}\alpha^2 m_p a^2/k$, is

$$T_s \sim \left(\frac{L}{aR^2}\right)^{1/4} \sim (\text{optical depth through star})^{-1/4} T_C \quad (55)$$

T_s exceeds $0.1\alpha^2 m_e c^2/k$ provided M exceeds

$$M_* \sim M_\odot \alpha_G^{-1/2} \alpha^6 \left(\frac{m_e}{m_p}\right)^2 \sim M_\odot \alpha_G^{-1/2} \alpha^{10} \quad (56)$$

using relation (45). The mass M_* thus divides the (convective) red dwarfs from the (radiative) blue giants. This lies in the range, around $M_\odot$, in which main-sequence stars exist only because

$$\alpha_G \sim \alpha^{20} \quad (57)$$

Were G (and hence α_G) slightly larger, all stars would be blue giants; if it were slightly smaller, all stars would be red dwarfs. Carter ascribes anthropic significance to relation (57) on the basis that the formation of planetary systems may be associated with convective stars. (This is supported by the observation that red dwarfs have much less angular momentum than blue giants and a loss of angular momentum may be a consequence of planet formation, but this is not a compelling argument. Alternatively, convective stars may lose their angular momentum through a wind, and this wind, by blowing away the gaseous envelope of planets close to the star, facilitates the formation of solid planets with non-hydrogen atmospheres.) Carter infers that no planets, and hence no life, would form if α_G were much larger than α^{20} . If α_G were much smaller, all stars would be chemically homogeneous due to convective mixing and one would not get the 'onion-skin' shell structure which characterises pre-supernova models. Carter's convective condition^{12,14} is also the condition that the number of stars in a galaxy be the same as the number of galaxies in the Universe.

The constants pertaining to nuclear physics are the strong (scalar) coupling constant $f^2 \approx 15$ and the mass ratios $m_\pi/m_N \approx 1/1837$, $m_\rho/m_N \approx 1/7$ and $\Delta_N/m_N \approx 1/730$ where $\Delta_N = (m_N - m_p)$. (Many more mass ratios are associated with high-energy physics but it is not clear which of them are fundamental.) The important features of nuclear physics depend²⁴ on the following four coincidences:

$$\begin{aligned} f^2 &\approx 2m_N/m_\pi & \Delta_N/m_\pi &\approx 2 & \alpha &\approx \Delta_N/m_\pi & f &\approx 1/3\alpha^{1/2} \\ (a) & & (b) & & (c) & & (d) \end{aligned} \quad (58)$$

Equation (58a) implies that strong interactions are only marginally strong enough to bind nucleons into nuclei. If f were

slightly weaker, only hydrogen could exist; if it were slightly stronger nuclei of almost unlimited size might exist. (58b) implies that neutrons are unstable to β -decay in isolation but not in the presence of relativistic degenerate electrons. (58c) implies that the electrostatic energy in light nuclei $\sim \alpha m_e$ is comparable to the neutron-proton mass difference. (58d) implies that the electrostatic energy is small compared with nuclear binding energy in light nuclei but comparable to it for nuclei with $Z \sim (f\alpha)^{-3} \sim 30$, so such large nuclei are unstable to electromagnetic disruption. If the relations indicated by equation (58) were not satisfied, elements vital to life would not exist, so one might also ascribe anthropic significance to these relations. Note that the combination of (58a), (b), (c) and (d) implies equation (45). Kahn²⁷ noted that $m_e \ll m_p$ may be a prerequisite for complex chemistry, as this ensures that the ions are located to a precision $\sim (m_e/m_p)^{1/4}$ times their mean spacing.

There may be other anthropic aspects to the value of f . For example, elements heavier than helium can form in stars through the triple- α reaction ($\text{He}^4 + \text{He}^4 \rightarrow \text{Be}^8$, $\text{Be}^8 + \text{He}^4 \rightarrow \text{C}^{12}$), and it is apparently accidental that this process yields a substantial amount of carbon^{25,26}. ^8Be is unstable (otherwise the 'helium flash' in giants would lead to a catastrophic explosion); but the reaction proceeds to ^{12}C because this latter nucleus happens to have an energy level just above the sum of the energies of ^8Be and ^4He . There is, however, no similar favourably placed resonance in ^{16}O ; otherwise almost all the carbon would be transmuted into oxygen. If f were even merely a few per cent larger, there would be 100% cosmological helium production because He^2 would be bound, and deuterium could form by $p + p \rightarrow ^2\text{He} + \gamma$, $^2\text{He} \rightarrow \text{D} + e^+ + \nu$, even if there were no frozen-out neutrons. A small increase in f would also affect the binding energies of heavier nuclei.

Few features of the physical world seem to depend on the actual value of the weak interaction coupling constant. There are, however, two processes, both crucial to nucleosynthesis, which are sensitive to neutrino cross-sections and thus imply a coincidence of 'anthropic' relevance.

The first of these is connected with the production of helium through cosmological nucleosynthesis. The helium abundance Y is essentially determined by the temperature T_F at which the neutron-proton ratio freezes out. This occurs when the weak interactions ($p + e^- \rightarrow n + \nu$, $p + \bar{\nu} \rightarrow n + e^+$), proceeding at a rate $\propto g_w^2 T^5$, where $g_w \sim 10^{-49} \text{ erg cm}^3$ is the weak coupling constant, become slower than the cosmological expansion rate $\sim (GaT^4/c^2)^{1/2}$ at a temperature

$$kT_F \sim G^{1/6} g_w^{-2/3} \hbar^{11/6} c^{-5/6} \quad (59)$$

As virtually all the frozen-out neutrons burn into helium, the resultant helium abundance is

$$Y \sim \frac{2n_N/n_p}{n_N/n_p + 1} \quad \text{where} \quad \frac{n_N}{n_p} \sim \exp\left(\frac{-\Delta_N c^2}{kT_F}\right) \quad (60)$$

The fact that $Y \sim 25\%$ rather than 0% or 100% results only because $kT_F \sim \Delta_N c^2 \sim m_\pi c^2$ and, from equation (59), this derives from the 'coincidence'

$$\left(\frac{Gm_\pi^2}{\hbar c}\right)^{1/4} \sim \left(\frac{g_w m_\pi^2 c}{\hbar^3}\right) \quad (61)$$

The dimensionless number on the right may be regarded as the weak fine structure constant ($\alpha_w \sim 10^{-11}$) and the number on the left as the quarter power of the electron gravitational fine structure constant. In the Weinberg-Salam unified theory of weak and electromagnetic interactions²⁸ α_w is related to α by

$$\alpha_w \sim \alpha \left(\frac{m_e}{m_W}\right)^2 \quad (62)$$

So equation (61) combined with equation (45) may be written as

$$\alpha_G \sim \left(\frac{m_p}{m_W}\right)^8 \left(\frac{\alpha^2 m_p}{m_n}\right)^2 \sim \left(\frac{m_p}{m_W}\right)^8 \quad (63)$$

It is unclear to what extent these coincidences can be interpreted anthropically. Relation (63), for example, may reflect some deep-rooted connection between gravity and the weak and electromagnetic interactions. However, life could probably not exist if Y were 100% (as would be the case if α_W were slightly smaller), as there would be no water. Also, the lifetime of a helium star is less than that of a hydrogen star and might not be long enough to permit the evolution of life. It is not obvious that 0% helium production (as would arise if α_W were slightly larger) would be incompatible with life.

Equation (61) may, however, be associated with another anthropic condition (which would limit α_W in both directions)—the existence of supernovae. What actually ejects the stellar envelope in a supernova explosion is still uncertain, but it may be the outward surge of neutrinos generated by the high temperatures in the collapsing core³⁰. For this model to work, one requires the timescale on which neutrinos interact with nuclei in the envelope to be comparable to the dynamical timescale. If it were much longer, the envelope would be essentially transparent to the neutrinos; if it were shorter, the neutrinos would be trapped in the core, and could not escape to deposit their momentum in the less tightly bound surrounding layers. The two timescales are comparable if

$$c^{-3} \hbar^{-4} g_W^2 n (kT)^2 \sim (G n m_p)^{1/2} \quad (64)$$

where n is the nucleon number density. For the neutrinos to be produced at all (by $e^+ + e^- \rightarrow \nu + \bar{\nu}$) one requires kT to be of the order of $m_\nu c^2$. One also expects the density at the bounce to be of the order of the nucleon degeneracy density which, for $M \sim M_\odot$ is of the order of the nuclear density: $n \sim (\hbar/m_p c)^{-3}$. Putting these values for n and T into equation (64) gives relation (61) apart from a $(m_p/m_n)^{1/2}$ factor. Hence, the condition that stars go through a supernova phase is essentially the same as the condition that there be an interesting amount of cosmological helium production.

Conclusion

The possibility of life as we know it evolving in the Universe depends on the values of a few basic physical constants—and is in some respects remarkably sensitive to their numerical values. Indeed, the various anthropic relations quoted above in principle determine the order-of-magnitude of most of the fundamental constants of physics. Equations (54) and (57) specify α and α_G ; equations (61) and (58d) specify α_W and f ; and equations (9), (45), (58) and (63) determine m_p , m_n , m_e , and m_W in terms of the Planck mass. From a physical point of view, the anthropic 'explanation' of the various coincidences in nature is unsatisfactory, in three respects. First, it is entirely *post hoc*: it

has not yet been used to predict any feature of the Universe (although some people have used it to rule out various cosmological models). Second, the principle is based on what may be an unduly anthropocentric concept of an observer. The arguments invoked here assume that life requires elements heavier than hydrogen and helium, water, galaxies, and special types of stars and planets. It is conceivable that some form of intelligence could exist without all of these features—thermodynamic disequilibrium is perhaps the only prerequisite that we can demand with real conviction. Third, the anthropic principle does not explain the exact values of the various coupling constants and mass-ratios, only their order of magnitudes. With enough anthropic conditions, one may be able to be more precise about the constants of nature, but the present situation is unsatisfactory.

On the other hand, nature does exhibit remarkable coincidences and these do warrant some explanation. Apart from Dirac's suggestion¹ (which only considers the age of the Universe coincidence), the anthropic explanation is the only candidate and the discovery of every extra anthropic coincidence increases the *post hoc* evidence for it. The concept would be more palatable if it could be given a more physical foundation. Such a foundation may already exist in the Everett 'many worlds' interpretation of quantum mechanics, according to which, at each observation, the Universe branches into a number of parallel universes, each corresponding to a possible outcome of the observation³⁰. The Everett picture is entirely consistent with conventional quantum mechanics; it merely bestows on it a more philosophically satisfying interpretation. There may already be room for the anthropic principle in this picture. Wheeler³¹ envisages an infinite ensemble of universes, all with different coupling constants and so on. Most are 'still-born', in that the prevailing physical laws do not allow anything interesting to happen in them; only those which start off with the right constants can ever become 'aware of themselves'. One would have achieved something if one could show that any cognisable universe had to possess some features in common with our Universe. Such an ensemble of universes could exist in the same sort of space as the Everett picture invokes. Alternatively, an observer may be required to 'collapse' the wave function³². These arguments go a little way towards giving the anthropic principle the status of a physical theory but only a little: it may never aspire to being much more than a philosophical curiosity. One day, we may have a more physical explanation for some of the relationships discussed here that now seem genuine coincidences. For example, the coincidence $\alpha_G \sim (m_p/m_W)^8$, which is essential for nucleogenesis, may eventually be subsumed as a consequence of some presently unformulated unified physical theory. However, even if all apparently anthropic coincidences could be explained in this way, it would still be remarkable that the relationships dictated by physical theory happened also to be those propitious for life.

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articles

The possibility of superheavy elements in iron meteorites

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The possible role of superheavy elements in the early Moon is reviewed. The siderophilic superheavy elements (possibly $Z = 114, 115, 116$) may have been the heat source in the Moon's iron core needed to generate the ancient lunar magnetic field. It is suggested that in the search for present day evidence of the existence of these hypothetical elements, iron meteorites may be a key. The available data on their trace elements are reviewed and future work to determine whether they are in part fission products from metallic superheavy elements is suggested.

WE proposed recently¹ that superheavy elements (SHE) were present in the Moon at its accretional origin, 4.6×10^9 yr ago, in sufficient abundance to melt it in its entirety—thus forming a small iron core as well as the anorthositic highlands. Geochemists at first thought of a differentiation of the outer Moon only², which it was plausible to suppose might have been brought about by accretion³ or solar wind heating⁴, processes which are most effective in the outer layers. Lunar palaeomagnetism, interpreted as being acquired in a lunar field of internal origin at the time of the cooling of the lunar rocks, $3.2\text{--}4.0 \times 10^9$ yr ago, requires the existence of a molten iron core during this time⁵. Palaeointensity experiments show the surface lunar field to have been about 1 G 4.0×10^9 yr ago, a surprisingly high value, especially as the lunar core cannot be more than 500 km in radius. Fundamental thermodynamics of the convecting core dynamo show that a heat source of 10^{12} W is required in the iron core at that time to generate the inferred magnetic field⁶. It is shown that the various conventional heat sources, proposed over the last 30 yr for the geodynamo and shown to be quantitatively sufficient for its working, are not powerful enough for the early Moon.

Indeed we¹ suggested that those superheavy elements which were siderophilic ($Z = 107\text{--}111, 114, 115, 116$) might provide the necessary heat source for the ancient lunar core dynamo. Supposing that the SHE completely melted the Moon, they could account for the differentiation, which was completed by 4.4×10^9 yr, forming the two outer lunar shells of the outer anorthositic highland layer and the source region of the mare basalts, and they could have then been incorporated into the iron core.

In Fig. 1, dN/dt is the number of SHE fissions per year per kilogram of the Moon. It was shown that 3×10^8 nuclei per year per kg are required⁷ to fission at 4.6×10^9 yr ago to melt the

Moon, and 1.1×10^7 , 6.9×10^5 and 1.5×10^4 fissions per yr per kg are required to run the dynamo at 4.0×10^9 yr, 3.6 and 3.2×10^9 yr respectively. As these values of dN/dt lie on an exponential curve of 10^8 yr half life, SHE of this half life would explain the decay of heat sources in the early Moon.

Figure 1 also shows the rate of fission of SHE nuclei in the Moon's core, assumed to be 500 km in radius, expressed per kilogram of iron for the period 4.0×10^9 yr— 3.2×10^9 yr for which the palaeointensity of the field is known. Assuming the half life of 10^8 yr previously obtained from these data, another ordinate scale can be drawn giving the abundances of SHE in the iron at various times. If the palaeointensity of the younger rocks is underestimated, as has been suggested, then it is possible to produce a plot in which the present day abundance of SHE is of the order of that claimed by Flerov⁸ in carbonaceous chondritic meteorites. The half life given by this procedure is 1.6×10^8 yr and an ordinate scale giving abundances can be drawn corresponding to this value. Also it was shown that this empirical relation gave as the original abundance of SHE in the Moon about 0.1 that of cosmic abundance of uranium.

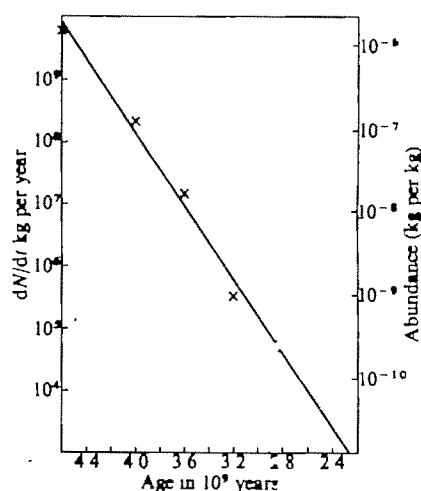


Fig. 1 Rate of fission of superheavy elements versus age in the early Moon (ordinate scale, left-hand side). Abundance by weight of the superheavy elements versus age in the Moon (ordinate scale, right-hand side).

Trace elements in iron meteorites

We have reviewed¹ the many efforts that have been made to search for evidence of SHE in nature. In this article we direct attention to the iron meteorites. We think that it is remarkable that bodies of all sizes in the Solar System (parent bodies of meteorites, the asteroid Vesta, the terrestrial planets, and the Moon) show evidence of early melting and it is a plausible hypothesis that the cause was the same. When early melting of orbiting bodies in the Solar System occurred, siderophilic elements would have accumulated in the iron phase. Half lives of 10^6 yr leave little chance of detecting SHE in iron meteorites today, but the fission products should still remain in the iron.

Figure 2 shows measurements⁹ made in iron meteorites of concentrations of elements heavier than iron. Peaks at $Z \sim 43$, $Z \sim 52$ and $Z \sim 78$ with maximum concentrations of about 10 p.p.m. (kg of element/kg of iron) are clearly present: we consider these peaks to be significant, despite the paucity of measurements and the difficulties in the mechanical separation of inclusions, such as sulphides and silicates, from the iron phase. Figure 3 shows that there is evidence for two of the peaks in the more primitive carbonaceous chondrites; they also occur (with much smaller amplitude relative to iron) in the Cameron¹⁰ curve of abundance of elements in the primitive solar nebula around $Z = 53$ and 78 (magic numbers) where the peaks have an abundance roughly 10^{-6} relative to iron. We must therefore conclude that the iron meteorites have not taken up, either in the process of condensation from the solar nebula or from any late processes of differentiation and melting in the 'parent body', any appreciable amounts of the other elements. This has been explained following Goldsmidt¹¹ by classifying the former elements as siderophile and the latter as lithophile; we attempt here a quantitative treatment. To establish whether the shapes of these peaks are entirely explained by the solubilities in iron of the elements originally present in the solar nebula we have made

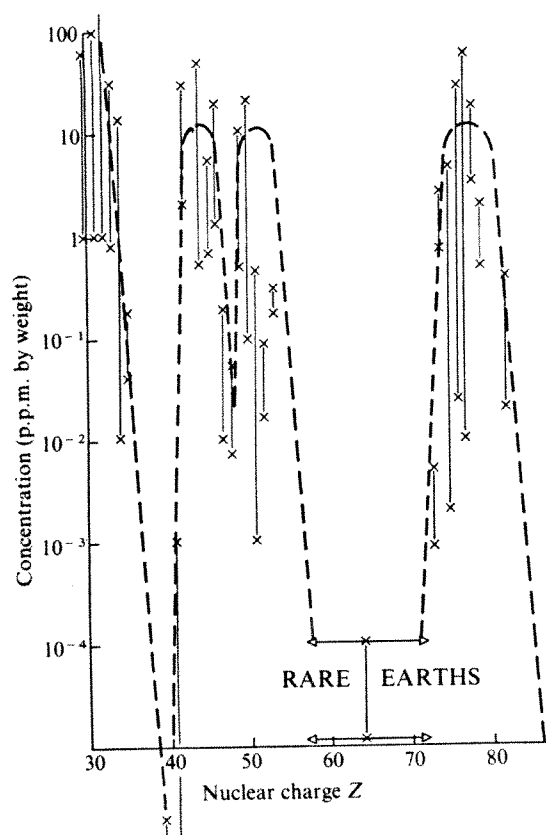


Fig. 2 Abundance of elements measured in iron meteorites versus Z (see ref. 9). Dashed line shows suggested fission product distribution.

the following computation. We assume that the liquid metals obey the perfect solution law, that is, the solubility S_s is equal to the reciprocal of the ratio of the vapour pressure of the pure solute to the ambient pressure over the solution. Based on this assumption we have computed the expected concentration of the metallic elements in iron at the freezing point of iron.

We further assume that if an element's oxide is stable at $1,535^\circ\text{C}$, the melting point of iron, it will not be miscible with iron and so its concentration in iron meteorites will be zero. This assumption produces zeros in the theoretical curve of concentration against Z , from $Z = 33$ to $Z = 39$ inclusive and from $Z = 52$ to 71 inclusive. For the light elements this assumption is also true, and, in particular aluminium oxide is very stable and will never be found in iron: thus ^{26}Al on our theory is not plausible as a heat source in an iron core as has been suggested¹². (In any case its short half life of 0.73×10^6 yr rules it out as a heat source for the Moon's dynamo.) Similarly, halogens form hydrogen halides in a hydrogen-rich solar nebula and are immiscible in iron therefore. We have further assumed that the rare gases are insoluble in iron.

For those elements which are not rare gases, not halogens, and which do not have very stable oxides, their heats of vaporisation ΔH are computed using Trouton's rule: $\Delta H = (25) \times (\text{boiling point, K}) \text{ cal mol}^{-1}$. Putting this value of ΔH into the Clausius-Clapeyron equation, P_s , the vapour pressure of the given element as pure liquid at the freezing point temperature of iron (chosen as the lowest temperature at which a solute element could dissolve in iron) is calculated. Similarly the vapour pressure P_{Fe} of iron at its melting point temperature is computed. Assume a solar nebula at 1 atm pressure, containing 10^{12} atoms H, $10^{6.9}$ atoms Fe (designated as $n_{\text{Fe-O}}$), and numbers of atoms of each element (designated as $n_{\text{S-O}}$) equal to its cosmic abundance relative to hydrogen at 10^{12} . Let the number of solute atoms in the vapour at the melting point of iron be $n_s = P_s V kT$ and the number of iron atoms in the vapour similarly be $n_{\text{Fe}} = P_{\text{Fe}} V kT$. If S_s is the mole fraction of solute in the condensed system at the freezing point of iron, $S_s = P_s / P_{\text{S-O}}$. Then, using conservation of numbers of atoms,

$$n_{\text{S-O}} = m_s + (n_{\text{Fe-O}} - n_{\text{Fe}}) \times S_s / (1 - S_s)$$

which, with some rearrangement, yields the relation (using $V/kT = 10^{12}$ and using $S_s \ll 1$),

$$S_s = n_{\text{S-O}} (10^{12} + (n_{\text{Fe-O}} - n_{\text{Fe}}) / P_{\text{S-O}})^{-1} \times (P_{\text{S-O}})^{-1}$$

where $(N_{\text{Fe-O}}) = (n_{\text{Fe-O}})$ is the number of condensed iron atoms.

Simple reasoning by analogy leads one to guess that the SHE elements of lower Z (114, 115, 116) would be siderophilic, whereas elements of $Z = 121$ to 138 probably would have a chemistry like that of actinium and lanthanum, and that they would form stable oxides in a water-rich nebula and could not have existed in iron meteorites. However, elements of $Z = 121$ –138 are g -orbital elements of which we have no chemical experience. So it is possible that our capability of predicting their solubility in iron fails in this range of Z . Elements 107–111 almost certainly will be iron soluble.

Table 1 lists the theoretical solubilities in iron versus Z , calculated by the method discussed above and the measured concentrations versus Z . In general the calculated solubilities in iron fit the observations in iron meteorites for about half the metallic elements, which have long been recognised as siderophiles, but they are lower than the observed ones in most other cases. The differences in concentrations—those found experimentally minus those calculated—provide evidence of strong peaks around $Z = 32$, $Z = 46$, $Z = 50$ and $Z = 77$. These differences, plotted in Fig. 4, agree well with the peaks in the experimental measurements of heavy elements in iron meteorites. Furthermore, the highest values of peak elements occur in those metal meteorites with the largest crystals having cooled the most slowly. In addition, we have evidence for the presence of Zn, the halogens, the noble gases, the rare earths, and the actinides, none of which should be present in an iron phase according to our theory. Experimental confirmation of the

Table 1 Computed solubility of heavy elements in iron compared with measured concentrations of heavy elements in iron meteorites (see ref. 9)

Element	Charge	Calculated kg per 10^6 kg Fe	Maximum observed kg/ 10^6 kg of Fe	Excess concentration of element*	Element	Charge	Calculated kg per 10^6 kg Fe	Maximum observed kg/ 10^6 kg of Fe	Excess concentration of element*
Zn	30	—	61	61	Ba	56	Zero	—	—
Ga	31	0.14	100	100	La	57	Zero	10^{-4} to 10^{-6} (?)	10^{-4} to 10^{-6}
Ge	32	8.2	520	506	Lu	71	Zero	—	—
As	33	Zero	30	30	Hf	72	2.8	—	—
Se	34	Zero	—	—	Ta	73	2.8	5×10^{-3}	-2.8
Br	35	Zero	0.15	0.15	W	74	5.3	2.7	-2.5
Kr	36	Zero	10^{-4}	10^{-4}	Re	75	1.7	4.8	3
Rb	37	Zero	—	—	Os	76	8.5	50	42
Sr	38	Zero	—	—	Ir	77	6.9	59	52
Y	39	Zero	—	—	Pt	78	17	19	2
Zr	40	52	—	—					
Nb	41	10	<20	Zero	Au	79	0.29	2.2	2
Mo	42	18	11	-7	Hg	80	8.2×10^{-9}	2.2 (?)	2.2
Tc	43	Zero	—	—	Tl	81	6.6×10^{-4}	0.25×10^{-3}	0.0025
Ru	44	7.7	35	27	Pb	82	6.7×10^{-4}	0.4	0.4
Rh	45	1.3	4.0	3	Bi	83	2.1×10^{-9}	—	—
Pd	46	4.6	19	14	Po	84	Zero	—	—
Ag	47	10×10^{-4}	0.2	0.2	At	85	Zero	—	—
Cd	48	13×10^{-3}	0.056	0.04	Rn	86	Zero	—	—
In	49	4.1×10^{-4}	0.02 (?)	0.02 (?)	Fr	87	Zero	—	—
Sn	50	0.013	20 (?)	20	Ra	88	Zero	—	—
Sb	51	12.6	0.5	-12.1	Ac	89	Zero	—	—
Te	52	Zero	—	—	Th	90	Zero	1.8×10^{-5}	1.8×10^{-6}
I	53	Zero	0.3 (?)	0.3	Pa	91	Zero	—	—
Xe	54	Zero	10^{-4} (?)	10^{-4} (?)	U	92	Zero	10^{-4} to 10^{-6} (?)	10^{-4} to 10^{-6}
Cs (?)	55	Zero	—	—					

* Excess is the difference between the measured and calculated concentrations in p.p.m. by weight.

theoretical calculations is lacking and is much needed.

From the point of view of physical chemistry, these results are only a first approximation. The basic consideration is whether the assumption of equilibrium with the nebular gases at the time of freezing of the iron rich phase is valid. Many workers on iron meteorites assume that further fractionation via formation of 'parent bodies' and subsequent break-up to form the meteorites occurs. We suggest that this may not be so and that the slow cooling required by the Widmanstätten etch patterns may have been due to the presence of superheavy elements furnishing heat by radioactive decay, thus locking the cooling rate into their radioactive lifetimes.

Fission of superheavy elements

The sum of the nuclear charges of the two middle mass peaks equals $Z = 94-96$; but because the oxides of uranium and plutonium are very stable, these elements cannot be present in iron meteorites in quantities sufficient to explain these two peaks as the products of their fission: nor does the measured concentration of uranium in iron meteorites permit this explanation as will be discussed below.

The sum of the nuclear charges for the peaks at $Z \sim 46$ and $Z \sim 78$ is 121, and the sum for those at $Z = 52$ and $Z = 78$ is 130. Thus, if two superheavy elements had produced these peaks by fission, their nuclear charges would have ranged from 110 to 120. The width of the peak at $Z \sim 78$ is equal to twice the width of the peak at $Z \sim 43$. For this reason we have drawn the second peak, at $Z \sim 52$, in such a way that it has the same width as the peak at $Z \sim 43$, to account for the enhanced width at $Z \sim 78$, suggesting that indeed two superheavy elements are the progenitors. However, some theorists^{13,14} prefer superheavy elements in the low Z end of the island of stability, $Z = 114$, 115, 116, to be the most stable and these, as we have seen, are the only ones, on present chemical reasoning, to be siderophile.

The sum of the charges of three peaks in Fig. 2 corresponds to $Z = 168-180$. Since nuclear shell theory predicts a further island of stability at $Z = 164-184$ (depending on which potential energy function is favoured)¹⁵, we must consider whether the three peaks originate from decay of super-superheavy elements

(SSHE) by ternary fission. Although uranium and plutonium decay by ternary fission with only very low probability, it is possible that the geometry of the super-superheavy elements is such that ternary fission becomes the most probable mode of decay. The shape of the nucleus in its ground state could then be deduced from the shapes of the peaks of the fission product distribution. The shape might, for example, be bent and lumpy, perhaps like a water molecule, or perhaps like a three-legged starfish. According to recent theoretical ideas¹⁶, such nuclei

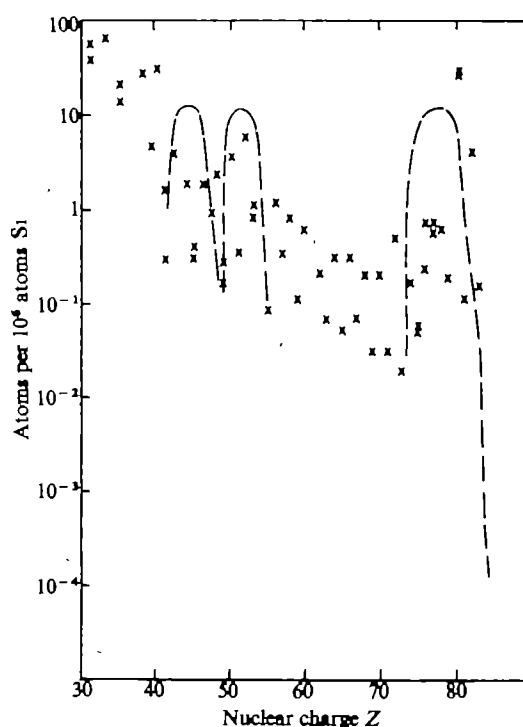


Fig. 3 Excess abundance of elements in iron meteorites above that expected on solubility theory.

($Z \sim 173$, $N \sim 249$) could have three axes of rotational symmetry.

Our hypothesis that the peaks result from the decay of SHE and SSHE in the early Solar System encounters some quantitative difficulties. If energies released on fission by the SHE and SSHE are ~ 250 MeV, then the peaks of concentration at about 10^{-5} g g $^{-1}$ would imply that in the early cores of the parent bodies a total energy release of about 2×10^4 J kg $^{-1}$, sufficient to vaporise iron. However, radiative cooling would prevent this. At the melting point of iron, 1,535 °C, and with a mean life of Y yr for spontaneous fission, an iron sphere taken as a black body would radiate at the fission rate for any values of Y longer than 1 Myr for spheres of radii less than 3,000 km. However, from the studies of the Widmanstätten patterns and kamacite bands¹⁷, we know that the cooling rates of iron meteorites must have been of the order of 1 °C Myr $^{-1}$. This probably requires the parent bodies of the iron meteorites to have been a loosely held aggregate in order to allow radiative cooling. Such a model for them may be required to explain why they were broken up by external forces, possibly tidal. We conclude that for iron meteorites early solidification is probable, with sealing-in of a substantial part of the fission products. Scott and Wasson¹⁷ discuss the classes of iron meteorites: only some may have originated as cores of small planetary bodies. The data we have used include all iron meteorites together; plots like that of Fig. 2 should be made for each class when sufficient measurements are available.

Another difficulty for the hypothesis is that the peak concentrations of the hypothetical fission products are about 10^{-5} kg kg $^{-1}$ and would imply, assuming a maximum fission yield for an element of 5%, that the initial abundance of SHE in the early Solar System, allowing that they became concentrated in iron by a factor of 10–100 would have been between 2×10^{-6} and 2×10^{-5} kg kg $^{-1}$. This is to be compared with a cosmic abundance of 10^{-7} for uranium. Recent calculations of the r process suggest a cosmic abundance of SHE within an order of magnitude that of uranium¹⁸. If this were so, and if the SHE most likely to have been present were those with $Z = 114$ for the two reasons already given, then the fission peaks expected by analogy with uranium should be near $Z = 50$ and $Z = 80$ and with abundance 5×10^{-8} – 5×10^{-7} . If the peaks which we have

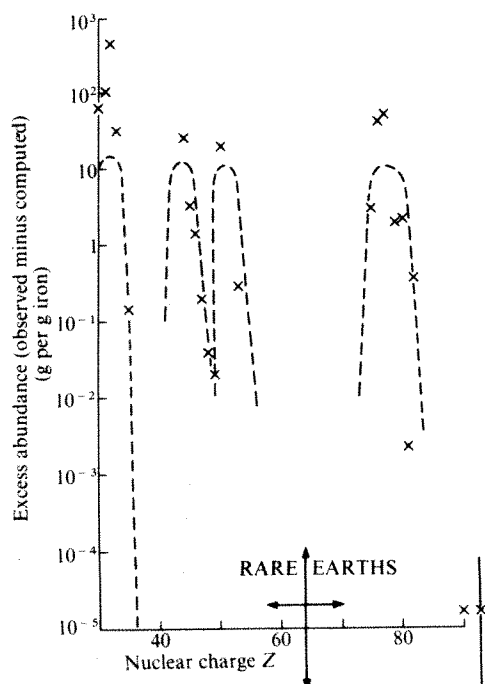


Fig. 4 Abundance of elements in carbonaceous chondrites. Dashed line shows suggested fission product distribution.

discussed are after all the result of elements being taken up from the solar nebula by fractionation processes and as a result of extraction of elements into the iron phase during iron-silicate fractionation, then, as there is a window around $Z = 55$ – 70 where the expected fission peaks should lie, better data on rare earth abundances should enable their presence to be determined. So far virtually no data are available.

In iron meteorites the abundance of uranium is 10^{-10} – 10^{-12} (see ref. 19), completely inadequate to explain the 10 p.p.m. of lead and the several parts per million of Sn, Sb, Te and I found in iron meteorites and cannot explain the Xe and Kr which has recently been detected^{20,21} in iron meteorites with concentrations of about 10^{-12} because their isotopic ratios are not those of fission products.

In the so-far unsuccessful attempts to make SHE in accelerators, collisions of heavy ions have been used²². We note that in collisions of ^{40}Ar (~ 300 MeV) and ^{84}Kr (~ 600 MeV) with ^{238}U , three peaks of heavy element concentrations were produced²² at about the same values of Z as those of the peaks for the iron meteorites (Fig. 2). It is as if perhaps SHE of $Z = 110$ – 120 were being produced but their neutron/proton ratio was too small and their excitations too large to allow them to live long after the collision.

If indeed the peaks are made by fission products of SHE in iron meteorites, the wide separations of the peaks indicates a very asymmetric mode of binary fission. Asymmetric fission was predicted by Rao²³ at the very values of Z and N which characterise the peaks in Fig. 2. They centre around the 'magic numbers': for the peak at $Z = 46$, the magic number is $N^* = 50$; the peak at $Z = 50$ centres around the two magic numbers, $N^* = 82$ and $Z^* = 50$; and the peak at $Z = 78$ centres around the two magic numbers $N^* = 126$ and $Z^* = 82$. We believe that decay of superheavy elements initially present in supernova remnants is partly responsible for the peaks mentioned above in the carbonaceous chondrite abundances. It would be strange if the abundance peaks observed in the iron meteorites were to be entirely explained by their siderophile character. Why would elements with magic numbers in their nuclear structure also have the chemical property of solubility in iron?

Classical theory of iron meteorites

E. R. D. Scott²⁵ states that "all major groups (of iron meteorites) were produced by fractional crystallisation of an iron melt. Meteorites did not form a single molten core. The cooling rates estimated from kamacite bandwidths and bulk nickel contents do not support a core origin from some groups. The wide range of cooling rates suggested formation of these meteorites in isolated regions of a parent body. Metre-sized molten iron pools formed. Unless it can be shown that other factors (such as the effect of trace and minor elements on diffusion rates) are responsible for the wide range of cooling rates estimated, such data are not compatible with core formation. The primary fractionation which established the chemical differences between the groups might have occurred during condensation of the solar nebula. It seems unlikely that the major differences could have been produced during separation of metal, silicate, and sulphide phases in parent bodies."

J. T. Wasson²⁶ suggests that "All currently available evidence favours the viewpoint that meteorites formed at relatively low ambient pressures, much less than those found in the interior of a moon-sized body, and consistent with an origin in parent bodies no larger than the largest asteroids. Now that a low-pressure origin of the meteorites is widely accepted, ... some, perhaps many, probably originated in the smaller asteroids, with central pressures less than 100 bar ... the minimum size of the parent body can be estimated, the total mass is 0.8×10^{15} g, 650 Myr ago. At a density of 7.9 g cm $^{-3}$, the minimum radius is 0.3 km. This lower limit is much smaller than parent body sizes necessary to account for estimated cooling rates." Thus while we acknowledge that many people have worked intensively to understand the mysterious properties of iron meteorites for at

least 40 yr, many of the properties of iron meteorites are inexplicable by the classical ideas of their origin. We hypothesise that the iron meteorites were never very large, condensed from a solar nebula in small masses containing superheavy elements soluble in iron-nickel melts, cooled very slowly because of a radio-half life of about 100 Myr, and produced solid iron-nickel chunks supersaturated in fission products which ranged from $Z = 30$ to 92, and perhaps an even wider distribution of elements of charge Z .

Conclusions

Our hypothesis that the heavy elements in iron meteorites are partly the fission products of SHE and perhaps of SSHE is put forward tentatively as befits our state of ignorance in this field. But we suggest some definitive measurements to test it: (1) measurements of heavy element concentrations in each class of iron meteorites and of halides, alkalis, alkaline earths, and rare gases in particular; (2) measurement of solubilities of heavy elements in iron versus pressure and at high temperatures, to test our crude calculations; (3) study of the theory of ternary fission in SSH elements; (4) bombardment of uranium with uranium ions²³ in the heavy ion accelerators to determine if the heavy element peak at $Z \sim 78$ increases in height relative to that observed in Ar-U and Kr-U bombardments²⁴, and bombardment of U with Xe ions, for the same purpose; and (5) further work on isotope ratio analyses of peak elements.

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Transfer of actinides from the English Channel into the southern North Sea

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Results are presented for ^{238}Pu , $^{239+240}\text{Pu}$, ^{241}Pu , ^{241}Am , ^{242}Cm and ^{244}Cm in waters of the English Channel and southern North Sea. Based on actinide concentration ratios in these waters compared with expected fallout values, the activities measured are considered to be mainly due to releases of low-level waste from the La Hague fuel reprocessing plant. Measurements made on ^{242}Cm indicate that any support by ^{242m}Am would be minimal.

As part of a study of the distribution, behaviour and sources of actinide isotopes in the North Sea, two series of surface water samples were taken during February-March and September-October 1975, concentrating especially on an area of the southern North Sea and the English Channel. This area is of particular interest because of the situation of a nuclear fuel reprocessing plant at La Hague near Cherbourg, as well as various nuclear power installations on coastal or estuarine sites in countries bordering this region. Previous work¹⁻³ has shown that important amounts of various fission radionuclides originating from releases of low-level wastes find their way into waters of the North Sea.

Murray and Kautsky⁴ have shown that waters entering the northern North Sea by the Pentland Firth and to the north of the Orkney Islands also transport detectable quantities of $^{239+240}\text{Pu}$ and ^{238}Pu resulting from discharge from fuel reprocessing plants at Windscale and to a much lesser extent, Dounreay. We report here the results of measurements of isotopes of three actinide elements, plutonium, americium and curium, in waters of the English Channel, whose activities are also believed to result

from waste discharge. These waters act as input source of several isotopes into the southern North Sea. Comparisons of various actinide activity concentration ratios for these waters with those that may be expected from fallout have been used to identify the source and the extent of their penetration.

Sampling and analysis

Samples of surface seawater, varying in volume between 60 and 200 l, were taken at 13 stations in the Channel and North Sea between 50° and 55° N during February-March, and September-October 1975. The samples were immediately acidified on collection and stored unfiltered in plastic drums until their subsequent analysis. Separation and measurement of the samples were carried out within three months of collection. The chemical separation and measurement by α spectrometry of the various actinide isotopes and the measurement of IAEA intercalibration samples have been described elsewhere⁵. After initial measurements of $^{239+240}\text{Pu}$, ^{238}Pu and ^{241}Am , the disks containing plutonium were stored for nearly two years so that ^{241}Pu (β emitter) could be measured from the ingrowth of its daughter isotope ^{241}Am (for a detailed discussion of this procedure see ref. 6). Due to the short time between sample collection and analysis, initially separated ^{241}Am is assumed to contain a negligible contribution of ^{241}Am due to ^{241}Pu decay between collection and analysis. Where activity levels were sufficiently high, measurements were also made of ^{242}Cm and ^{244}Cm . The decay of ^{242}Cm was traced on a single sample for nearly three years to determine whether some fraction of it was supported by ^{242m}Am .

Ratio of actinides in fallout

Hardy *et al.*⁷ have discussed the global inventory and distribution of fallout plutonium caused by atmospheric nuclear-weapon testing; from their data an average $^{238}\text{Pu}/^{239+240}\text{Pu}$ ratio

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Table 1 Seawater activities of actinides (f kg^{-1}) in surface waters of the English Channel and southern North Sea

Cruise	Local station no.	^{238}Pu	$^{239+240}\text{Pu}$	^{241}Pu	$^{241}\text{Am}^\dagger$	$^{242}\text{Cm}^\ddagger$	^{244}Cm
Feb–March 1975	52	2.0 ± 0.1	10.6 ± 0.3	364 ± 31	1.48 ± 0.09	7.48 ± 0.7	ND
	53	1.4 ± 0.1	10.5 ± 0.4	493 ± 42	NM	NM	NM
	56	1.5 ± 0.2	8.4 ± 0.7	78 ± 17	0.28 ± 0.04	2.9 ± 0.3	ND
	60	ND	2.3 ± 0.4	NM	0.20 ± 0.04	ND	ND
	62	0.2 ± 0.05	1.9 ± 0.2	53 ± 15	0.10 ± 0.03	ND	ND
	63	0.3 ± 0.1	1.6 ± 0.2	39 ± 17	0.10 ± 0.03	ND	ND
	73	$0.03 \pm 0.01^*$	0.4 ± 0.1	11 ± 9	0.04 ± 0.02	ND	ND
	1	$0.06 \pm 0.02^*$	0.8 ± 0.1	14 ± 12	0.04 ± 0.01	ND	ND
Sept–Oct 1975	30	0.98 ± 0.19	3.7 ± 0.3	113 ± 25	0.76 ± 0.08	1.68 ± 0.23	0.07 ± 0.02
	31	0.65 ± 0.10	2.8 ± 0.3	140 ± 20	0.36 ± 0.05	0.39 ± 0.07	0.03 ± 0.01
	34	0.35 ± 0.04	2.5 ± 0.1	100 ± 15	0.32 ± 0.04	0.46 ± 0.02	ND
	22	0.33 ± 0.05	1.5 ± 0.1	88 ± 13	0.13 ± 0.03	ND	ND
	40	0.26 ± 0.05	1.8 ± 0.1	75 ± 14	0.23 ± 0.06	ND	ND

Error, 1σ propagated; NM, not measured; ND, not detected.

*Estimated activity for coastal water (ref. 17).

†No correction for ingrowth of ^{241}Am from ^{241}Pu as sample collection date to analysis was ≤ 3 months only.

‡Corrected for decay to date of collection.

of 0.04 can be calculated for integrated soil samples for 1970–71. They estimate that the accidental release of ^{238}Pu from the SNAP 9A satellite almost tripled the global deposition of this isotope by 1970.

Livingston *et al.*⁶ have reported that the $^{241}\text{Pu}/^{239+240}\text{Pu}$ ratios for fallout-contaminated marine samples are similar to those from fallout-contaminated soils. They showed, however, that these ratios are clearly different in samples from planned or accidental plutonium releases. Whereas the global fallout activity $^{241}\text{Pu}/^{239+240}\text{Pu}$ ratio on 1 January 1970 was calculated to be 8.2, the ratio in IAEA intercalibration samples, which are known to originate from areas contaminated by waste effluent from a major fuel reprocessing plant, was found to be about 30.

Fukai *et al.*⁸ measured the activity ratio of $^{241}\text{Am}/^{239+240}\text{Pu}$ in Mediterranean surface waters sampled in 1975 as 0.055 ± 0.007 . Results¹⁰ for North Atlantic surface water gave a range of ratios of 0.05–0.28, which agree with the estimate of Krey *et al.*¹¹ for 1974 for integrated global fallout of 0.22. Livingston *et al.*⁶ calculated the increase of the $^{241}\text{Am}/^{239+240}\text{Pu}$ ratio due the decay of fallout ^{241}Pu with time and for 1975 a value of about 0.18 was determined.

Holm and Persson⁹ and Bowen and Livingston¹² have discussed the occurrence of ^{242}Cm and ^{244}Cm in the environment. The former report that the average $^{242}\text{Cm}/^{239+240}\text{Pu}$ ratio in lichen for the period 1961–72 was $(0.3 \pm 0.1) \times 10^{-4}$. They also estimated the $^{244}\text{Cm}/^{239+240}\text{Pu}$ activity ratio to be about 0.2×10^{-4} , which agrees well with the observations of Bowen and Livingston¹². Both groups of workers assumed that ^{242}Cm was supported by ^{242m}Am , due to the age of the samples and the relatively short half life of ^{242}Cm (163 d).

On the basis of these expected fallout ratios an attempt has been made to distinguish the probable source and dispersion of actinides entering the southern North Sea from the English Channel.

Results and discussion

Figure 1 shows the positions of the sampling stations measured during February–March, and September–October 1975. Results for surface water activities of ^{238}Pu , $^{239+240}\text{Pu}$, ^{241}Pu , ^{241}Am , ^{242}Cm and ^{244}Cm are presented for both cruises in Table 1; all activities are corrected to the date of sampling. The concentrations for ^{238}Pu , $^{239+240}\text{Pu}$ and ^{241}Am for the February–March cruise have been presented elsewhere⁴ but these results are needed for calculation of isotopic ratios and are, therefore, included.

Kautsky¹³ has shown that patches of water of greatly varying ^{137}Cs activity enter the southern North Sea from the English

Channel at irregular intervals, rather than as a steady input. He proposed that this probably reflected irregular discharge by the La Hague fuel reprocessing plant. Such irregular discharges probably characterise the input of actinides as well.

As there are no available data for the variations in amounts of radionuclides discharged by the French plant as a function of time, a difficulty arises in trying to separate differential actinide behaviour due to chemical reactivity (towards biological or sedimentological processes) from discharge compositional fluctuations and contributions from fallout.

An indication of the pulse-like nature of the actinide discharge in the English Channel may be obtained by comparing

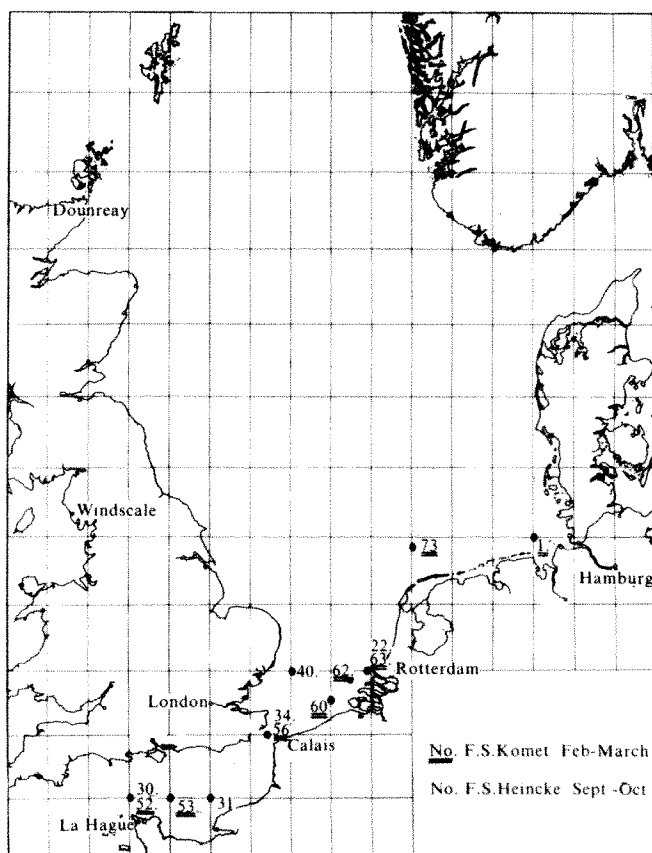


Fig. 1 Sampling stations February–March, and September–October 1975 cruises.

Table 2 Variations in channel water activity ratios occurring between February–March, and September–October 1975 cruises

Stations	^{238}Pu	$^{239+240}\text{Pu}$	^{241}Pu	^{241}Am	^{242}Cm
March–October					
52/30	2.0 ± 0.4	2.9 ± 0.3	3.2 ± 0.8	2.0 ± 0.2	4.5 ± 0.7
53/31	2.2 ± 0.4	3.8 ± 0.4	3.5 ± 0.6	–	–
56/34	4.3 ± 0.7	3.4 ± 0.3	0.8 ± 0.2	0.9 ± 0.1	6.3 ± 0.7
Average decrease factor	2.8 ± 0.5	3.4 ± 0.3	2.5 ± 0.5	1.5 ± 0.2	5.5 ± 0.7

Error, 1σ propagated.

concentrations at stations 52–56 (February–March 1975) and 30–34 (September–October 1975): these results are shown in Table 2. Although dilution occurs during the movement of water through the Channel, the variation in activity for ^{238}Pu , $^{239+240}\text{Pu}$ and ^{241}Pu , calculated as isotope activity in March divided by isotope activity in October (assuming that the dilution will be similar for both cruises), shows a remarkably similar pattern; the activity decrease for the isotopes being 2.8, 3.4 and 2.5 respectively. Thus the plutonium isotopes' water activity in the English Channel for March 1975 seems to have been some three times greater than that occurring for October 1975.

In the case of ^{241}Am a change in activity of about 2 occurs in the region of Cap de La Hague during the same period. The activity ratio in the Straits of Dover (stations 56/34, March–October), however, is about 0.9. As the ingrowth of ^{241}Am from ^{241}Pu will affect both concentrations equally, the difference may be considered real.

The results for ^{242}Cm are more complicated because of its relatively short half life of 163 d. Kautsky¹³ estimated the average speed taken for water to pass from Cherbourg to the Straits of Dover as about 1.7 nautical miles per day. The time it would take from Cherbourg to the Straits of Dover, a distance of ~160 nautical miles, would thus take some 100 d or somewhat less than one half life of ^{242}Cm . Assuming that both dilution and decay will affect the samples from the two cruises equally, it can be seen that ^{242}Cm activity in the English Channel in March 1975 was some 5.5 times greater than in October 1975.

It thus seems that the average concentration fluctuations for plutonium isotopes in the English Channel during March–October 1975, were reasonably constant. However, when one compares the fluctuations between the elements, large variations can be seen to occur; with decrease in the order $\text{Cm} > \text{Pu} > \text{Am}$. That the activities measured in the English Channel are not primarily due to fallout but may be attributed to the discharge of low-level waste from La Hague is shown by comparing the various isotope activity ratios in Table 3 with those that can be expected from fallout. In the case of plutonium isotopes the fallout $^{238}\text{Pu}/^{239+240}\text{Pu}$ ratio for integrated samples is about

0.04 ± 0.01 (2σ error) based on the results of Hardy *et al.*⁷. The ratios found in the channel region are about 0.20 for both cases. Even in the southern North Sea as far east as about $53^\circ 00' \text{N}$, $04^\circ 00' \text{E}$, plutonium activities are clearly due to sources other than fallout.

The results for the activities of ^{241}Pu found during both periods in 1975 are considerably higher than those of $^{239+240}\text{Pu}$. The values near Cap de La Hague are 30–50 times greater and are significantly different from those expected from fallout. The ^{241}Pu activity for station 56, February–March 1975 is 3–4 times lower than for other stations in the region. The reason for this anomalously low result is not clear, however, the $^{241}\text{Pu}/^{239+240}\text{Pu}$ ratios are generally comparable with those reported for 1972–74 by Hetherington *et al.*¹⁴ who also reported a large variability in the Windscale effluent ratio of 38.

The absolute activities of ^{241}Am in Channel water for both cruises are 5–10 times greater than those for Mediterranean surface water contaminated only by fallout. Average activities of about 0.06 fCi l^{-1} have been reported by Fukai *et al.*⁸ and Livingston *et al.*¹⁵. East of the Straits of Dover it becomes difficult to characterise the source of americium in surface seawater although it is clear from the $^{238}\text{Pu}/^{239+240}\text{Pu}$ ratio that plutonium entering the southern North Sea can be seen at least as far as $52^\circ 00' \text{N}$. The significantly higher ^{241}Am activities at stations in the Channel can, however, be attributed to discharges by the French plant.

An indication of a difference in behaviour of americium and plutonium may be deduced if one considers the $^{241}\text{Am}/^{239+240}\text{Pu}$ ratio. If on the basis of Kautsky's¹³ estimated transport velocity of about 1 nm d^{-1} for water moving from Cherbourg to the German Bight, a period of about 0.7 yr is required for activity to reach $52^\circ 00' \text{N}$. The ingrowth of ^{241}Am from ^{241}Pu for a $^{241}\text{Pu}/^{239+240}\text{Pu}$ isotope ratio of 40 would produce, in this time, an *in situ* increase in the measured $^{241}\text{Am}/^{239+240}\text{Pu}$ ratio of about 0.04, supplementing that due to direct input of ^{241}Am from La Hague. Taking expected fallout $^{241}\text{Am}/^{239+240}\text{Pu}$ ratios in surface waters to range between 0.05 and 0.28, no significant differences from fallout for either the Channel or the southern North Sea can be detected for the two cruises. However, the absolute ^{241}Am activities are clearly significantly different from fallout in the Channel, compared with those in the southern North Sea where plutonium activities due to waste discharge are to be seen. Thus the rate of disappearance of americium introduced from fuel reprocessing plants into surface waters is apparently considerably faster than for plutonium. This behaviour has also been reported¹⁶ for americium discharged into the Irish Sea. In the case of fallout for Mediterranean water Livingston *et al.*¹⁵ have also shown that a more rapid loss of ^{241}Am occurs.

Only a small number of samples from the English Channel showed detectable levels of ^{242}Cm and ^{244}Cm . On the basis of a comparison of ratios of $^{242}\text{Cm}/^{239+240}\text{Pu}$ and $^{244}\text{Cm}/^{239+240}\text{Pu}$

Table 3 Activity ratios of actinides in surface waters of the English Channel and southern North Sea

Date	Local station no.	$^{238}\text{Pu}/^{239+240}\text{Pu}$	$^{241}\text{Pu}/^{239+240}\text{Pu}$	$^{241}\text{Am}/^{239+240}\text{Pu}$	$^{242}\text{Cm}/^{239+240}\text{Pu}$	$^{244}\text{Cm}/^{239+240}\text{Pu}$
Feb–March 1975	52	0.19 ± 0.01	34.2 ± 3.1	0.14 ± 0.01	0.71 ± 0.07	–
	53	0.13 ± 0.02	47.0 ± 4.4	–	–	–
	56	0.18 ± 0.03	9.3 ± 2.2	0.03 ± 0.006	0.35 ± 0.05	–
	62	0.11 ± 0.03	27.6 ± 5.9	0.05 ± 0.02	–	–
	63	0.19 ± 0.07	24.9 ± 11.1	0.06 ± 0.02	–	–
	73	$*0.06 \pm 0.03$	26.7 ± 27.0	0.10 ± 0.05	–	–
	1	$*0.06 \pm 0.02$	16.9 ± 15.2	0.05 ± 0.01	–	–
Sept–Oct 1975	30	0.26 ± 0.06	30.5 ± 7.2	0.21 ± 0.03	0.45 ± 0.09	0.02 ± 0.007
	31	0.23 ± 0.04	50.0 ± 9.0	0.13 ± 0.02	0.14 ± 0.03	0.01 ± 0.003
	34	0.14 ± 0.02	40.0 ± 6.2	0.13 ± 0.02	0.18 ± 0.01	–
	22	0.22 ± 0.04	58.6 ± 9.5	0.09 ± 0.02	–	–
	40	0.14 ± 0.02	41.6 ± 8.1	0.13 ± 0.02	–	–

Error, 1σ propagated.

*Ratio estimated for coastal water (ref. 17).

Table 4 Decay of separated ^{242}Cm , sample station 52 February–March 1975

Decay period (d)	^{242}Cm (fCi kg $^{-1}$)	^{241}Am (fCi kg $^{-1}$)
0	7.48 $\pm$ 0.70 (calculated)	1.48 $\pm$ 0.09
206	3.66 $\pm$ 0.47 (measured)	1.48 $\pm$ 0.09
339	1.94 $\pm$ 0.20 (measured)	1.48 $\pm$ 0.09
434	1.34 $\pm$ 0.17 (measured)	1.48 $\pm$ 0.09
628	0.63 $\pm$ 0.12 (measured)	1.48 $\pm$ 0.09
880	0.26 $\pm$ 0.04 (measured)	1.48 $\pm$ 0.09

^{242}Cm , half life = 163 d; ^{241}Am , half-life = 433 yr; $^{242\text{m}}\text{Am}$ = 152 yr. Error, 1 σ propagated.

from fallout of around 0.3×10^{-4} and 0.2×10^{-4} respectively, it is clear that these samples contain curium originating from the French fuel reprocessing plant: ratios of greater than 0.1 and 0.01 respectively were determined. Although ^{244}Cm was not detected during February–March 1975, small amounts were measured during September–October 1975. In the case of ^{242}Cm the decrease in activity was much larger than for the other actinide isotopes; the September–October activities in the Channel being about 20% of those for February–March.

The non-detection of ^{244}Cm during February–March 1975 is remarkable, especially in view of the fairly high ^{242}Cm activities in the Channel, and seems to confirm the variability, both compositionally and quantitatively, of the discharges from the French fuel reprocessing plant.

It is difficult to estimate the degree of actinide penetration eastwards into the southern North Sea. From $^{238}\text{Pu}/^{239+240}\text{Pu}$ ratios it seems that the region about 53° 00' N, 04° 00' E is the furthest point where the source of plutonium is known to be due to waste discharge. The somewhat increased ratios of $^{241}\text{Pu}/^{239+240}\text{Pu}$ in this area compared with those reported for fallout⁶ might indicate the appearance of actinide activity from nuclear fuel reprocessing operations moving slowly eastwards.

Murray and Eicke¹⁷ have shown that although actinide activity levels along the German North Sea Coast in 1975 were mainly due to fallout, over 75% of the measured ^{137}Cs could be attributed to fuel reprocessing plants in both the UK and France. As they point out, base-line values obtained for the German North Sea and Baltic coastal waters may be valuable in showing possible changes in concentrations of actinides in these areas that might occur in the future, as well as helping to identify the source.

An attempt has been made to determine whether ^{242}Cm was supported by $^{242\text{m}}\text{Am}$. The decay activity of ^{242}Cm was measured in sample 52 taken on the February–March 1975 cruise, at intervals over a period of about 3 yr. The results are shown in Table 4. Using the procedure outlined by Dutton and Lovett¹⁸, a regression line calculated by the method of least squares gave a half life of 179 ± 25 d. This compares to a 163-d value for the

pure isotope. The fact that the 1 σ error in the half life includes the standard half life of ^{242}Cm precludes the possibility of determining the existence of $^{242\text{m}}\text{Am}$. However, the establishment of its existence using the method described by Dutton and Lovett¹⁸ would require samples containing very much higher activities (to obtain small measurement errors) than those of the seawater measured in the present case. The possibility remains, nevertheless, that measurement of these samples after a period of more than 10 half lives of ^{242}Cm might reveal some residual activity.

Conclusions

The variations of activity concentrations in the English Channel between March and October 1975 have demonstrated the pulse-like nature of actinide contamination entering the southern North Sea. Comparing actinide ratios in these waters with those expected from fallout, it has been shown that the major input of the isotopes is the French nuclear fuel reprocessing plant at La Hague.

Although plutonium water activities probably only represent a small percentage (<5%) of the total released in low-level wastes, this fraction shows a surprisingly high degree of mobility. Considering the various differences in behaviour of these elements it is clearly necessary to develop methods for the analysis of their chemical forms.

Finally, as the European nuclear generating and reprocessing capacity increases over the next few decades, the level of discharge could also increase. Actinide activities in the North Sea, although at very low concentrations, may reflect this evolution in nuclear technology.

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Nerve fibre topography in the retinal projection to the tectum

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The orderly layout of visual fibres in the optic nerves of cichlid fishes suggests that they are guided most of the way to the brain by contact with their neighbours. Before they reach the visual map in the tectum, however, their arrangement in the cross-section of the nerve is reorganised in a way which requires longer-range guidance.

RETINAL nerve fibres in vertebrate animals grow out of the eye during development, cross the body mid-line, and spread to terminate on the surface of the opposite midbrain tectum. Their synaptic arborisations become laid out in the tectum to form an accurate neurological map of the retina, orientated in rough alignment with the visual field of the eye concerned. Surgical disturbance of the growing visual pathway has little effect on the disposition of the map which optic nerve fibre terminals

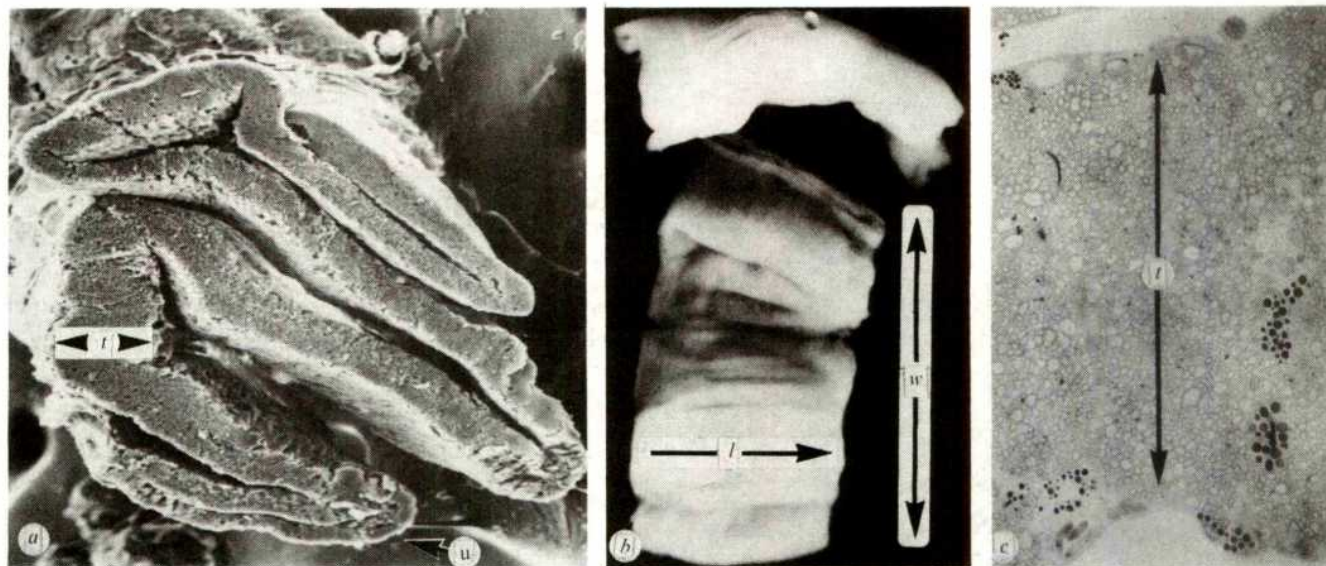


Fig. 1 *a*, Scanning electron micrograph of the cut central stump of the optic nerve in a cichlid fish, *Astronotus ocellatus*. The arrow (u) points to the unmyelinated fibre edge of the ribbon (compare Fig. 2*a*), and *t* is the ribbon thickness, $\sim 100 \mu\text{m}$. *b*, Lengths of the left and right optic nerves from *Astronotus*. Both are desheathed, and the ribbon of one has been spread out flat. The arrow is the direction of fibre growth (*l*), and *w* is the width of the spread ribbon, here $\sim 7 \text{ mm}$. *c*, Transmission electron micrograph of a part of a ribbon optic nerve (*Tilapia* sp.). It comprises myelinated fibres exclusively in this region. Ribbons from different sized fish are roughly equal in thickness ($t = 75 \mu\text{m}$) but vary widely in width to accommodate up to 80,000 myelinated fibres.

establish in the brain, and this finding¹ has suggested that retinal axons are guided into their synaptic array by specific affinities for predetermined loci in the tectal surface¹⁻⁴.

Nevertheless, common sense suggests that mechanical constraints to axonal growth, like neighbour contact and substrate guidance, may also contribute to the normal development of spatial order in the tectal map⁵. How they might do so is easily seen from the normal mode of retinal fibre growth within the eye. The majority of ganglion cells destined to connect with the brain accumulate serially throughout life^{6,7} in retinal tree rings, by cell division around the iris margin of the eye⁸⁻¹⁰. Thus, new optic neurites continually extend inwards from the retinal circumference to the head of the optic nerve, growing alongside their neighbours and over a template surface formed by previously differentiated axons and their satellite cells.

My purpose here is to establish whether neighbour relationships between retinal axons, laid down in the eye^{11,12}, are normally preserved throughout the remaining course of the visual projection to the tectum. To answer this question, and to understand the topological continuity of the visual pathway, I studied an informative optic nerve structure (Fig. 1*a*)^{13,14} found in certain fishes (many of the Cichlidae, and various other percoid fish). Visual axons are laid out in the cross-section of this nerve to form an accurate map of the retina¹⁴. I will show that the map is of a strikingly simple form which suggests that it is shaped by very short-range constraints (such as physical contact) to the growth of single fibres. However, at a definite point along the length of the optic nerve, almost at its central end, the map becomes topologically re-ordered in a manner which involves systematic neighbour exchanges between fibres, and therefore can only be mediated by some longer-range mechanism of guidance. I will argue that this re-ordering—the only fibre manoeuvre in the normal projection which manifestly requires long-range guidance—must also be a general feature of other lower vertebrate retinotectal pathways. Moreover, both the nature of the re-ordering, and the fact that, as discussed later, it is apparently completed before fibres arrive at the tectum, seem to preclude that its mechanism involves adhesive axonal recognition^{1,4,15} of loci within the tectal surface.

Retinotopic maps in ribbon optic nerves

Figure 1*a* shows the cut central stump of the optic nerve in a cichlid fish. Within a cylindrical outline, it contains a folded

internal ribbon of nerve fibres¹⁴, easily flattened out into a continuous sheet (Fig. 1*b*). Apart from one edge, whose relatively small cross-sectional area is filled by numerous (up to 25% of the total) closely packed fine unmyelinated fibres, the ribbon comprises myelinated axons only (Fig. 1*c*). The myelinated fibres are laid out in the cross-section of the ribbon to form an accurate map of the retinal surface, but one whose topology is not directly related to the circular outline of the eye. Instead, as shown below, the polar coordinates of the retina become laid out orthogonally in a Cartesian fibre map which is bounded by the rectangular (though folded) cross-section of the ribbon.

Figure 2*a* shows the layout, in the nerve, of (degenerating) axons from a dorsal sector of the retina, distributed as a thin continuous sheet¹⁴ throughout most of the width (*w*) of the ribbon, and accurately located halfway through its thickness (*t*). These degenerating fibres were severed inside the eye by a small cut through the retina, close to the optic disk, and parallel to the circumference of the eye, which interrupted a sector-shaped beam of peripheral dorsal retinal axons. Retinal lesions of this sort always produced sheet-like degeneration patterns extending from the unmyelinated edge of the ribbon through some fraction of its width directly related to the area of retinal surface whose fibres were severed. Thus, the *w* axis of the ribbon maps the retinal radius, with equal increments (Δw) representing retinal annuli of roughly equal area. The myelinated edge of the ribbon is the origin (w_0), representing the centre of the retina. This suggests that the unmyelinated fibres, numerous though they are, represent a queue waiting for consolidation into the extended myelinated ribbon of an older fish. In agreement, their degeneration patterns (detectable only by electron microscopy) extend those in the rest of the ribbon, and they comprise, as growth proceeds, a diminishing fraction of all axons in the nerve. If it is assumed that the rate of addition of new retinal cells during growth is roughly constant^{1,2}, then equal increments to the width of the myelinated part of the ribbon (for example, Δw in Fig. 2, and see below) represent equal growth periods.

Conversely, and contrary to an earlier analysis¹⁴, radial sectors of the retina are mapped in circumferential order, across the thickness (*t*) of the ribbon. The ribbon's two surfaces, boundaries for this linear representation of the eye circumference, correspond with a radial and ventral discontinuity in the otherwise closed outline of the retinal disk. This is the embryonic choroid fissure¹², which persists throughout adult life in

fishes with ribbon nerves. Axons from retinal cells along each edge of the fissure are guided to run along one or other surface of the ribbon (Fig. 2b).

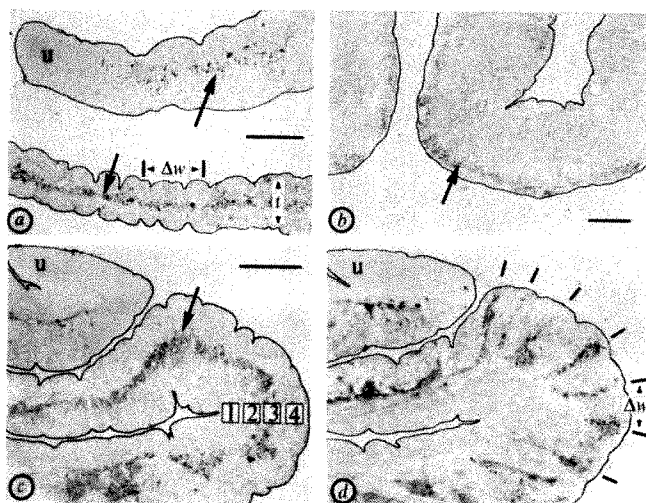


Fig. 2 Transverse sections of ribbon nerves, showing osmiophilic myelin degeneration patterns which result when populations of axons from narrow sectors of the retina are severed close to the optic disk. The sectors were mapped in animals killed after 3–4 weeks survival. Primary fixation was with aldehydes in 0.3M buffer with added divalent cations, followed by OsO_4 then epoxy embedment. The light micrographs are of unstained 2–5- μm sections, with the ribbon outlines emphasised by pen. Parts *a* and *b* are before the chiasm, and *c* and *d* beyond it. *a*, Right eye ribbon from the cichlid *Aequidens maronii* after severing a 30° sector of dorsal retinal fibres (compare positions 2–3 in Fig. 3). Degeneration debris (arrows) forms a sheet halfway through the thickness (*t*) of the ribbon, which could only be followed into the unmyelinated region (*u*) by electron microscopy at shorter survival times. Δw matches part *d* of this figure, and the scale bar is 50 μm . *b*, Similar ribbon (*Tilapia* sp.) where a retinal lesion interrupted fibres from the temporal edge of the choroid fissure (position 4, Fig. 3). Myelin debris is located along the corresponding surface of the ribbon. Scale bar, 50 μm . *c*, Part of the same ribbon as in *a*, cut further upstream almost at the left tectum, and viewed in the direction of fibre growth. Because the nerve has now wrapped around the ventral and contralateral surface of the brain, the unmyelinated edge of the ribbon (*u*) faces dorsolaterally (upwards here) rather than ventrally as at the exit from the eye (compare Fig. 3). Degenerating fibres still form a sheet. The numbered boxes define the position in the nerve of the fibre strips depicted in Fig. 4, and refer also to Fig. 3. Scale bar, 50 μm . *d*, The same ribbon approximately 70 μm closer to the tectum, at a location intermediate between the two numbered levels of the projection depicted in Fig. 4. The sheet of fibre debris has separated into blocks, one to each ribbon cross-lamina (lines), which are exchanging position with fibres at the lateral surface of the ribbon. Δw contains the same number of fibres as in *a*, showing the relationship between the fibre map grain and the size of the cross-laminae.

Changes in the outline of the fibre map

The extension of the ribbon, which I define as the ratio of width to thickness (w/t), varies along the length (l , Fig. 1*b*) of the optic nerve, having its smallest value ($w/t \approx 5$) where retinal axons leave the eye through the apex of the choroid fissure as a broad vertically orientated strap, not yet folded up. Extension rises to remarkable values ($w/t \approx 200$; $w > l$) in older fish as the ribbon folds up shortly behind the orbit. It condenses again to between 10 and 20 where the optic nerves cross (bodily) at the mid-line and approach the contralateral tecta. But retinal fibres remain in constant positions relative to w and t throughout, almost to the tectum, so these changes of outline are fluid deformations, only minimally affecting the local neighbour relationships of fibres within the ribbon (compare Fig. 2*a* and *c*).

In principle, the more radial transformation between circular retinal topology and the layout of axons within the nerve could arise by a process of fluid deformation similar to that involved in ribbon extension and condensation. This is most easily seen in the following way. Imagine the retinal disk is a fan, opened through 360° so that its edges meet at the position of the ventral choroid fissure. Now picture closing the fan. This manipulation compresses retinal points into an internal order similar to that assumed by corresponding fibres in the ribbon. If allowance is made for the fact that the ribbon maps the retinal radius as r^2 rather than linearly, the description below, summarised in Fig. 3, shows that axons enter the optic nerve in a projection pattern broadly isomorphous with this model transformation, which resembles a conformal mapping¹⁶.

The retinal projection into the optic nerve

In retinal whole mounts, axons of ganglion cells recently added in the periphery are seen clearly to run over the outer, vitread, surface of older established fibres, and they converge radially upon the most ventral edge of the ribbon as it leaves the eye. The more centrally the fibres originate, the more deeply they are stratified in the layer of retinal axons and the more their course is directed towards the dorsal apex of the slit-shaped optic disk. These points, true also in embryonic chick retina¹¹, are shown schematically in Fig. 3. The diagram shows how retinal fibres project into the ribbon outline without changing their local neighbour relationships; to verify that this is so, account must be taken of fibres of retinal eccentricities between the two extremes depicted (*c* and *p*).

This interpretation suggests how retinotopic order in the nerve could be generated by rather minimal physical constraints to the growth of new fibres extending from the retinal margin. Sufficient growth rules could be that individual neurites, (1) can only extend over a substrate formed by their predecessors in differentiation, and (2) cannot freely exchange neighbour relationships with others growing at the same time.

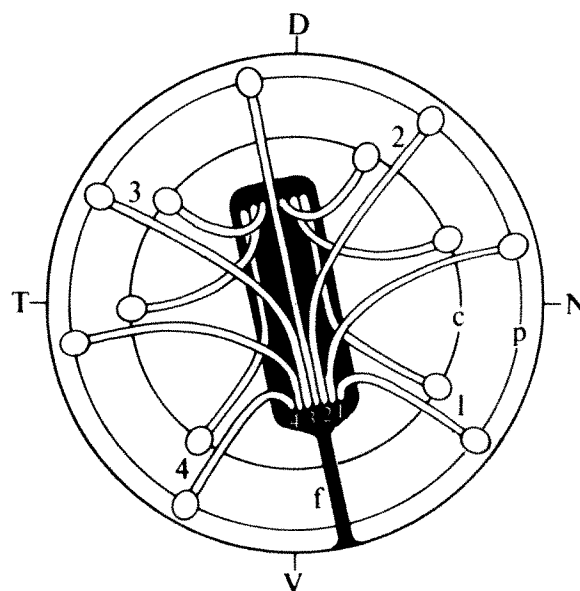


Fig. 3 A schematic picture of how fibres from an extreme peripheral annulus of retinal ganglion cells (*p*) and an extreme central one (*c*) project into the cross-section of the ribbon nerve as it leaves the right eye (viewed as if through the pupil), yet to extend and fold into the outline shown in Fig. 1*a*. Along each retinal radius, axons originating between these two extremes are ordered in the depth of the fibre layer according to eccentricity and take intermediate courses to the head of the nerve. Nasal, dorsal, temporal and ventral retinal quadrants are N, D, T and V respectively, and *f* is the choroid fissure. Numbered circumferential positions refer to Figs 2*c* and 4.

Mature patterns of axonal fasciculation in the cichlid retina and nerve, as elsewhere^{11,17}, involve frequent local exchanges between nearby fibres. If these exchanges were cumulative, it is arguable that any nascent retinotopic order should become scrambled before axons reach the brain, but degeneration patterns in the nerve show no signs of diffusing. Fasciculation patterns must, therefore, represent entirely local segregations of fibres into bundles—a kind of random bracketing which could well be mediated by glial ensheathment and continued tissue growth.

How fibres leave the ribbon

Cartesian retinotopic order persists throughout almost the entire length of the ribbon (Fig. 2a, c), to the point where its outlines begin to merge into the marginal fibre brachia of the contralateral tectum. This prompted the question whether considerations shaping the form of the fibre map also play a part in the accurate assembly of optic axon terminals into the visual map on the tectum itself. First of all, does the fibre map in the ribbon unfold again conformally like a fan, during radiation onto the tectum, as it became folded (Fig. 3) at the optic nerve head? The short answer is no.

Just before (150–300 μm) the folded outline of the ribbon coalesces into the tectal brachia, its entire width (w) sub-divides into a series of cross-laminae, each comprising a few hundred fibres. The laminae have very sharp boundaries, visible in thick sections of normal nerves as shearing planes which separate countercurrents of fibres running extremely obliquely in opposite directions across the thickness (t) of the ribbon. Within laminae, however, fibres change their positions in the ribbon by more fluid movements, for the most part preserving neighbour relationships.

Particularly for axons at the lamina boundaries, these manoeuvres abruptly disrupt neighbour relationships between fibres, otherwise preserved throughout the entire retinotectal projection. Their major effect is to re-order, piecewise, the fibre map of the eye circumference laid out across the t axis of the ribbon. The movements are illustrated in Fig. 2c and d, showing how degenerating fibres from a dorsal retinal lesion, first ordered in a continuous sheet (Fig. 2a, c), separate into blocks and move onto the lateral surface of the tract (Fig. 2d). Figure 4, compiled from similar data for other retinal sectors, shows schematically how all fibres within a single cross-lamina (representing a retinal annulus) become re-ordered by these exchanges, which were identified many years ago in the roach¹⁸.

Because axons in each lamina of the ribbon move independently in the same way, the outcome is to reverse the handedness of the entire fibre map, and to re-allocate its boundaries to a new point on the eye circumference. Handedness here means the direction, relative to the growth axis (w), in which the eye circumference is mapped across the thickness of the ribbon (t). To specify handedness, a viewpoint must be defined, and the natural one, adopted in Figs 2 and 4, is the direction along which fibres grow and conduct impulses. As visual fibres originate on the outer surface of the retina, this convention dictates that the layout of ganglion cells be viewed, as in the keys to Fig. 4, from behind the eye. Thus, Fig. 4 shows that, in an otherwise continuous projection, a coarse-grained reversal of map handedness at the cross-laminae enables fibres to proceed to their tectal loci without further exchange of their neighbour relationships.

Irrespective of retinal eccentricity, established myelinated fibres complete their re-ordering together within a very short distance (for example, 200 μm) compared with the pathway length (for example, 1 cm) accrued by bodily growth since the first embryonic fibres arrived at the tectum. Thus, although it was not possible to trace, by degeneration methods, the small minority of unmyelinated fibres engaged in growth at any instant, extrapolation suggests they also re-order at the same level.

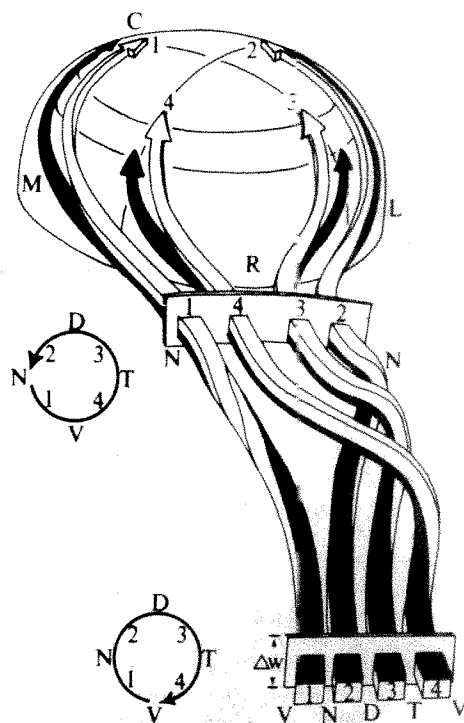


Fig. 4 Schematic interpretation of how retinal axons within a single cross-lamina of the ribbon nerve become internally re-ordered as they approach the rostral (R) pole of the left tectum. The strips represent blocks of axons from different sectors of an annulus of ganglion cells in the right retina, numbered and labelled as in Fig. 3. Their starting configuration corresponds with the numbered blocks superimposed on the micrograph of Fig. 2c. Fibre populations in each cross-lamina of the ribbon (compare Fig. 2d) re-order independently and in the same way. Each maps an increment (Δw , Fig. 2a and d) of the retinal radius. Accordingly, the black surfaces of the strips are fibres most recently added to the cross-lamina, depicted rather conjecturally as they could not be traced fully. The course of the strips onto the tectal map is consistent with the fibre radiation pattern seen in whole mounts, although the twist handedness depicted for strip 1 is arbitrarily chosen. The keys on the left are back-projections of the lamina to its origin in the right eye (shown as if viewed from behind) to show how re-ordering reverses the circumferential sequence (arrows) of the fibre map, and re-allocates its boundaries (gaps) to a nasal position. M, C, L, and R are medial, caudal, lateral and rostral poles of the tectum respectively; other labels as in Figs 2 and 3.

Other retinotectal projections

It is important to realise that the cross-laminar fibre re-ordering in the cichlid ribbon is not related to any basic mismatch of handedness between retinal image and tectal map. They are, as in other lower vertebrates, superimposable when properly viewed from a single frame of reference, for example the standpoint of axonal growth. It relates instead to a general mismatch between the orientation of the tectal map and the direction of optic tract approach. This is best understood from Fig. 4 by noting that the positional exchanges depicted would be unnecessary were the pathway to approach the tectum medially rather than rostrally.

Because the mismatch is a consequence of the relative layout of the tract and the tectum, it must be a general characteristic of other lower vertebrate retinotectal pathways, which the cichlid projection resembles in all particulars except the unusual ribbon structure of its optic nerve. Data in the literature make it possible to state this argument more explicitly. In amphibia, the axis along which new fibres accrue to the optic tract²¹, and the direction through which the tract approaches the tectum, are the same as in the cichlid pathway at a comparable level. Moreover, orthogonal to the growth axis of the optic tract, amphibian visual fibres are laid out in a retinal circumferential sequence (NVTDN, Fig. 4)²² matching that into which cichlid axons

emerge from the ribbon cross-laminae (Fig. 4), similarly bounded and of the same handedness. As the arrangement is of opposite handedness to the layout of retinal fibres as they originate in the eye (Fig. 4, keys), some reorganisation of the fibre map similar to that achieved in the cichlid cross-laminae must already have been mediated before the amphibian optic tract emerges onto the lateral wall of the diencephalon, possibly as far downstream as at the optic chiasm. Indeed fibre interdigitation patterns in fused chiasms have a certain resemblance of appearance to the cross-laminae located further upstream in the visual pathway of cichlids, whose optic nerves, as in fish generally, cross bodily at the mid-line.

Conclusions

It is hazardous to infer prior developmental mechanisms from differentiated structures. Within their limits, however, these observations allow certain useful conclusions. First, an extremely short-range mechanism, possibly no more sophisticated than physical contact between adjacent retinal axons, is evidently consequential enough to determine the layout of optic nerve fibres throughout most of their course to the tectum, albeit a layout incompatible with that which their axon terminals must adopt in the tectal map. Second, the topological re-ordering necessary for axons to reach their correct terminal sites in the tectum involves neighbour exchanges and must, therefore, be

guided by longer-range constraints to fibre growth. This re-ordering, which I have argued must be a general feature of lower vertebrate retinotectal projections, seems to be mediated, particularly in Amphibia, some distance before growing fibres reach the tectum. It is unlikely, therefore, to be the outcome of exploratory axonal growth towards putative neuronal recognition sites within this tissue. Finally, the piecemeal nature of the re-ordering means that the number of retinal fibres which exchange their neighbours is, in fact, a small fraction of the total. It can be seen, therefore, that the mechanism which guides re-ordering of the fibre map needs in principle only to act on a fraction of the fibres which are involved in the movements; the rest might follow by neighbour guidance.

Current understanding of the ontogeny of the retinotectal projection is that the accretion of new retinal fibres is a continuous process^{7,23,24}. One urgent question raised by the findings described here is how the continual growth of the pathway is punctuated to enable the large blocks of fibres, comprising single cross-laminae (Δw , Figs 2 and 4), to reorganise as independent units.

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letters

The accuracy of predicted radioastronomical frequencies and the spectrum of hydroxyl

IF radioastronomers are to use predicted frequencies to search for new interstellar species, they require some fairly precise indication of the accuracy of the theoretical calculations. Calculations of rotational constants are not very accurate by the standards of radiofrequency spectroscopy, but computations of Λ -doublet splittings have seemed remarkably accurate¹⁻³. However, these calculations were presented without error limits and as it is not possible to produce these from pure theory we have used the hydroxyl radical to probe the limits of accuracy. The experimental frequencies are known from observations^{4,5} of the interstellar maser action in OH to a high precision, and it is a particularly suitable case for computation. We show here that the prediction for the lowest rotational level is accurate to 1 MHz; an accuracy higher than conventional terrestrial experimental spectroscopy.

By considering Λ -doubling of rotational levels of $^2\Pi$ states to be caused by second order perturbations, Van Vleck⁶ showed

that the splitting may be expressed in terms of the parameters $(\frac{1}{2}p+q)$ and q :

$$\Delta\nu = -(\frac{1}{2}p+q)[1 \pm (2-Y)/X](J+\frac{1}{2}) \mp (2q/X)(J+3/2)(J+\frac{1}{2})(J-\frac{1}{2})$$

Here the upper signs refer to F_2 sublevels and the lower to F_1 ;

$$X^2 = Y(Y-4) + 4(J+\frac{1}{2})^2 \quad \text{and} \quad Y = A_v/B_v$$

The parameters p and q depend on integrals over vibronic wave functions of the operators $\hat{B}(\hat{L}^+ + \hat{L}^-)$ and $\hat{H}_{so}$. These matrix elements are factorised into vibrational and electronic integrals so that the contribution of a single $^2\Sigma^+$ electronic state to the splitting in the v th vibrational level of a $^2\Pi$ state is given by

$$p_v = 4 \langle ^2\Pi | \hat{H}_{so} | ^2\Sigma \rangle \langle ^2\Pi | \hat{B}(\hat{L}^+ + \hat{L}^-) | ^2\Sigma \rangle \times \sum_v \frac{\langle v | \hat{B} | v \rangle}{E_{v,v} - E_{\Sigma,v}}$$

and

$$q_v = 2 \langle ^2\Pi | \hat{B}(\hat{L}^+ + \hat{L}^-) | ^2\Sigma \rangle^2 \sum_v \frac{\langle v | \hat{B} | v \rangle^2}{E_{\Pi,v} - E_{\Sigma,v}}$$

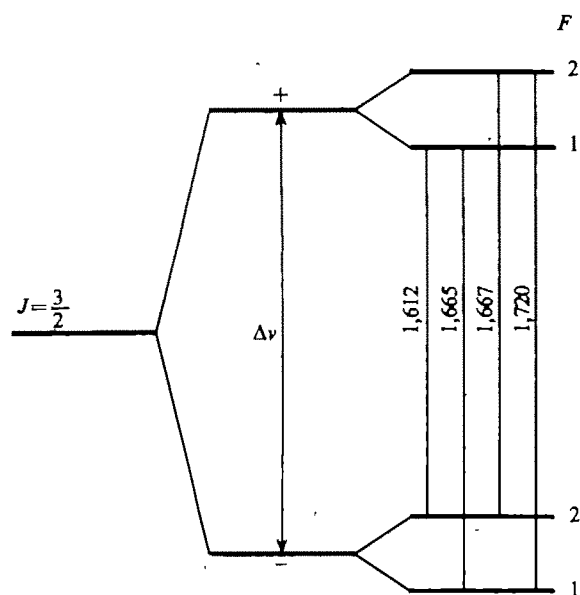


Fig. 1 Λ -doubling ($\Delta\nu$) in the lowest rotational level of OH ($^2\Pi_{1/2}$; $J=3/2$) showing the observed radioastronomical transitions in MHz (not to scale).

where the summation should be taken over all vibrational levels, v' . Contributions from all $^2\Sigma$ states are then added with $^2\Sigma^-$ states giving negative terms.

An earlier study¹ calculated p and q for OH assuming that molecular orbitals were invariant between electronic states and restricted summations by assuming Van Vleck's case of pure precession. Here we have calculated separate wave functions for $X^2\Pi$ and $A^2\Sigma^+$ using Cade and Huo's atomic orbital basis set⁷ in the ALCHEMY program⁸. The electronic functions were then improved by configuration interaction (CI) using 15 Π configurations and 11 of Σ symmetry. This was done in stages to ensure that a limit had been achieved; further additions did not change the computed values of p and q .

Morse functions were used for vibrational wave functions after showing that these give identical results to those obtained with numerical solutions from RKR curves. The vibrational summations over v' extend to $v'=5$; terms for $v'=5$ and above do not contribute significantly.

The effects of $B^2\Sigma^+$ were calculated to be less than those of $A^2\Sigma^+$ by a factor of about 10^9 , indicating how good Van Vleck's pure precession approximation is in this case.

Figure 1 indicates the observed astronomical transitions with epicentres separated ($\Delta\nu$) by 1,666 MHz. Table 1 shows calculations of p and q and of this separation.

This lowest rotational level is the one of prime importance astrophysically and the agreement between theory and experiment is essentially exact, being better than 1 MHz. However, the splitting in this particular level depends mostly on the value of the constant q . A slightly more difficult case for theory is provided by the manifold excited rotational states, where p as well as q has an influence and errors are magnified by multiplication of the constants by higher powers of the rotational quantum number.

Table 1 Λ -doubling in the $X^2\Pi$ ($v=0$; $J=3/2$) state of OH

	p (cm^{-1})	q (cm^{-1})	Splitting (MHz)
Hinkley <i>et al.</i>	0.242	-0.0391	
This work without CI	0.2308	-0.03807	
This work with CI	0.2378	-0.03982	1,666
Terrestrial expt	0.2348	-0.0387	1,654
Astronomical expt			1,666

For $J=1/2$ of $^2\Pi_{1/2}$ the separation of epicentres of the observed astronomical lines is 4,730 MHz, for which our calculations predict, 4,795 MHz; $J=5/2$ of $^2\Pi_{3/2}$ has a terrestrially measured separation of 6,033 MHz and we calculate 5,946 MHz, whereas the even more highly excited level $J=5/2$ of $^2\Pi_{1/2}$ has an epicentre separation of 8,153 MHz and our result is 8,444 MHz.

These results suggest that the realistic limits for our calculations, if done with care, are a few megahertz. This is better than conventional spectroscopy. We intend to recompute the Λ -splitting in SiH to this accuracy so that calculations of this type can be used to predict as yet unobserved interstellar molecules and ions.

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A nonspherical thermal instability in young stars

NONSPHERICAL thermal instabilities have been shown¹⁻⁴ not to be excited during the hydrogen-burning phase of stellar evolution. However, this conclusion holds true only for models with a homogeneous distribution of heavy elements. We show here that in nonhomogeneous young stars more massive than, or of the same mass as, the Sun, large-scale nonspherical thermal instability can be excited during the short period of time when carbon just starts capturing protons but before convection has set in.

The non-homogeneous structure is expected to form in the case of a dusty protocloud. In this case gravitation can collect the dust at the centre of gravity of the cloud^{5,6} or the dust can fall into the centre during cloud contraction⁷. We assume here that, although heavy elements are concentrated near the centre, their mass fraction (Z) is small everywhere and Z is proportional to Z_{12} , the mass fraction of carbon. Convective mixing of the central region before the ignition of nuclear reactions is assumed not to occur. This is true if the mass of the star is sufficiently large⁸⁻¹⁰. For simplicity, effects due to rotation and radiative pressure are ignored in the following.

For thermal instability to occur ϵ , the rate of nuclear energy generation, must be highly sensitive to T , the temperature. We take ϵ to be proportional to $\rho X Z_{12} T^\nu$, where ν is assumed to be constant. The dependence of ϵ on Z_{12} makes this sensitivity greatly enhanced in our case of a small Z , that is, of large h_μ/h_Z , where μ is the molecular weight, while scale height $h_\mu = -(\partial \ln \mu / \partial r)^{-1}$, and so on. (Here $h_\mu > 0$, and $h_Z > 0$, but h_X is likely to be negative.) In other words, for this rapidly growing instability any displacement leads to a change in mass fraction of heavy elements which is much larger than, and of the same sign as, the change in molecular weight. Furthermore, the excitation of the nonspherical thermal instability leads to negligibly small distortions in pressure and density distributions, we find¹⁻⁴ that

$\mu^*/\mu = T^*/T$, that is, the relative eulerian perturbations of molecular weight and temperature are equal. Thus, provided that the time of instability growth is much shorter than the timescale of change of the unperturbed energy generation rate, we find that a large increase (decrease) in this rate corresponds to a small increase (decrease) in the temperature. This conclusion follows from an equation^{2,4} generalised to include variations in $Z_{12} = \text{const}$. Z :

$$\varepsilon^* T (\varepsilon T^*)^{-1} = \nu_{\text{eff}} = \nu + h_\mu (h_Z^{-1} + h_X^{-1})$$

As $h_X \approx h_\mu$ and $h_\mu \gg h_Z$, we have $\nu_{\text{eff}} \approx h_\mu/h_Z$. Usually, $\nu \approx 15$ – 20 , while Richstone's⁴ estimate shows that some mean value of $\nu_{\text{eff}} \approx 20$ makes the nonspherical thermal instability likely. If Z equals a few per cent then $\nu_{\text{eff}} \approx 100$. Such a value of ν_{eff} is so large that the probability of instability excitation is high even though, in our contracting model, most of the energy liberated is due to the contraction of the model. This instability excitation certainly occurs in models with a sufficiently large mean value of ν_{eff} , in which case the perturbed opacity can also produce a destabilising effect. For the most rapidly growing perturbation, the point of the highest temperature is displaced while the distributions of pressure and density remain almost unaltered.

Here we are considering the phase of evolution before the formation of the convective core; this means that f , the radial component of unperturbed entropy gradient, decreases and tends to zero. Therefore, rapid motions should be excited to ensure the necessary change in entropy to fulfil the energy balance in the perturbed state, which is characterised by almost the same entropy distribution as the unperturbed state. In other words, the time of growth can be very short in the model under consideration^{2,4}. The minimum value of the time of growth (τ_{min}) is determined by when perturbations of pressure and density fields become significant. This gives $\tau_{\text{min}} \approx (r^2 \varepsilon^{-1} h_p h_Z h_*^{-2})^{1/3}$, where $h_* = |\nabla \ln p^*|^{-1}$. It is easy to take the time dependence of τ into account if f can be modelled by a linear function of time.

Detailed computation is necessary to find that mean value of h_μ/h_Z above which a rapidly growing instability is really excited in the model chosen. When $\nu_{\text{eff}} \approx 100$, the instability apparently does exist in many models. Using Iben's¹¹ models of contracting stars we find that time τ_{min} is usually of the order of 0.1 yr. As the molecular weight of each moving elementary volume is conserved, we expect, as a result of the instability, that large-scale relative spatial variations $T^*/T \sim \mu^*/\mu \sim h_p/h_\mu$ are formed, whereas $v_r^* \sim h_*/\tau$, and $p^* \leq r^2 \rho/\tau^2$; for a $3M_\odot$ model, $v_r^* \sim 10^4 \text{ cm s}^{-1}$, and $p^*/p \leq 10^{-7}$, the nonspherical motions produced by the instability are large enough to transfer all the heat produced by the energy source. Also, these motions can effectively amplify the magnetic fields which are expected to be present in young stars. A field-induced instability followed by emergence of frozen-in fields on the surface can be responsible for the erratic activity usually observed in young stars.

Considering outbursts in FU Ori type stars¹², an interesting model would be one undergoing recurrent phenomena involving a temporal increase of the energy generation rate by more than two orders of magnitude on a scale of some months. If this increase were caused by the nonspherical thermal instability excited at the moment when the equilibrium is nearly adiabatic, then the time of growth can be as short as a month. However, to ensure that ε increases we have to accept that the instability can lead to the formation of a nonspherical structure containing points at which the temperature is up to 1.5 times higher than the unperturbed temperature. In this case the unperturbed model must have a large central concentration of heavy elements. More thorough study is needed to clarify whether such a model can be unstable. If the instability is possible, and its excitation produces large variations in the temperature field, the pressure field will also be disturbed. In such circumstances local convection is likely in some regions with the convective heat flux depending on the horizontal coordinate. If periods of liberation of considerable energy are followed by the energy source zone

expanding slightly at the same time as local convection reduces due to the horizontal temperature differences, then the contraction phase which was terminated by the instability excitation can resume. In this rather speculative sequence of recurrent outbursts, the local convection (perhaps coupled with the magnetic field induced instability) is supposed to be effective in transporting heat but not in causing full mixing of the energy source zone.

Nuclear reactions seem to maintain and amplify the horizontal temperature variations of the type in question¹³. That is because the ratio T/μ remains spherically symmetric, the temperature and hence the nuclear burning rate are higher within those regions where the burning element is more exhausted and, hence, the molecular weight is larger. Thus, we cannot exclude the possibility that the structure with the horizontal temperature variations can survive the stage of hydrogen burning at least in models not having convective cores, or in models where the convection is modified (in the presence, for example, of a magnetic field) to such an extent that the convective mixing does not destroy the horizontal variations mentioned. I have suggested elsewhere that a similar state is realised in magnetic stars¹³, in which case, the dimensions of the model seem to be larger and, hence, the rotation to be slower. These facts apparently agree with the observational data¹⁴.

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Drifting spikes—a new species of solar radio bursts

OBSERVATIONS of solar radio bursts of spectral type IV have revealed the presence of a complicated fine structure in the emission at metric wavelengths^{1–4}. It would be interesting to extend high resolution spectral observations to decimetric wavelengths to explore the structure of radiation which is generated at lower heights in the solar atmosphere, and is, therefore, closer to the site of the associated optical flare. We report here a new species of fine structure bursts, drifting spikes, which have been observed in the 500 MHz frequency region.

A radio spectrograph covering frequencies $490 \leq f \leq 550$ MHz has been in operation at the Oslo Solar Observatory since 1972. The instrument sweeps across the observed band at a rate of 50 times per s, and has a frequency resolving power of 0.3 MHz.

Drifting spikes were recorded on 18 September 1974, 11.14–11.17 UT, 23 September 1978, 10.14–10.18 UT and 27

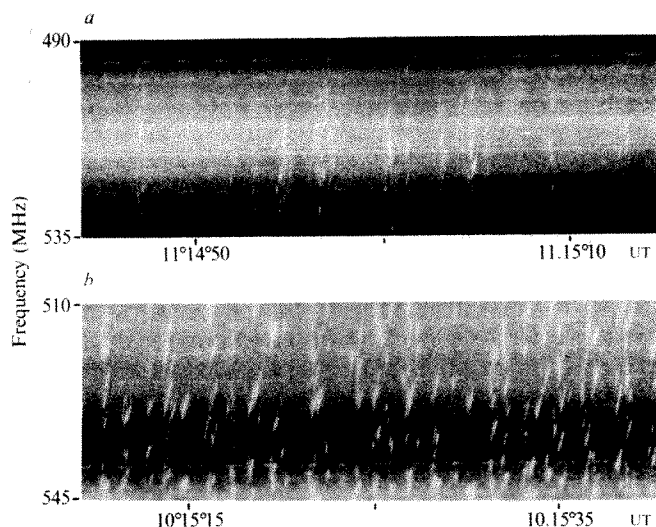


Fig. 1 Dynamic spectra showing drifting spikes observed at: *a*, 18 September 1974; *b*, 23 September 1978. Time marks, each lasting for 1 s, are seen as weak horizontal dashes. They occur at frequencies separated by 5 MHz.

September 1978, 14.45–14.47 UT. In all cases type IV bursts were in progress and flares of importances 2N, 2B and 2B showing two-ribbon structure were observed in H α at the respective times.

Simultaneous observations with radio spectrographs in the bands 140–170 MHz and 310–340 MHz revealed no drifting spikes. But bursts of this type were present on records obtained with the Dwingeloo radio spectrograph on 23 September 1978. The instrument was then tuned to cover the bands 509–533 MHz, 574–614 MHz and 655–666 MHz. The phenomenon may thus be typical at decimetric wavelengths. It is not known whether drifting spikes appear at even shorter wavelengths.

Two examples of drifting spikes are given in Fig. 1 which shows that a large number of tiny bursts are superimposed on a smooth continuum emission. Each elementary burst has a short duration, narrow bandwidth and a frequency drift from higher to lower frequencies.

Several parameters typical of individual bursts were measured on the record from 23 September 1978, which contained the

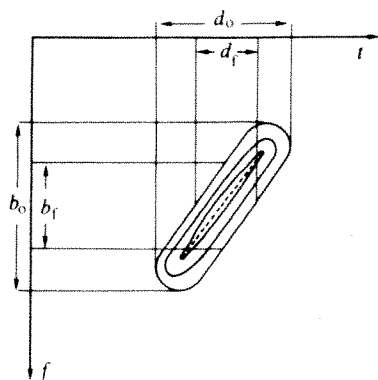


Fig. 2 The definition of some relevant burst parameters in the time-frequency plane: b_i , instantaneous bandwidth; b_o , frequency range (total range of frequency occupied by the burst at all times); d_f , duration at one frequency; d_o , lifetime (total duration of the burst at all frequencies). The slope of the burst is indicated by a dotted line, from which the frequency drift rate, df/dr , is determined.

most spectacular event. (Denotations in the following are as in Fig. 2.) The frequency range covered by single bursts (b_o) was small, the average value being 2.7 MHz. It was difficult to determine the instantaneous bandwidth b_i with good precision. Only an upper limit, $b_i \leq 1.5$ MHz can be determined. Thus drifting spikes are very monochromatic, $b_o/f = 0.5\%$ and $b_i/f \leq 0.3\%$.

For the lifetime, d_o , the average value was 0.12 s. Another quantity of interest is the duration at one frequency (d_f), but as most bursts were not adequately resolved with our sweep repetition rate of 50 Hz, it is concluded that $d_f \leq 50$ ms.

All spikes showed a frequency drift. The average value of df/dr was -23 MHz s^{-1} , giving $(1/f) df/dr = -0.04 \text{ s}^{-1}$. This is larger than, but of the same order of magnitude as, that found for intermediate drift bursts (fibre bursts) in the same frequency range. The scatter in burst slopes was surprisingly small, and positive drifts were not seen.

Drifting spikes frequently appeared as pairs at roughly the same frequency with the elements separated in time by 0.15 s. There was also a second and very distinctive type of ordering of the bursts. On the records they formed columns because drifting spikes were generated almost simultaneously at many frequencies separated by intervals of 5–10 MHz. This was demonstrated even more clearly by a comparison between our records and those obtained with the radio spectrograph at Dwingeloo. The effect is similar to that seen when stria bursts are ordered into decametric-type IIIb bursts.

Another important property was a regular repetition of the columns of drifting spikes. This is seen in Fig. 1b. The drifting spikes are generated at time intervals which have an average value of 1.2 s. It is interesting that before the occurrence of drifting spikes, the continuum intensity showed oscillations (pulsating structure).

If the emission frequency is equal to, or near to the plasma frequency, the occurrence of drifting spikes between 500 and 666 MHz implies electron densities $N_e = 3\text{--}5.5 \times 10^9 \text{ cm}^{-3}$ at the place of generation. New observations at higher frequencies may lead to an increase of the upper limit. The quoted electron densities correspond to heights below 50,000 km in active regions. Single frequency durations $d_f \leq 50$ ms are of the same order of magnitude as the electron collision time in these regions.

The phenomenon described here is of particular interest in connection with other observations of decimetric and centimetric fine structure^{5–7} and a theoretical discussion of mechanisms for the production of millisecond bursts. A possible quasi-periodic generation of such bursts by plasma turbulence in flare regions has been envisaged⁸.

Further explorations of the finest structures in solar radio bursts at short wavelengths may lead to the detection of basic, small scale acceleration processes in solar flare regions.

A comprehensive analysis of drifting spikes will be given elsewhere.

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Detection of circularly polarised light from noctilucent clouds

NOCTILUCENT clouds are very tenuous clouds that appear in the high latitude mesosphere during the summer¹. They are seen by scattering of sunlight from cloud particles near the mesopause. They are known to be at altitudes of ~82 km, more than 50 km higher than the next highest clouds in the atmosphere, the nacreous or mother-of-pearl clouds. They are usually seen after midnight in June and July in the north-east twilight arch at latitudes of 55–65° and appear silvery-blue² in the red twilight glow. Observations have been made from ground level^{3,4}, rocket⁵ and satellites^{6,7}. Studies of sunlight scattered from the clouds have concentrated on measurements of polarisation of the scattered light to allow estimates of the size distribution of the cloud particles to be made. These estimates agree in that only submicron particles with an upper radius limit in the range 0.05–0.3 μm are present. Recent measurements⁴ of scattered light have shown the presence of a small proportion of circular polarisation, indicating that the cloud particles are probably non-spherical, and estimates of the particle size, being based on the assumption that scattering is from homogeneous spheres (Mie scattering), may need revision. The revision might be enough to force reappraisal of the amount of water condensed to form the clouds and consequent reappraisal of the relative humidity in this part of the atmosphere. A small amount of circular polarisation can be produced in the scattering of natural (unpolarised) light from elongated particles⁸ but a greater proportion of circular polarisation will arise if the incident light is partly polarised and if the axes of the elongated particles show some directional preference. However, light scattered from spherical particles will also show some small amount of circular polarisation, if the incident light were to be linearly polarised in a direction skewed with respect to the plane of scattering. If the circular polarisation is produced by elongated cloud particles, it is possible that polarisation of the scattered light will show marked differences from place to place due to different proportions and directions of alignment of particles being caused by

updraughts or vertical wind shears present in different parts of the cloud region. This would not be expected if the circular polarisation was due to scattering of linearly polarised light from spherical particles. Apart from early photographic observations³, recent measurements of the polarisation in noctilucent clouds have been photo-electric and there has been no simultaneous measurement at different points spread over an appreciable part of the cloud display. We have developed and report here a method which allows us to test how circular polarisation varies over the area of display⁹.

A fixed-polariser/rotating-retardation plate assembly was placed in front of a sensitive television camera (with a silicon-intensified target (SIT)), fitted with a 25 mm, $f/0.78$ lens with blue-green filter. The period of rotation of the retarder was 24 s. The polariser and retarder were Polaroid thin-sheet (cellulose acetate butyrate) and allowed wide field viewing; the SIT photocathode gave a field of view of $22^\circ \times 29^\circ$ horizontally. The noctilucent cloud images recorded on videotape can be analysed on playback using a video-integrator¹⁰ which allows the sum of video signals from any area selected in the television field of view to be displayed on a chart recorder. The amplitude of the signal is proportional to the average light intensity in the selected area.

Analysis of the video signal is best handled using Stoke's vector to describe the state of polarisation of light from the noctilucent clouds. The Stoke's vector consists of a set of four components (the Stoke's parameters I , M , C and S). Each parameter is a time-averaged intensity: I is the light intensity; M contains the 'horizontal preference' of the polarised part of the light, C the '45° preference' and S the 'circular preference'. These parameters may be written as a column vector and the transmission of polarised light through the Polaroid/retardation plate assembly is estimated using Mueller calculus¹¹. The state of polarisation of the light emerging from the Polaroid/retardation plate combination is found by successive multiplication of the Stoke's vector of the incident light by the two matrices representing the Polaroid and the retardation plate. The intensity I of light falling on the television camera varies with time such that

$$I(t) = X + Y \sin 4(\beta(t) + \beta_0) - Z \sin 2\beta(t)$$

where $\beta(t)$ is the instantaneous angle between the fast axis of the retarder and the (fixed) polariser axis. If the Stoke's parameters

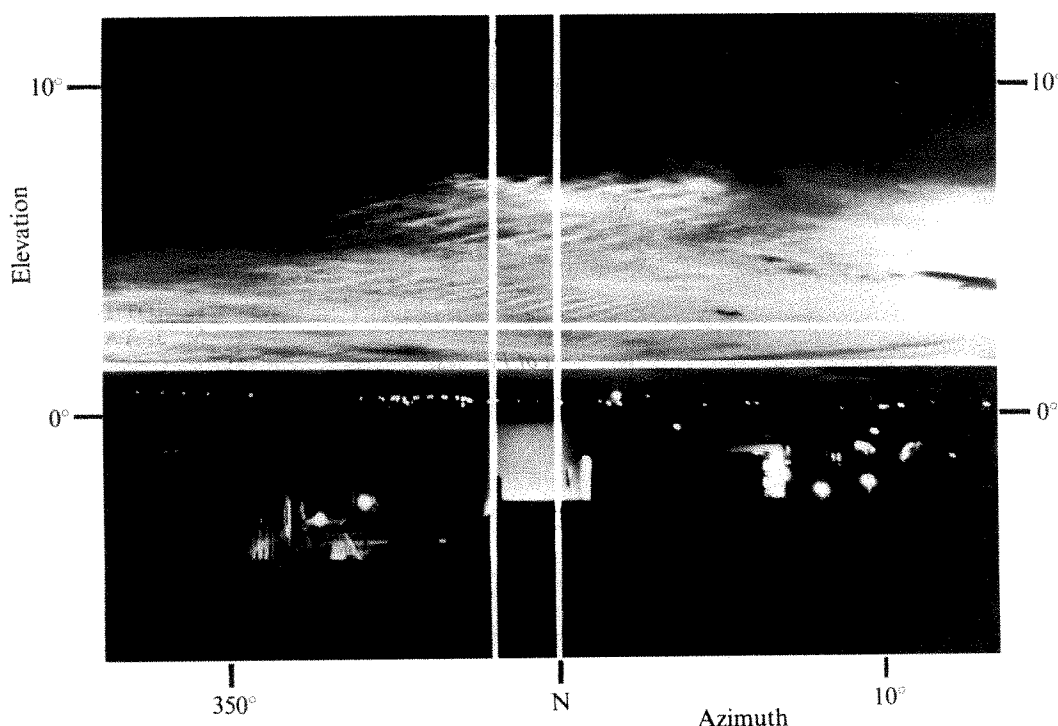


Fig. 1 Noctilucent cloud display seen from Aberdeen on the night of 28–29 July 1978. The print is from a monochrome copy of a 15-s ($f/2.8$) exposure on high-speed Ektachrome film. White lines enclose an area of sky where circular polarisation was detected.

of the light from the clouds are I_0 , M_0 , C_0 , S_0 , the expression for $I(t)$ contains the following:

$$\beta_0 = \frac{1}{2} \tan^{-1} (M_0/C_0);$$

$$2X = (k_1 + k_2)I_0 + \frac{1}{2}(k_1 - k_2)(1 + \cos \delta)M_0;$$

$$Y = (1/4)(k_1 - k_2)(1 - \cos \delta)(M_0^2 + C_0^2)^{1/2} \quad \text{and}$$

$$Z = \frac{1}{2}(k_1 - k_2) \sin \delta S_0$$

k_1 and k_2 are the principal transmittances of the polaroid; the retardance of the retarding plate is δ . In the simple case of partly polarised incident light which contains no circular polarisation ($S_0 = 0$), the video signal is a constant level X with a superimposed 4β sinusoidal modulation of amplitude Y . Addition of a small amount of circular polarisation ($S_0/I_0 \approx 0.01$) has the effect of adding in a 2β modulation on top of the 4β modulation and the presence of this shows up mainly as a half-frequency modulation Y . If the light from the clouds is predominantly linearly polarised, with only a small amount of circular polarisation added, then the detection of circular polarisation is simple, as extrema of the 4β signal will alternate in size.

Figure 1 shows a picture of the sky taken during the noctilucent cloud display of 28–29 July 1978. The Sun is 11° below the horizon at an azimuth of 24° . The videotape recording indicates that the degree of linear polarisation increases systematically from the clouds to the right of Fig. 1 (scattering angles $< 20^\circ$) to those shown in Fig. 1 (scattering angles 20° – 50°) as is to be expected both from scattering theory and from all previous reports of polarisation measurements of noctilucent clouds. However, regions in which the maxima of the 4β signal alternate in size have been found only in restricted parts of the cloud display and one such area is indicated in Fig. 1. Figure 2 shows part of the chart record obtained when integrating the video recording in this area. The alternation in height of the maxima is clearly seen, and analysis of the data from Fig. 2 shows the proportion of circular polarisation, S_0/I_0 , to be 0.005 ± 0.0013 . This is less than that of earlier measurements⁴, when S_0/I_0 was estimated to be typically 0.05 in the blue part of the visible spectrum. However, the noctilucent clouds that were seen on this occasion from Aberdeen were unremarkable in brightness and development, and the observation night was towards the end of the useful season.

The presence of a noticeable amount of circular polarisation in only certain parts of the display (adjacent regions showing, as far as can be seen, none) shows the effect is not produced locally by tropospheric scattering or absorption even at the great zenith distance of that area showing circular polarisation. There is no reason that the part of the noctilucent cloud that does show circular polarisation should have this difference from other parts of the display at the same zenith distance. It is also difficult to reconcile the appearance of localised circular polarisation with its being caused by partly polarised light incident on spherical particles. The cloud enclosed by the lines in Fig. 1 is at a latitude of 64.5° (no allowance being made for refraction) and there the Sun is only 4.4° below the horizon. The sunlight shining on the cloud passes the sunset line at a height of over 60 km and there is negligible absorption in the direct path. Any linear polarisation in the illumination of the cloud must come from tropospheric scattering near the sunset line, from that part of the atmosphere below the direct solar rays.

The relationship between degree of circular polarisation and amount of asphericity of the particles scattering the light cannot be described easily without knowing the distribution of sizes and shapes among the cloud particles. However, some estimate can be made by assuming that the low-contrast (or Rayleigh-Gans) approximations⁸ are applicable and that only unpolarised sunlight is shining on the clouds. If the scattering particles are cylinders of length exceeding two light wavelengths ($1 \mu\text{m}$), circular polarisation of $S/I = 0.005$ will arise if the radius of all the particles is close to $0.01 \mu\text{m}$. Alternatively, there could be a mixture of 10% of cylinders with radii $0.04 \mu\text{m}$ in a cloud of otherwise spherical scatterers.

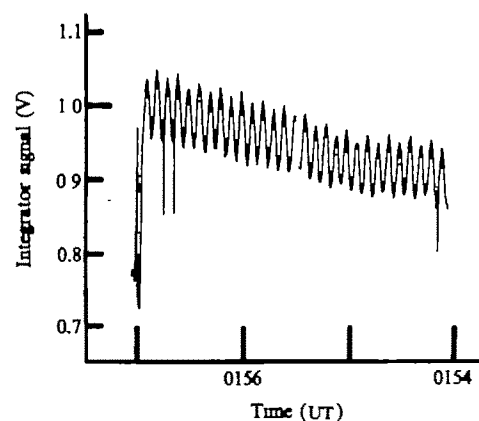


Fig. 2 Section of chart record made using a video-integrator on the area of sky indicated in Fig. 1. Note the depressed zero of the ordinate scale; the proportion of linear polarisation present is ~ 0.16 . The presence of a small amount of circular polarisation is shown by the alternation in height of the maxima.

Little is certain about the way in which ice crystals will grow in the low temperature and low pressure conditions near the mesopause. By analogy with the efficient screw-dislocation growth of crystals in the laboratory, it is possible that a significant proportion of the cloud particles will grow into needles rather than into spheres and this is certainly seen in the laboratory at low temperatures which are, however, much higher than the temperatures of noctilucent clouds. The fall speeds of needle-shaped crystals will be less than those of spheres. The amount of light scattered by a single particle increases very quickly as it grows in a supersaturated layer in the atmosphere. Spherical particles, however, will fall out of this layer more quickly than needle-shaped particles and so have less time for growing. It could be that there will be an aerodynamic advantage for growth of non-spherical particles relating to the spherical ones and that these non-spherical particles, being large, will individually scatter more light than the spheres and so seem to be relatively more important when observing the light scattered from a cloud.

The relative humidity of the atmosphere in the cloud region controls the optical thickness of the cloud that can form. Needle-like scatterers with their longer residence times and eventually greater relative sizes will give thicker (that is, brighter) clouds than spherical scatterers. For a given cloud brightness, the atmosphere does not have to be as wet with needles as with spheres.

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Isotopically light carbon in diamonds from some kimberlite pipes in Lesotho

THIS study of the isotopic composition of carbon has been carried out on six diamond crystals from kimberlite pipes in Lesotho. One crystal came from the Kao pipe, one from the Liqhobong and four from the Kolo pipe. We consider here how their colour and location are related to their isotopic composition.

The Kao and Liqhobong kimberlite pipes are located in the highlands of the northern Lesotho where they cut through the thick basaltic lavas of the Drakensberg Beds^{1,2}. The Kolo pipe is situated in the lowlands of western Lesotho and it cuts through the shales and sandstones of the Beaufort series and the Karoo dolerites. Diamonds from these deposits are characterised by a large quantity of fragments and angular forms (up to 65% for the Kao pipe and 80% for the Liqhobong and Kolo pipes).

Generally among the crystals, rounded dodecahedra prevail^{3,4} but a tendency towards an increase of octahedral forms in the Liqhobong-Kao-Kolo direction is noticeable.

Table 1 A brief description of the diamonds and analytical results of isotopic composition of carbon

No.	Pipe	Brief description	¹³ C‰ PDB
1	Kao	A flattened rounded twin of dodecahedra, smoky, transparent fissured. Faces 110 are unevenly pitted. The lines of plastic deformation and traces of etching are observed. Weight 40.62 mg.	-4.6
2	Liqhobong	A fragment of a lemon-yellow transparent octahedron with triangular cavities and hillock of irregular or rounded shape on the relics of faces III. Weight 30.5 mg.	-20.5; -10.6
3	Kolo	An irregular intergrowth of 3 flattened colourless transparent octahedra with development of the curved dodecahedral surfaces. The surfaces are badly etched with cavities. The diamonds have several graphite inclusions. Weight 30.95 mg.	-16.2; -15.9
4	Kolo	A rounded badly elongated on L ₃ yellow-transparent dodecahedron with several cavities. Weight 30.95 mg.	-6.9
5	Kolo	A rounded flattened colourless dodecahedron with several graphite inclusions. Faces 110 are roughly pitted with some hillocks. Weight 30.81 mg.	-6.5
6	Kolo	A rounded flattened smoky dodecahedron. The faces are irregularly developed and have deep canals of etching. An inclusion of orange garnet is observed inside the crystal. Weight 50.83 mg.	-14.7; -15.1

Serial no. *	Crystal habit	Colour	¹³ C‰ PDB
1	Twin or rounded dodecahedron	Smoky	-4.6
2	Fragment of octahedron	Lemon-yellow	-20.5; -19.6
3	Intergrows of octahedra	Colourless	-16.2; -15.9
4	Rounded dodecahedron	Yellow	-6.9
5	Rounded dodecahedron	Colourless	-6.5
6	Rounded dodecahedron	Smoky	-14.7; -15.1

* The diamonds are described in the same succession as in the text.

Many of the stones are of different colours and varying intensity: for the Liqhobong pipe, yellow is dominant: Kolo diamonds are mostly smoky-grey whereas Kao diamonds are brownish. Most of the diamonds have black inclusions of graphite. A brief description of the diamonds which have been analysed is given in Table 1.

Analysis of the isotopic composition of carbon of the diamonds has been done by oxidation in a stream of oxygen on copper oxide. The relative contents of isotopes of carbon have been measured by a mass spectrometer (Varian MAT -230) with precision of $\pm 0.01\%$ ($\pm 0.1\%$). Analytical data in Table 1 are in values of ¹³C ($\pm 0.1\%$). Analytical data in Table 1 are in values of ¹³C (‰) which are deviations in ‰ of ¹³C/¹²C of samples with respect to ¹³C/¹²C of the PDB standard.

Table 1 shows that three of the six diamonds (nos. 2, 3 and 6) are characterised by somewhat light intermediate composition of carbon (group B of the Galimov *et al.*⁵ classification). Note that the analysis of these diamonds has been done twice and the data obtained have been rechecked.

Diamonds with a similar carbon isotope composition have not been reported either in kimberlite rocks of Africa^{6,7} or in Yakutia^{5,8}. Therefore, it had been thought until recently that the ratio ¹³C in kimberlites is rather constant and is characterised by a value ¹³C = -4 to -9‰ PDB the value assumed for mantle carbon carbonatites as also fall in this range⁹. The only exception were carbonados of Brazil with ¹³C = -27 to -30‰ PDB^{5,10} as well as some coloured diamonds from placers of the northern Yakutia¹¹. However, it has been established recently that among the diamonds from the placers of the Urals, Sayans, Ukraine and from other areas of the USSR there are monocystals with light ¹³C = -20 to -25‰ PDB and intermediate ¹³C = -10 to -20‰ PDB isotopic composition of carbon⁵. Kaminsky *et al.*¹² have even suggested that such diamonds are of non-kimberlitic origin. In this connection, the discovery of isotopically light diamonds in Lesotho kimberlites is very significant. From these descriptions of the diamonds and from Table 1, neither the habitat of the stones nor their colours are related to a particular isotopic composition. Among the isotopically light diamonds there are rounded dodecahedra and octahedra which are colourless or have a yellow or smoky colour. This isotopic composition of diamonds probably depends on their genetic peculiarities but further study on this is required.

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New data on natural todorokites

TODOROKITE has been found as a product of terrestrial weathering, in sedimentary manganese ore, in deep-sea iron and manganese nodules and crusts. It is considered as an important source of Ni, Co, Zn, Cu, Mg in marine manganese nodules.¹⁻³

We show here that besides previously described todorokites with parameters $a_0 = 9.75$, $b_0 = 2.84$ and $c_0 = 0.6$ Å there are others, one with $a_0 = 14.6$ Å (The Pacific Ocean, Bakal) and one with $a_0 = 24.38$ Å (Bakal, Sterling Hill), b_0 and c_0 being unchanged. Morphologically the todorokites with different a_0 are very similar—their a_0 -values being multiples of 4.88.

According to Straczek *et al.*⁴, the approximate formula of todorokite might be $(\text{Na}, \text{Ca}, \text{K}, \text{Mn}^{2+})(\text{Mn}^{4+}, \text{Mn}^{2+}, \text{Mg})_6\text{O}_{12} \cdot 3\text{H}_2\text{O}$. The extent of variations of Mn^{4+} , Mn^{2+} , and Mn^{3+} are still unknown. The symmetry of the mineral is orthorhombic (or monoclinic, β about 90°), with $a_0 = 9.75$, $b_0 = 2.84$ and $c_0 = 9.59$ Å. In electron microscope preparations todorokite occurs in the form of thin lathes (001) frequently elongated along the b axis.

Burns and Burns^{1,5} assumed that the todorokite structure was similar to that of hollandite and psilomelane and had common features with the structure of rutile, this being shown by their ability to produce planar net trillings of elongated crystals. The intergrowths of todorokite form nets of hexagonal, triangular and rhombic cells. The electron micrographs of the treble intergrowths show three superimposed diffraction patterns orientated to each other with a relative rotation at an angle of 120° (Fig. 1). From selected area electron diffraction (SAD) todorokite has been shown to occur in the form of lathes with a basal face (001).

A study of SAD-patterns revealed that besides the todorokite having $a_0 = 9.75$, $b_0 = 2.84$ and $c_0 = 9.59$ Å found in many deposits, species with $a_0 = 14.6$ (Fig. 1c) and 24.38 Å (Fig. 1d), the b_0 and c_0 parameters being unchanged, occur in the natural environment. Under the microscope and on the electron micrographs these species look similar to todorokite with $a_0 = 9.74$ Å. The results, obtained from an analysis of individual lathes of todorokites with different values of the a_0 parameter carried out with an EM 100 C microscope and X-ray energy spectrometer (Kevex), indicate the similarity of their chemical composition. The X-ray powder patterns of these todorokite species display an insignificant difference between the parameters, which is reflected in slight variations of spacings for some reflections, hkl . The X-ray patterns failed to show weak reflections indicative of a true value of the a_0 parameter of todorokite. The strong reflection $hk0$ and 001 of structural varieties of todorokite largely correspond to the same lattice spacings.

It is easy to see that the values of a_0 characteristic for the members of the todorokite group (9.75, 14.6, 24.38 Å) are

divisible by 4.88 Å. The variations of this parameter are shown by the frequency of the reflections occurring along the axis a^* . For example on an electron micrograph showing the todorokite basal plane (001) with $a_0 = 9.75$ Å (Fig. 1b) there are three less-intense reflections along the a^* -direction between the strong reflections forming a pseudohexagonal net. The first strong reflection along the a^* axis has indices 400 ($d = 2.4$ Å). In the SAD pattern of the todorokite with $a_0 = 14.6$ Å (Fig. 1c) there are five weak reflections between the strong ones. In this case, the reflection at $d = 2.44$ Å along the a axis has indices 600. On the pattern of todorokite with $a_0 = 24.40$ Å (Fig. 1d) there are, between the strong reflections along the a axis, four weak reflections on each side, which, provided other reflections are absent, would determine a base-centred unit cell and the extinction law for the plane (001)*— $h+k=2n$, where n is an integer. However, on the diffraction pattern of the todorokite species with $a_0 = 24.38$ Å, midway between the strong reflections, there is the fifth, weak, reflection which violates the symmetry of the unit cell and shows it to be really primitive. In this case, the strong reflection at $d = 2.44$ Å occurring on the a^* axis has indices 10.0.0.

Under the microscope todorokite with the parameter $a_0 = 14.6$ Å is seen to occur in the nucleus of an iron-manganese nodule collected from the Pacific Ocean floor. Flaky todorokite from the Bakal deposit (USSR) seems to consist of aggregates of two todorokite species with the a parameters 14.6 and 24.4 Å. Todorokite with $a_0 = 25$ Å has been identified in a sample from Sterling Hill (USA) and a specimen from the Takhta-Karacha deposit in the Middle Asia.

Thus, todorokite species may be characterised by unit cells having different parameters a_0 which is divisible by 4.88 Å (the parameter along the a axis being determined by strong reflections located in accordance with the pseudohexagonal law, if there is a base-centred orthogonal unit cell). We assume that todorokites with other a_0 parameters, but still divisible by 4.88 Å, do also exist in nature.

The variations of the a parameter and formation of the todorokite superstructures will probably be attributed to certain difference in their composition and the environment of their crystallisation.

The data available show that todorokite is displaced by vernadite (δMnO_2). Even after a complete displacement of todorokite by vernadite, the former retains its fibrous or flaky appearance. Therefore, the products of partial or complete replacement of todorokite by vernadite can be erroneously taken for todorokite, without comprehensive electron microscopic examination. Partially vernadite-replaced todorokite as well as the pseudomorphs of vernadite after todorokite are common.

In view of the possibility of a new species of todorokite family being found in nature as well as in synthetic products, we recommend the following nomenclature: todorokite I with $a_0 = 4.88$ Å; todorokite II with $a_0 = 4.88$ Å $\times 2$; todorokite III with

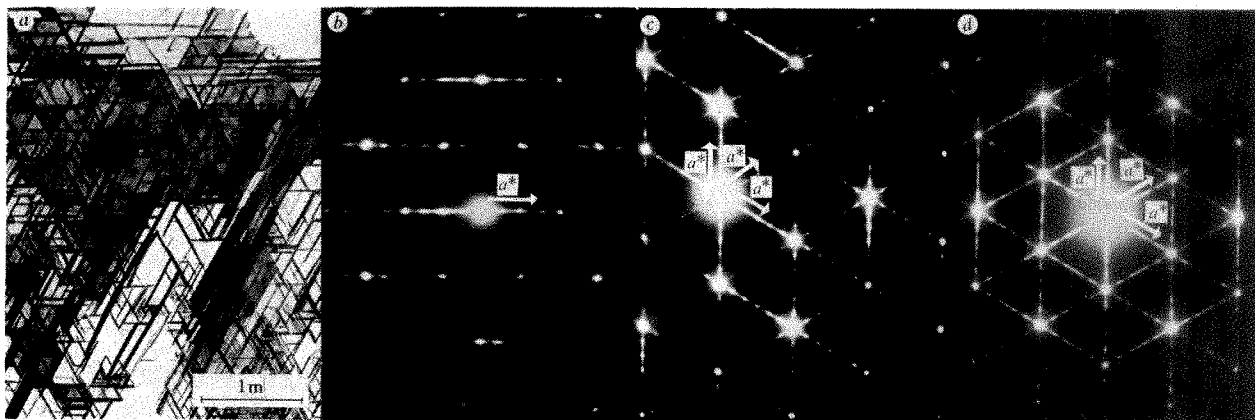


Fig. 1 a, electron photomicrographs of the todorokite treble intergrowths. b, c, d, Electron diffraction pattern of monocystal of todorokite with parameter $a_0 = 9.75$ Å (b), and of treble intergrowths of todorokite with parameters $a_0 = 14.6$ Å (c) and 24.4 Å (d).

$\iota_0 = 4.88 \text{ \AA} \times 3$; todorokite IV with $a_0 = 4.88 \text{ \AA} \times 4$; todorokite V with $a_0 = 4.88 \text{ \AA} \times 5$. (Todorokites II, III and V have been already found in nature.)

Predictions made by other authors have a direct bearing on the discovery of todorokite polymorphs. They deal with pure crystalline manganese oxides and hydroxides (see refs 5, 6). According to Turner and Buseck⁷ mixtures of polymorphs found recently have also been known for other manganese oxides—hollandites and cryptomelanes.

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Rates of Holocene folding in the coastal Zagros near Bandar Abbas, Iran

FOR the study of recent crustal processes, seismological and geodetic surveys need to be complemented by measurements of Earth movements over periods long enough for secular trends to emerge¹. The one such item available for the Zagros was obtained in 1952, when Lees and Falcon used archaeological remains to trace the growth over the past 1,700 yr of the Shaur anticline near the head of the Persian Gulf². Lees and Falcon also observed that recent marine terraces on the shores of the Gulf had been deformed by anticlinal growth². I report here

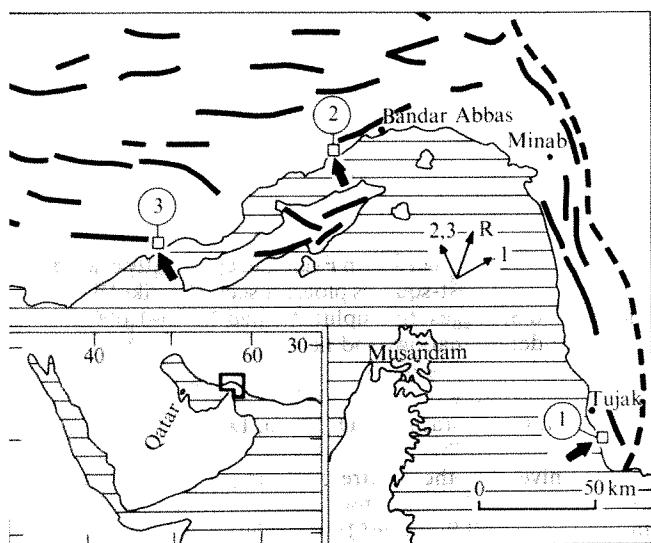


Fig. 1 Location of sections. Main fold axes (heavy lines) after Berberian¹⁷. Broken heavy line shows approximate position of Minab Fault. Arrows are normal to generalised strike of corresponding anticline and denote shortening vectors; bearing of resultant **R** (Table 2) based on vectors at sections 1 and 2, which are at right angles.

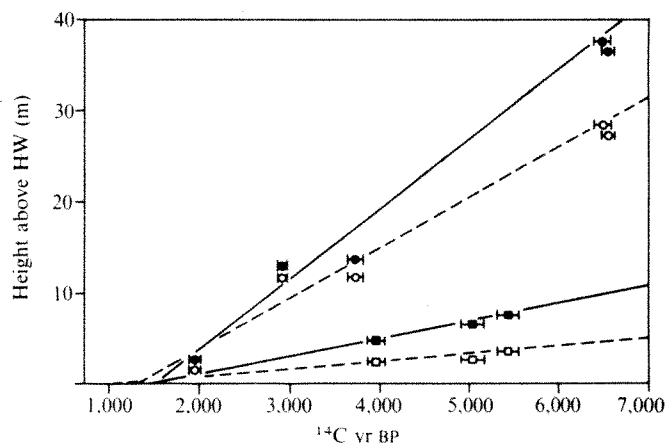


Fig. 2 Age/height plots for section 1 (●) and sections 2 and 3 (■) corrected for eustatic factor after Flint⁷; corresponding open symbols (○, □) corrected after Clark et al.⁸. Solid lines are least-squares fits for former, dashed lines for latter. Age error bars denote 1 s.d. No correction has been made for 'apparent age' at death of molluscs, which would shift all points to the left.

preliminary ¹⁴C dates on shells from fossil beach deposits associated with three fold structures on the eastern Zagros coast of Iran near Bandar Abbas, and derive Holocene deformation rates from them. The results, though not necessarily applicable to other parts of the folded belt, are compatible with estimates of the current rate of rotation of Arabia, to whose northeasterly drive the Zagros Range has been ascribed³.

The samples were collected for a survey of recent coastal movements⁴ which, in view of the uncertainty that surrounds global (eustatic) sea-level history, is confined to the relative displacement of isochronous shorelines⁵. For the present work, however, absolute values of vertical displacement are needed and hence allowance must be made for fluctuations in sea level. Data from other parts of the Gulf cannot be used for this as they may embody the tectonic effects we are trying to isolate. For this reason, and also to facilitate eventual comparison with data from the Makran⁶, the corrections adopted below are based on the empirical global curves favoured by Flint⁷, which indicate progressive if decelerating submergence during the Holocene, and on the model formulated by Clark et al.⁸, which for the latitudes at issue yields slow emergence over the past 7,500 yr.

The folds of the southern Zagros east of Bandar Abbas swing round parallel to the coast. The first section considered here, 2 km south of Tujak (1 on Fig. 1), consists of a succession of beaches cut into marls of the Mio-Pliocene Agha Jari Formation which were exposed by erosion of an anticline striking N 160° E. The beaches are capped by 0.5–1.5 m of fossiliferous sands whose lithology, form and faunal content reflect intertidal conditions⁹. Three of the beaches were sampled for dating. Each sample consisted of a single species of mollusc; thin-section analysis, X-ray diffraction and inspection under the scanning electron microscope were used to identify unaltered specimens.

The second set of dates refers to two anticlines west of Bandar Abbas which are also composed of late Tertiary rocks. Unrecrystallised shells were obtained from the summit of a beachrock on the southern flank of one of the anticlines, which strikes roughly N 60° E and consists of sands and marls of the Miocene Mishan Formation (3 on Fig. 1), and from an abandoned offshore bar and a beach terrace on the southern flank of the other, which strikes N 70° E and includes both Agha Jari and Mishan deposits (2 on Fig. 1). The results from sections 2 and 3 are treated together; additional dates should make it possible to establish differences between the histories of the two structures.

The ¹⁴C ages obtained for both coasts are listed in Table 1 and, after eustatic correction, plotted against height in Fig. 2. The presence of successive discrete beaches, notably at section 1,

Table 1 Radiocarbon ages for coasts east and west of Bandar Abbas

Sample no.	Laboratory no.	Location	Age (yr BP)*	$\delta^{13}\text{C}(\text{T})$	Species	Elevation above HW
Section 1						
ET Ia	SRR 1258	26°00' N, 57°14' E	1,940 ± 45	+0.40	<i>Scapharca inaequivalvis</i>	2.0
ET IIb	UM 1268	"	3,735 ± 85	+0.79	<i>S. inaequivalvis</i>	11.8
ET IIc	SRR 1257	"	2,925 ± 40	+1.90	<i>Asaphis deflorata</i>	11.8
ET IIIb	UM 1269	"	6,525 ± 95	+0.46	<i>S. inaequivalvis</i>	28.6
ET IIIx	UM 1255	"	6,580 ± 155	+0.83	<i>A. deflorata</i>	28.6
Section 2						
76/1	MC 1574	27°09' N, 56°08' E	3,960 ± 100	+2.86	<i>Turritella fultoni</i>	2.3
76/4a	MC 1572	"	5,410 ± 110	+2.96	<i>T. fultoni</i>	4.0
Section 3						
IBR/1	MC 1099	26°47' N, 55°10' E	5,040 ± 115	+1.42	<i>Barbata cf. fusca</i> and <i>A. deflorata</i>	3.0

* Corrected for isotopic fractionation by normalising to a $\delta^{13}\text{C}$ value of -25‰ relative to PDB.

suggests that movement was spasmodic, but average uplift rates can still be informative. Table 2 lists the values obtained both by assuming uniform uplift since the formation of the highest sea-level indicator and by calculating least-squares lines for the data. The maxima obtained are 7.4 mm yr⁻¹ for section 1 and 1.9 mm yr⁻¹ for sections 2 and 3. These figures lie between the minimum average of 1 mm yr⁻¹ that has been proposed for general uplift of the Zagros Mountains³ since the early Pliocene, and the rate of about 12 mm yr⁻¹ obtained for the Shaur anticline². A Holocene rate of 6 mm yr⁻¹ has been calculated for subsidence of the Musandam Peninsula at the mouth of the Gulf¹⁰; data from Qatar¹¹ give uplift rates of 1.1 and 0.3 mm yr⁻¹ according to which eustatic correction is adopted.

A preliminary assessment of the horizontal shortening represented by the observed uplift can be made using purely geometrical methods and assuming that the folds approximate

The values for ΔX for sections 2 and 3, which represent northward compression, are probably minima, as shortening is likely to be taken up in part by other anticlines inland and offshore. Folding due to eastward shortening at section 1 is confined to a narrow belt by the Minab Fault to the east¹², but evidence at the Chah Shirin Fault near Minab for 3.5 m of north-eastward thrusting over the past 7,000 yr (ref. 15) suggests that on this coast also the calculated shortening is a minimum. At all events the ΔX values give results (Table 2) which range between 16.6 and 31.9 mm yr⁻¹ and bear roughly NNE. Palaeomagnetic evidence from the Red Sea and the Gulf of Aden indicates separation between Africa and Arabia at a rate of about 20 mm yr⁻¹ during the past 5 Myr and a spreading axis in the western Gulf of Aden with a strike of N 110° E (ref. 16). The relationship between the two sets of results needs to be scrutinised.

Table 2 Uplift and compression rates

	$L(\times 10^5 \text{ mm})$	$s(\times 10^5 \text{ mm})$	$\omega \text{ (rads)}$	Average (mm yr ⁻¹)				Least-squares plot (mm yr ⁻¹)			
				a		b		a		b	
				Δy	ΔX	Δy	ΔX	Δy	ΔX	Δy	ΔX
Section 1	245.0	61.25	1.3788	5.7	20.0	4.3	15.1	7.4	26.0	5.4	19.0
Sections 2 and 3	167.5	15.0	0.4887	1.4	13.6	0.7	6.8	1.9	18.4	0.8	7.8
Resultant (mm yr ⁻¹)				24.2		16.6		31.9		20.5	
Bearing				N 26° E		N 36° E		N 25° E		N 38° E	

Heights corrected for eustatic factor after Flint⁷ (a) and Clark *et al.*⁸ (b).

symmetrical curves of intrinsic sine form. Let L be the length of the fold surface between two troughs and ω the maximum slope of the limbs. During compression with horizontal displacement ΔX , the vertical displacement Δy of a point P on the fold at a distance s along the fold limb from one of the troughs can be approximated (C. D. Mann, personal communication) by the expression

$$\Delta y = \Delta X \times 2\pi/\omega \times (s/L)^2$$

where s/L is small and the folded zone is bounded at the base by a surface of discontinuity (décollement)^{3,12}. Known values for L , ω , s and Δy , and calculated values of ΔX , are given in Table 2. L , s and ω were derived from field measurements and air photographs for sections 1 and 3, and from geological cross-sections constructed from published maps¹³ by the Busk method¹⁴ for section 2. The many minor faults associated with all three structures have been disregarded.

Movement on the Chah Shirin Fault had apparently ceased by 1,250-yr BP¹⁵; the least-squares plots for section 1, like those for sections 2 and 3, suggest that uplift stopped 1,300–1,000 yr BP. Further age determinations and field mapping are required to establish whether this is an artefact of dating and to refine the uplift plots so that the underlying mechanisms can be explored.

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Ordovician fish spines from Girvan, Scotland

THE Upper Ordovician Lady Burn Starfish Beds¹ of the Drummuck Group² in the Craighead inlier have long been known for their well-preserved, diverse and abundant invertebrate faunas^{3,4}. The trilobite⁵ and brachiopod (D.A.T.H., unpublished data) faunas indicate a late Rawtheyan (Ashgill) age for these predominantly green medium grained sandstone beds. Moreover, a study of the brachiopod associations and sediments of these beds together with the rocks above and below suggests that, although the Starfish Beds contain essentially 'shelf' faunas, final deposition was in a relatively deep-water, proximal slope environment⁶. I present here geochemical and morphological evidence of fish spines from these deep-water, siliciclastic marine rocks of Ordovician age at Girvan, south-west Scotland.

The occurrence of a pair of fish spines in the oldest and most fossiliferous of these marine strata at the South Threave locality (GR NS 250 038) is an unusually early appearance of this type of vertebrate material in the fossil record. The spines (Fig. 1) are subparallel and lie on a very fossiliferous bedding plane, which is crowded by disarticulated and randomly orientated valves of the Ashgill brachiopod *Eochonetes advena* Reed, 1917. The better preserved spine is 13 cm long, broken proximally and slightly curved. It is composed of predominantly black, apparently fibrous and lustrous material. Although now somewhat flattened, the spine seems to have been crudely rectangular in cross-section and has a hollow central canal.

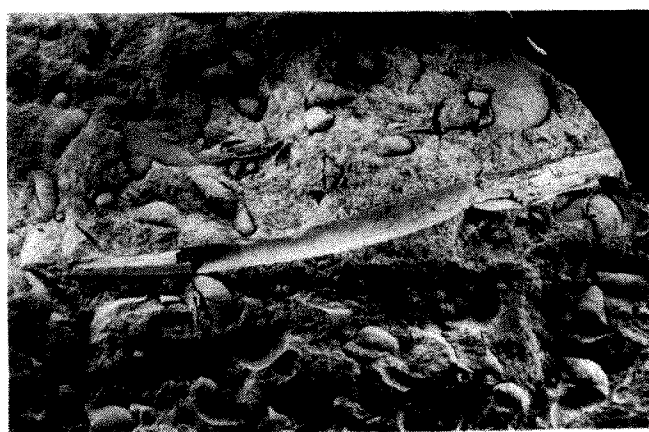


Fig. 1 Ordovician fish spines embedded in fossiliferous bedding plane.

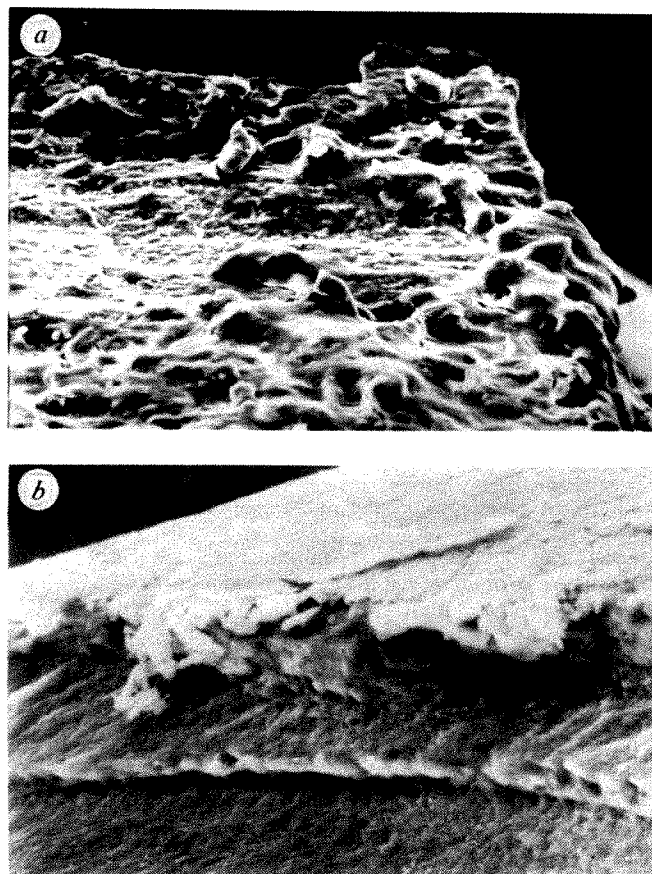


Fig. 2 SEM photomicrographs of spine fragment showing relatively simple bone histology. a, Oblique transverse view; b, oblique longitudinal view.

Electron microprobe analysis (D. C. Johnston, personal communication) indicates the composition of the material to be close to that of francolite, a carbonate-hydroxyfluorapatite commonly found as vertebrate bone.

Scanning electron microscope (SEM) photomicrographs (Fig. 2) of fragments of the material show the bone histology to be relatively simple; the oblique transverse (Fig. 2a) and longitudinal (Fig. 2b) views demonstrate the microstructure to be fairly massive, but with well developed growth rings about 20 μm apart. Interpretation of the cellular structure is difficult but in transverse section numerous minute pores perforate the substance; these presumably link with the central canal.

The morphological and geochemical evidence thus clearly demonstrate that these fossils are composed of vertebrate bone material and are best interpreted as fish spines; unfortunately no fish scales have been recovered.

Fish material is rare in rocks of pre-Silurian age, and when such remains are found they tend to be fragmentary⁷. The Ordovician occurrences, for example the Lower Ordovician Valhallfonna Formation of Spitzbergen⁸ and the Middle Ordovician Harding Formation of Colorado⁹, are usually represented by disarticulated heterostracan scales and cartilage. Similarly the earlier Cambrian record¹⁰ is marked by heterostracan plate fragments and tubercles. Although the similarly fragmentary nature of the later Girvan material does not allow an unequivocal determination, it cannot at present be related to any known fossil of Ordovician age. Most probably the spines are those of an acanthodian (spiny shark); note that the acanthodians are particularly characterised by paired fin spines¹¹. The close association of the spines suggests that the fish, or at least that part of the fish bearing the spines, had undergone negligible transportation since falling on to the substrate.

Recent studies of Palaeozoic fish¹² record the earliest appearance of acanthodians as Upper Silurian; in rocks of this age these fish are again represented by disarticulated spines and scales. The evidence from the Drummuck material, which strongly suggests that the pair of spines was part of a large specimen of acanthodian, significantly extends the stratigraphical range of these fish back into the Upper Ordovician.

The small amounts of the present material and lack of any comparable pre-Silurian occurrences do not allow much discussion on the origins of these spiny fish. It is, however, probable that by the later Ordovician the first marine vertebrates included at least both acanthodian and heterostracan fish.

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Growth rings in dinosaur teeth

ANNUAL growth rings are often found in the skeletal tissues of Recent and fossil ectothermic vertebrates^{1,2}. These rings are usually attributed to the inability of ectotherms to maintain high levels of activity, feeding and growth during adverse times, such as dry seasons in tropical areas and cold seasons at higher, more temperate latitudes¹. In endothermic vertebrates, however, the efficient production of endogenous heat results in a relatively constant body temperature, and high activity and feeding levels are more easily sustained. Consequently, the growth of skeletal tissues in these animals is less sensitive to seasonal variations than in ectotherms, and annulations are rare except in species living under severe seasonal regimes^{3,4}. Many workers have proposed that dinosaurs were endothermic, as are all living mammals and birds, rather than ectothermic, as are all living reptiles^{3,5–10}. In accordance with this hypothesis, pronounced seasonal growth rings have not been observed in the calcified tissues of dinosaurs^{5,6}. However, here I report the discovery of such rings in the teeth of diverse Late Cretaceous dinosaurs.

Underwater screening for small vertebrate fossils has led to the recovery of hundreds of dinosaur teeth from the non-marine Milk River (early Campanian), Oldman (middle Campanian) and Edmonton (Maestrichtian) formations of Alberta, Canada. The teeth include those of Saurischia (Tyrannosauridae (Fig. 1) and Coeluridae) and Ornithischia (Hadrosauridae (Fig. 2a), Ceratopsidae (Fig. 2b) and Ankylosauridae). Ground and polished sections, as well as acetate peels of etched surfaces, have revealed conspicuous rings in the dentine in all dinosaur groups examined. Two main types of rings occur (Fig. 1): first, contour lines of Owen, which in Recent species mark minor nutritional and metabolic variations during the deposition of

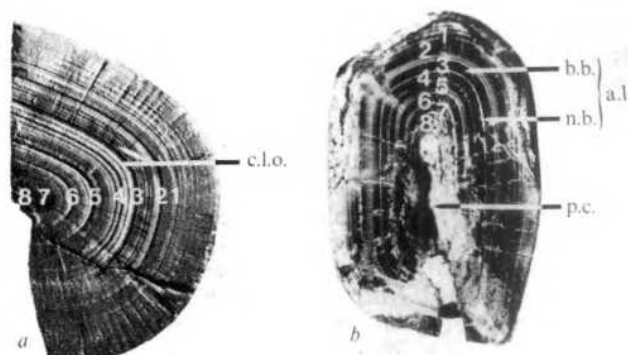


Fig. 1 *a*, Cross-section of a tyrannosaurid tooth, Milk River Formation, Alberta; c.l.o., contour lines of Owen; 1–8, annual layers in order of deposition ($\times 7.2$). *b*, Cross-section of a tyrannosaurid tooth, Oldman Formation, Alberta; n.b., narrow band; b.b., broad band; a.l., annual layer; 1–8, annual layers in order of deposition; p.c., pulp cavity ($\times 4.2$).

dentine and are accentuated according to the degree of disturbance experienced¹¹; and second, larger rings matching those of various Recent species^{4,12}, in which a narrow hypercalcified band (winter's growth) and a broad hypocalcified band (summer's growth), produced by seasonal variation in growth rate of the organic stroma of dentine, constitute an annual layer¹². Histologically, then, the structure of the dentine of dinosaurs indicates a periodicity in growth; the significance of this for the controversy concerning dinosaurian physiology is apparent when the clarity and pattern of the rings are compared to those in Recent and fossil organisms of known thermal physiology.

The only living terrestrial endotherms with annual layers of dentine as evident as those in Alaskan dinosaurs are mammals of Arctic or temperate regions^{4,12,13} subject to marked seasonal contrasts. Of tropical and subtropical mammals, only certain Australian marsupials^{4,12} and various African ungulates^{14–17} have been extensively investigated and no distinct annual layers in the dentine or bone have been reported, although layering in the cementum of some of the ungulates may correlate with wet and dry seasons. Consequently, if dinosaurs were endothermic and comparable to mammals in their thermal physiology, seasonal contrasts in Alberta during the Late Cretaceous must have been comparable to those of temperate and Arctic North America and Eurasia today to have produced conspicuous annual layers in the dentine.

However, severe seasonal contrasts do not seem to have characterised Alberta during the Late Cretaceous: palaeobotanical¹⁸ and palaeontological studies¹⁹ indicate a warm, subtropical climate without extreme daily or seasonal temperature change throughout the western interior of North America. If dinosaurs were endothermic in these conditions, how are the pronounced growth rings in their dentine to be explained? The

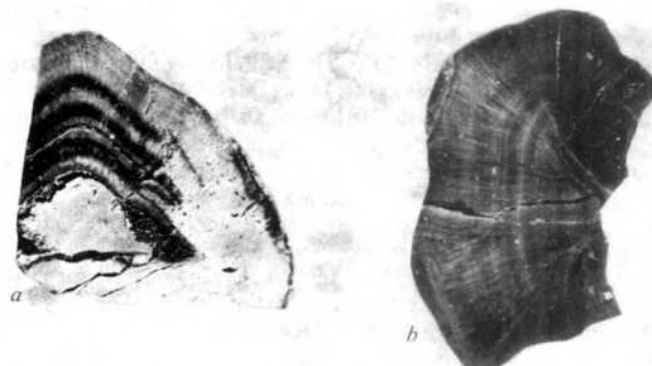


Fig. 2 *a*, Cross-section of a hadrosaurid tooth, Oldman Formation, Alberta, Canada ($\times 3.15$). *b*, Cross-section of a ceratopsid tooth, Oldman Formation, Alberta, Canada ($\times 5.04$).

origin of the rings is clearer, however, if dinosaurs were ectothermic, and thus presumably sensitive to relatively mild seasonal contrasts of temperature.

Fossil crocodile teeth (Fig. 3a) from the same localities as the dinosaur teeth have well developed rings, particularly similar to those of tyrannosaurids. No appreciable difference in pattern or clarity of annual layers or contour lines of Owen was found between the dentine of crocodiles (which are known ectotherms) and that of dinosaurs, suggesting that these organisms had a similar physiological tolerance to environmental fluctuations and hence a similar thermal physiology. Crocodile teeth from different geological settings, from Palaeocene and Eocene beds in Saskatchewan and Wyoming, also have growth rings like those of the Upper Cretaceous crocodiles and dinosaurs, thereby eliminating preservational bias.

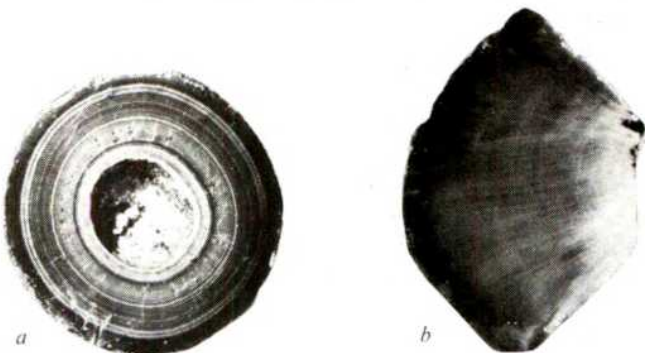


Fig. 3 a, Cross-section of a crocodile tooth (probably *Leidyosuchus* sp.), Oldman Formation, Alberta, Canada ($\times 3.6$). b, Cross-section of the root of an incisor of *Entelodon* sp., Oligocene, Cypress Hills Formation, Saskatchewan, Canada ($\times 3.0$).

Undoubted endotherms of large body size had not evolved by the Late Cretaceous and thus cannot be compared with dinosaurs. In teeth of small Late Cretaceous endotherms (mammals such as *Protungulatum*, *Didelphodon* and *Gypsonictops*) dentinal rings were not observed. Teeth from Tertiary mammals (representing four orders—Condylarthra, Amblypoda, Perisodactyla and Artiodactyla—and from formations interpreted as representing subtropical to warm temperature regimes) did show contour lines of Owen (Fig. 3b); however, apparent annual banding was rare and poorly developed.

As annual layers in most dinosaur and crocodile teeth are appreciably more conspicuous than in the Tertiary mammalian specimens, these reptiles were evidently affected to a greater degree by environmental factors influencing their growth rates. Furthermore, in the dinosaur and crocodile teeth, but not in the Tertiary mammal teeth, contour lines of Owen were often accentuated and tightly packed near and within the narrow bands of the annual layers: perhaps the crocodiles and dinosaurs were more sensitive to minor metabolic and nutritional stresses, particularly near and during adverse seasons, than were the Tertiary mammals examined.

In summary, the growth rings in the dentine of dinosaurs are more like those of contemporary crocodiles (known ectotherms) than those of fossil or Recent mammals from comparable climatic regimes. Evidently, growth and calcification of dentine in Late Cretaceous dinosaurs and crocodiles were affected similarly by both short-term and seasonal environmental variations, suggesting that these animals had similar thermal physiologies. If dinosaurs were ectothermic, their annual layers (as well as those of crocodiles and other organisms such as *Champsosaurus*, *Lepisosteus* and *Taxodioxylon* (unpublished data from the Late Cretaceous of Alberta)) seem best explained by Dodson's hypothesis²⁰ of seasonally distributed rainfall; by analogy, Recent crocodiles in Ngamiland exhibit annual layers in the periosteal bone apparently due to temperature fluctuations associated with wet and dry seasons characteristic of that tropical area¹.

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Analogous motion illusion in man and fly

SINCE Wertheimer's classic paper¹, research in motion perception has been concerned with the study of visual illusions such as ϕ -motion. Various phenomena of this type are easy to elicit by successive changes of the light flux in spatially distinct photoreceptors, and easy to explain by the specific properties of the motion detectors, although there are reports to the contrary². The present account deals with a less easily comprehensible illusion which is elicited by simultaneous changes of the light flux in differently illuminated receptors³⁻⁵. The phenomenon has previously been ascribed to the prolonged latency of the 'light-on' responses at lower levels of illumination which converts simultaneous stimuli into successive signals⁶, but this does not explain the illusion satisfactorily³. MacKay and co-workers were the first to attribute the apparent motion to the adaptive properties of the input channels of the motion detectors⁴. We show that the illusion can be induced in the fruitfly, *Drosophila melanogaster*, as well as in man. The course control response to motion provides a quantitative assay of the illusion in the fly. The results suggest that the illusion originates in the distortion of the visual signals before motion detection.

To elicit the illusion in humans we illuminated transparent optical wedge filters of linearly graded density with a variable light source. The observer of the circular array in Fig. 1a usually perceives a spurious 'propeller' behind the pattern which rotates a few degrees towards the less transparent side of the wedge filters during light increase, and in the reverse direction during light decrease. The predominant sensation is oscillatory rotation at modulation frequencies of 0.3–2 Hz, continuous rotation towards the less transparent side of the filters at 5–12 Hz and ambiguity at 2–5 Hz.

Motion around a fly evokes vigorous turning in the direction of the horizontal displacement. The response counteracts involuntary deviations from a straight course in a stationary environment. Turning is induced by a regular network of elementary motion detectors. The network accepts stimuli from all parts of the visual field, and acts on the difference between the

propulsive forces on either side. With the fly held in a stationary position the course control responses of wings⁷ or legs⁸ can be measured quantitatively. Figure 1*b* illustrates the paradigm used to assay apparent motion during fixed flight. The results in Fig. 2 reveal the existence of a motion illusion in *Drosophila* which is strikingly analogous to the corresponding illusion in man. The illusions coincide with respect to the phase and direction of the oscillatory component at low modulation frequencies, and with respect to the direction of the continuous component at high modulation frequencies of the illumination. Maximum intensity and spectral composition of the light source do not seem to be critical in the range 0.003–300 cd m^{-2} .

In the fly the illusion originates within the network of bidirectional motion detectors, each comprising motion-specific interactions between neighbouring input units of the visual system⁸. The steady-state responses of the detectors are adequately described by the correlation model in Fig. 3, where the interaction is achieved by multiplication of two input signals. The model is representative of all 'simple' motion detectors which do not require more than the theoretical minimum of two input units in second-order nonlinear interaction⁹. Models of this type are equivalent in their steady-state responses, and seem to be sufficient to describe the properties of entirely different motion detection systems, such as the direction-selective retinal ganglion cells in vertebrates^{10,11}. The models respond to successive stimulation of their input units and cannot discriminate true motion from the corresponding φ -motion. Figure 3 shows the simultaneous sinusoidal stimulation of the receptors in the present paradigms. The visual input is characterised by the transmission-dependent mean intensities $\bar{I}_1 > \bar{I}_2$, and by the transmission-independent relative amplitude, or modulation, $\Delta I/\bar{I} = \Delta I_1/\bar{I}_1 = \Delta I_2/\bar{I}_2$. In the linear range of signal processing at the receptor level (N) the gain factor of the response, R , of the model is $\bar{I}_1 \bar{I}_2 (\Delta I_2/\bar{I}_2 - \Delta I_1/\bar{I}_1) = 0$. This is easy to show by application of the operations specified in Fig. 3. The absence of responses at the level of motion detection excludes the generation of the expected responses at subsequent levels. Nonlinear processing of the simultaneous signals before motion detection becomes necessary to elicit non-zero responses from the detector model.

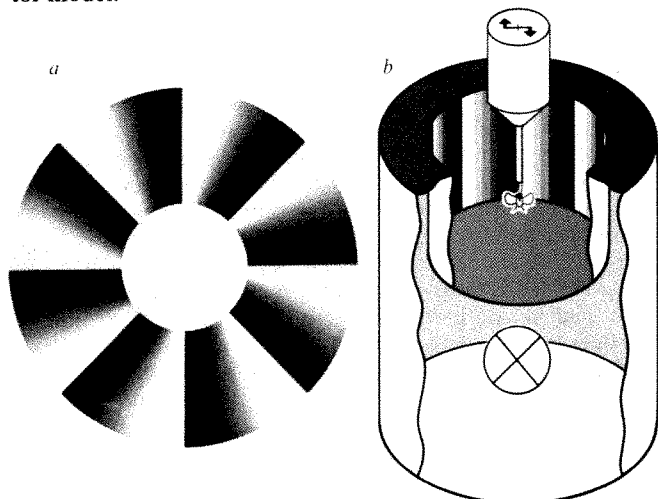


Fig. 1 *a*, Circular array of wedge filters used to elicit, stroboscopically, the illusion of oscillatory (0.3–2 Hz) or continuous (5–12 Hz) rotation in the human observer. The best results were obtained with a transparency of the pattern (absorbance 0–2), illuminated from behind by a sinusoidally modulated light source of almost arbitrary colour and intensity. It may be difficult to see the illusion when the figure is directly exposed to flickering light. *b*, Cylindrical array of wedge filters surrounding the fruitfly *Drosophila* during fixed flight on a torque meter which is used to record the course control response. The stationary pattern was illuminated from outside by a programme-controlled central light source in the lower compartment. The panorama was inverted between experiments and was presented in arbitrary angular positions to minimise lateral disparities of stimulus and response.

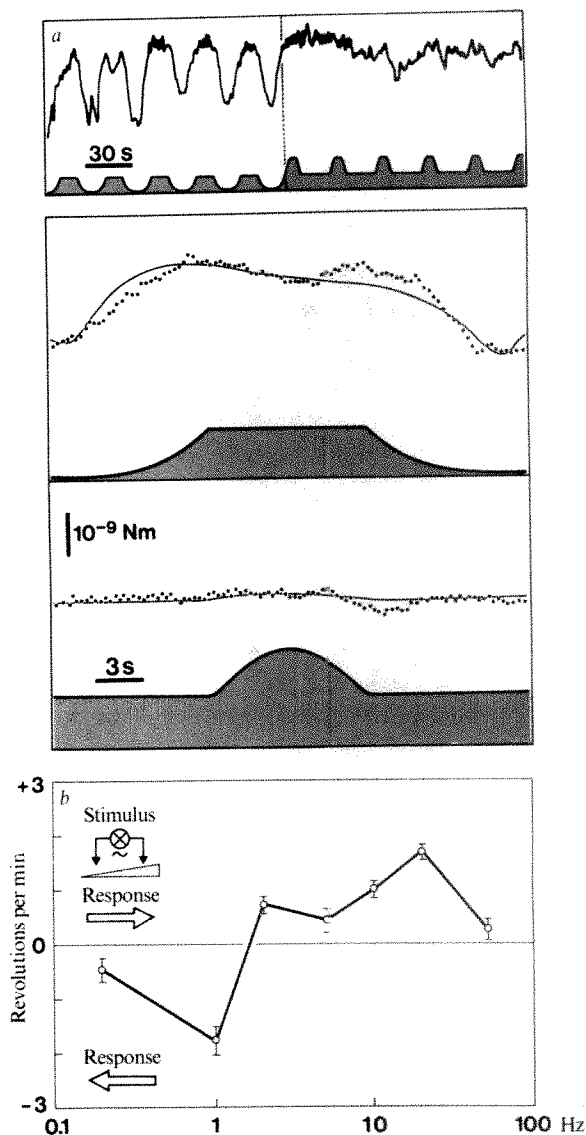


Fig. 2 *a*, Oscillatory motion illusion response of the fruitfly during fixed flight in the experimental set-up of Fig. 1*b*. The shaded area shows the actual light programme ($I_{\max} = 0.003 \text{ cd m}^{-2}$). The upper diagram shows the torque about the vertical axis produced in a single experiment. Increasing torque indicates the increasing tendency to turn towards the less transparent side of the filters, and vice versa. The dots in the lower diagrams refer to the responses of 12 flies, or the averages of 257 subsequent cycles of stimulation. The solid curves represent the motion illusion response of the model in Fig. 3. The oscillatory motion illusion of the fly is predictable and resembles the illusion induced in the human observer. *b*, Continuous motion illusion response of the stationary walking fruitfly as a function of the frequency of sinusoidal light modulation in the panorama of Fig. 1*b*. The tendency to turn was measured in revolutions per metre pathlength. The means and s.e.m. refer to the time averages of the responses of 11 flies which covered a total pathlength of 2,200 m in 250 h.

Surprisingly, the characteristics describing the apparent saturation of photoreceptors in quasi-static conditions, $X(t) \sim I(t)/(I(t) + I_0)$; $I_0 = \text{constant}$ (refs 12, 13), are apparently sufficient to elicit the expected oscillatory responses. To calculate R , the receptor output, $X(t)$, may be developed into a power series about $\bar{I}$. The first-order term accounts for a positive gain factor $I_0(\bar{I}_1 - \bar{I}_2)\Delta I/\bar{I} > 0$ and describes the illusion qualitatively. The results of a quantitative simulation are shown in the lower sections of Fig. 2*a*. The solid curves approximate the oscillatory responses of the fly with respect to (1) the light-on effect, (2) the light-off effect, (3) the phase lead on the periodic stimulus¹⁴, and (4) the decrease of the response with

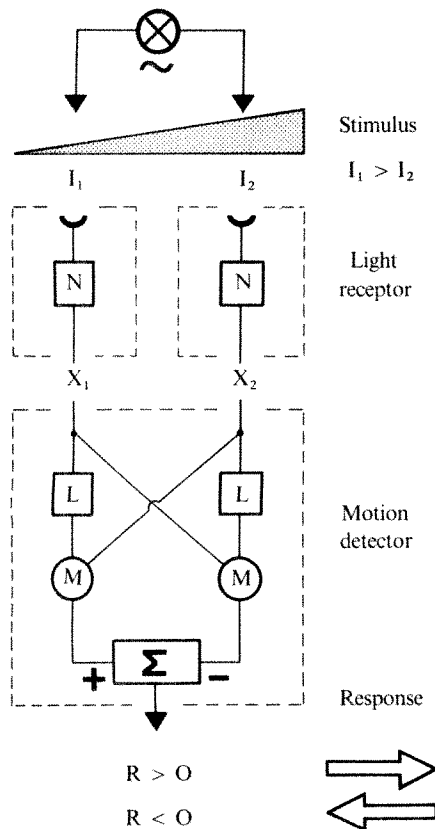


Fig. 3 Motion detector model used to describe the perception of both true and apparent motion in man and fly. The figure shows the attenuation of a sinusoidally modulated light source by a wedge filter. The simultaneous signals, I_1 and I_2 , are nonlinearly transformed (N) into the input signals, X_1 and X_2 , of a motion detector of the Reichardt-Hassenstein type⁹. The model consists of linear elements (L) for the retardation, and nonlinear elements (M) for the multiplication, of the input signals. It produces responses R of opposite sign if the direction of motion is reversed. Partition of the bi-directional motion detector into uni-directional antagonists imposes no constraints on the present results. The motion illusion obtained with the model is due to the nonlinear properties of N . The responses disappear if N becomes linear.

increasing background illumination. The matched parameters $I_0 = 0.04 I_{\max}$ of the receptor characteristics (N), and the matched time constant $T = 10$ s of the low pass filters (L), are also appropriate for the simulation of the response to true motion.

The simulated illusion decreases with progressing linearisation of the receptor characteristics at higher frequencies of stimulation. Concurrently, dynamic receptor nonlinearities become important, in particular, the prolonged latency of the light-on signal of the receptor output at lower levels of illumination^{15,16}. Latency differences between the light-on signals in the present experiments lead to successive stimulation of the input units of the motion detectors in the expected direction, towards the less transparent side of the wedge filters. The oscillatory motion illusion cannot be explained by prolonged latency. Qualitatively, there is no resemblance between the rebound of the transient light-on response $R > 0$ of the model and the marked light-off effect shown in Fig. 2a. Quantitatively, latency differences of 0–25 ms in man⁶, or 0–5 ms in the fly¹⁷, seem to be too small to contribute significantly to the oscillatory motion illusion³. However, accumulation and fusion of latency-induced transients $R > 0$ seem to account for the continuous motion illusion at higher frequencies of stimulation in Fig. 2b. The negative responses at medium frequencies are possibly due to the inversion of the brightness gradient at the edges between neighbouring wedge filters.

The results obtained permit the classification of various types of motion illusion according to their origin within the visual system. Unlike ϕ -motion² the present illusion requires nonlinear signal processing before motion detection, and is adequately, though not necessarily⁴, explained by the intensity dependence of gain and latency at the receptor level.

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Activity of an identified wind interneurone in a flying locust

LOCUST flight has been intensively studied by behavioural physiologists. The basic flight rhythm seems to be generated within the thoracic ganglia¹, although its timing is modified from wing beat to wing beat by phasic input from the wing sense organs². In this way, the basic motor score is possibly tuned to operate at the resonant, most energetically efficient frequency of the individual insect, and adjusted to accommodate changes in wing movements caused by variations of the external air current. Sensory influences on the flight motor neurones do not only originate at the wings. Weis-Fogh has demonstrated that stimulation of the wind-sensitive head hairs causes tonic excitation of these motor neurones³. Here, we have recorded in the locust during flight, the activity of two of the interneurones that convey wind information from the head hair afferents to the thoracic ganglia. These interneurones, the tritocerebral commissural giants (TCG)^{4,5}, receive their afferent wind hair input in the brain and make excitatory connections with flight motor neurones in the thoracic ganglia⁶. We show that these cells are rhythmically active during flight, synchronous with the wing beat, as a result of periodic stimulation of the head hairs. It is therefore possible that the wind-hair sensory system has a phasic influence on the flight motor as well as the already clearly established tonic influence.

We were able to record the activity of the TCG neurones during tethered flight because they take a curious path through the tritocerebral commissure, a nerve of small diameter (60 μ m) which loops under the gut and links the tritocerebral hemispheres of the brain (Fig. 1). With an axon diameter of 20 μ m, these interneurones are much larger than any of their neighbours in the commissure. This situation was exploited to make long-term recordings of the TCG at the commissure, using a chronically implanted hook electrode.

We used adult male *Locusta migratoria* from laboratory culture, selecting animals that demonstrated a strong willingness to fly. Insulated steel wires (diameter 30 μ m) were used to record from the interneurone and hind wing depressor muscle 129 in the thorax. Animals were supported at the sternum near

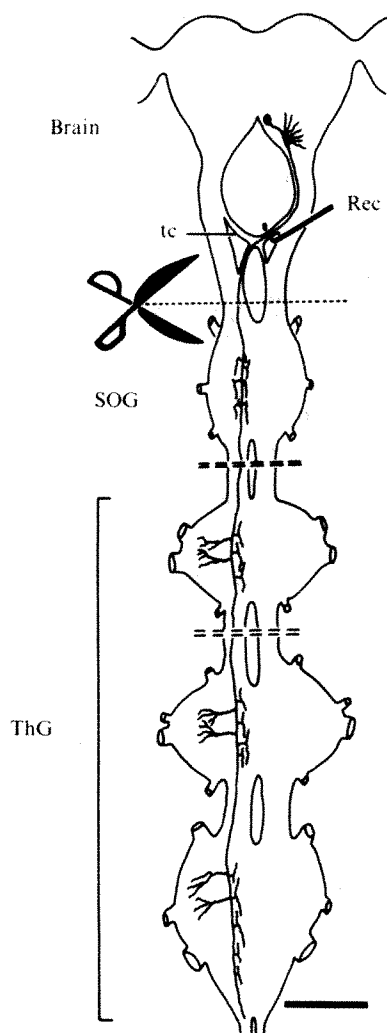


Fig. 1 Schematic view of the CNS in the head and thorax of the locust to show the structure of one of the paired TCG wind interneurons. The TCG is seen to pass through the tritocerebral commissure (tc) at which point it can be recorded with a hook electrode (Rec). The dotted line indicates the position at which the connectives were cut (see text). SOG, suboesophageal ganglion; ThG, thoracic ganglia. Scale bar, 0.5 mm.

the mouth of a wind tunnel producing a laminar air stream of 3.5 m s^{-1} .

Figure 2a shows the low level of spikes in the TCG interneurone in a resting animal exposed to a laminar air stream. In contrast, during flight the neurone displayed a much higher level of spiking activity which was rhythmical (Fig. 2b) and synchronised with the firing of a wing depressor muscle in the thorax (Fig. 2c). This effect persisted in animals flying in the dark, thus excluding the possibility that it arises from visual input produced by the movements of the wings.

The rhythmicity in the TCG neurone during flight may occur simply because the insect can sense phasic changes in turbulence around the head created by its own wing or head movements. Alternatively, the cell may receive rhythmical input from the flight motor or sense organs in the thorax, neck or abdomen. As the TCG has been observed to carry only descending information, such input would first have to reach the brain through ascending interneurons.

To investigate these two possibilities, we recorded the activity of the cell in an animal in which we had cut both circumoesophageal connectives behind the tritocerebral commissure (dotted line, Fig. 1). In these conditions of complete cessation of excitatory input from the head hairs to the flight motor neurones

in the thorax, flight was difficult to initiate and the wing beats were irregular. However, in a few cases continuous flight lasting up to 5 min could be induced. All lesions were verified after the experiment.

Figure 3 shows the results for an animal flying straight ahead. The activity of the cell was much diminished as a result of the lesion (Fig. 3a) but the infrequent spikes that occurred were synchronised with the firing of a wing depressor muscle in the thorax (Fig. 3b, c). Removing the antennae had no effect on this synchronisation.

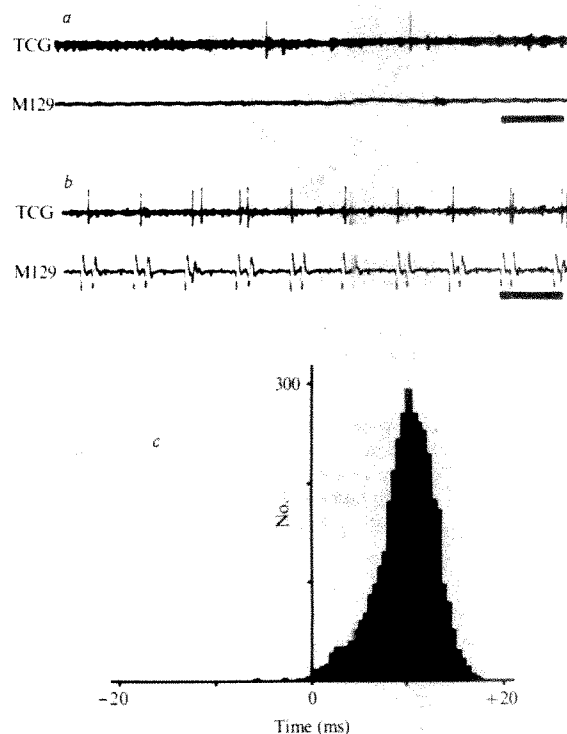


Fig. 2 Activity of the TCG interneurone (top trace) and wing depressor muscle 129 right (bottom trace) in a locust suspended in a laminar air stream. *a*, The weak response of the TCG in a resting animal. *b*, During straight flight the neurone shows rhythmical activity. Timescale, 50 ms. *c*, Histogram showing time of firing of the TCG in relation to the activity of the muscle. Abscissa: time of occurrence of interneurone spike relative to muscle spike; ordinate: number of interneurone spikes. The histogram is a record of 3,695 TCG spikes sampled during 3,500 successive wing beats.

Firing of the TCG and muscle are well synchronised.

This experiment demonstrated clearly that the TCG receives rhythmical input from the wind-sensitive hairs on the head. In addition, the lower level of firing of the TCG in this preparation, compared with that in the intact animal, showed that ascending information is also a significant input to the cell. However, other experiments in which interneurone recordings were made from animals with their heads completely waxed over (but connectives intact) showed that this ascending input contributes none of the rhythmicity during flight. We therefore conclude that the flying locust is sensitive to the phasic air currents caused by the rhythmical flight movements of its own body.

Intracellular recording has revealed that this interneurone makes functionally direct excitatory connections with flight motor neurones in the thoracic ganglia⁶. Therefore these motor neurones receive a phasic excitatory input, through the TCG, from the wind-sensitive hairs during flight. It remains to be seen, however, whether the phasic information transmitted by the TCG (and presumably by other wind-sensitive interneurons) is a behaviourally significant fast-feedback loop for the flight

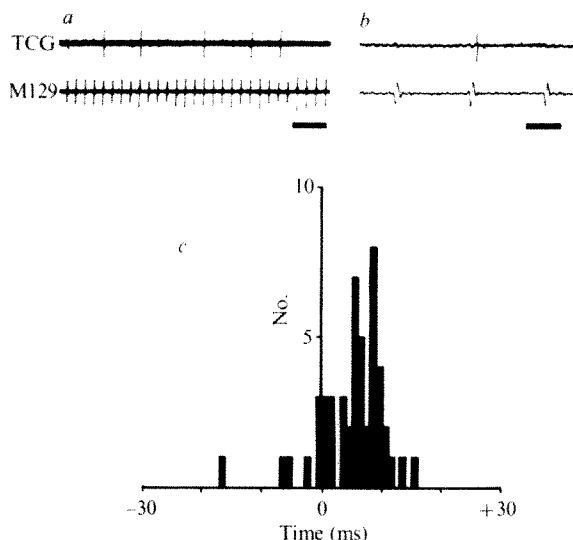


Fig. 3 Results obtained during straight flight in an animal in which both circumoesophageal connectives had been cut posterior to the tritocerebral commissure. *a*, Recordings of the interneurone (top trace) and the wing depressor muscle 129 right (bottom trace). Timescale, 200 ms. *b*, Expanded time axis showing interneurone spike in time with the contraction of the muscle. Timescale, 20 ms. *c*, Histogram showing time of firing of the interneurone in relation to the activity of the muscle, axes as for Fig. 2*c*. The data are a record of 48 TCG spikes sampled during 772 successive wing beats. Firing of the cell is fairly well synchronised with the contraction of the wing depressor muscle.

motor. Certainly, descending light-sensitive interneurons, when stimulated with a stroboscopic light, can entrain the flight frequency⁷. Perhaps some visual interneurons, able to detect the up-and-down movements of the wings, and wind-sensitive interneurons, work with the sensory inputs from the wings to set the optimum flight frequency for the insect. Alternatively, the phasic excitation that arises at the wind hairs, when conducted down the cord by many interneurons working in parallel, may arrive at the flight motor neurones as an integrated tonic excitation, and so function simply as a positive feedback mechanism to maintain flight. Further investigations using this preparation present exciting possibilities for a more detailed analysis of the neural basis of flight.

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The segmentation of axons from the segmental nerve roots to the chick wing

THE chick wing is innervated by adjacent spinal roots which meet at the brachial plexus and then redistribute their axons to the peripheral nerves. Both the anatomical arrangement of nerves within the wing and the innervation territories supplied by each root are constant among animals of the same strain¹⁻³. We describe here the pathways taken by the axons from each of the main roots to their particular territories in the normal wing, and also in wings which have dorso-ventral and antero-posterior axes reversed relative to the host axes. Our observations suggest that the characteristic innervation territories of the segmental roots may arise from two basic rules: that axons travel in parallel over long distances without crossing, and that the branching pattern of nerves within the limb is determined by the local environment^{3,4}.

The motor and sensory innervation territories of the three segmental roots were mapped electrophysiologically *in ovo* at 17 d of incubation. Sensory innervation maps were constructed by mechanically or electrically stimulating localised regions of skin while recording from each nerve root in turn (Fig. 1). The motor innervation was mapped by stimulating each root in turn

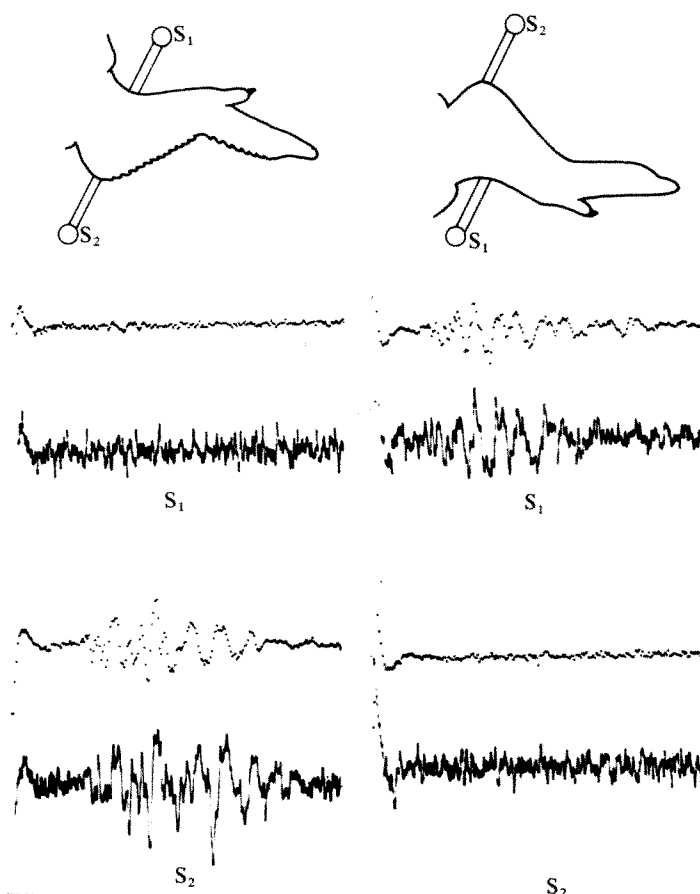


Fig. 1 Responses, recorded from the peripheral cut end of n16 using suction electrodes, to electrical stimulation of the skin in the normal (to the left) and rotated (to the right) wings of 578. Positions of the bipolar stimulating electrode (0.5-ms pulse) are shown at the top of each column. The top trace in each pair shows the averaged response to 16 consecutive stimuli, the bottom trace the response to a single pulse. Calibration bar, 10 ms.

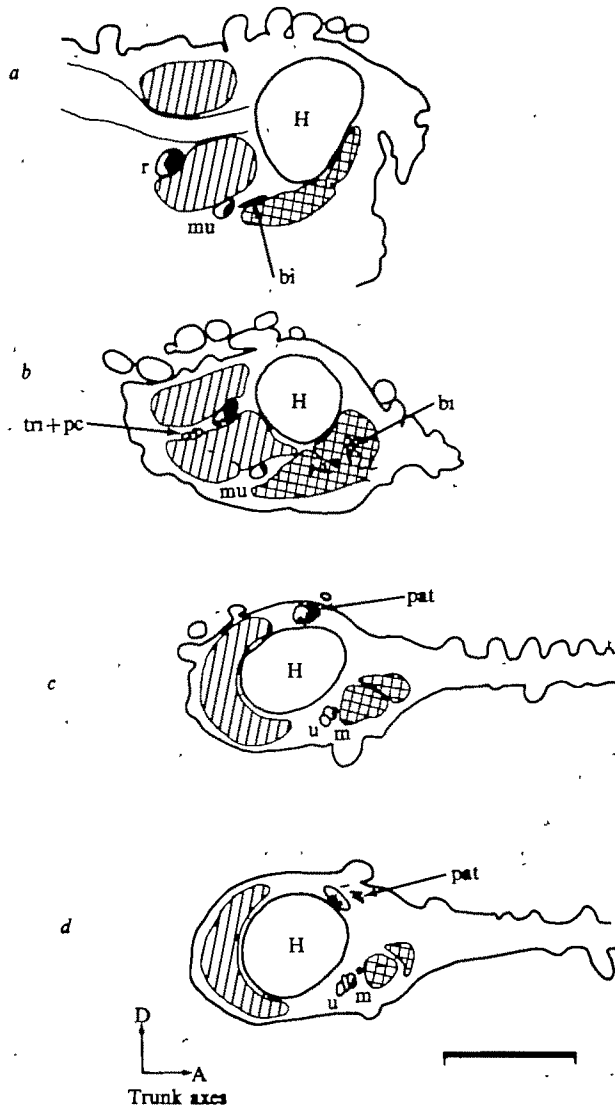


Fig. 2 Normal wing, n14 filled. Camera lucida drawings of transverse sections at four levels, all viewed from the elbow. Filled axons are shown in black; they occupy the anterior sectors of the radial and medio-ulnar nerves. Trunk axes shown, A, anterior; D, dorsal; calibration bar, 1 mm. *a*, Biceps nerve contains axons from n14 (arrowed). *b*, Triceps nerve (arrowed) has no filled axons, but n14 axons are clearly visible in the biceps muscle (arrowed). *c*, Filled axons leave the radial nerve at the patagial nerve. A photograph of this is shown in Fig. 4*a*. *d*, Patagial nerve with filled axons going to the web of the wing (arrowed). Biceps muscle cross-hatched, triceps muscle hatched. LAT DORS, latissimus dorsi muscle; EMR, extensor metacarpalis radialis; H, humerus; r, radial nerve; tri, triceps nerve; pc, posterior cutaneous nerve; m, median nerve; u, ulnar nerve; pat, patagial nerve.

while observing electrical events accompanying muscle contraction. In all animals of our strain (White Leghorns), the 14th segmental root (n14) innervates the proximal anterior region including the biceps muscle, and the 16th segmental root (n16) supplies the posterior and distal regions including triceps. The territory of n15 lies in between and overlaps both.

In our experimental animals the wing buds were rotated 180° at stage 19, a significant time before the axons invade the bud. These limbs develop autonomously^{3,6} with dorso-ventral and antero-posterior axes reversed with respect to the host. For simplicity we will refer to the axes of trunk and wing separately; thus, in control animals these axes are the same and in operated animals the wing axes are opposite to those of the host trunk. When we map the sensory and motor innervation pattern of these animals we find that n14 now goes to the posterior and

distal parts of the wing (including triceps and the hand), whereas n16 innervates the anterior region of the grafted wing (including biceps and the alar web). The pattern of innervation of the rotated limbs is therefore (within the limits of variation found in control animals) the converse of that in the normal wing (Fig. 1).

Dissection of animals after recording shows that the spinal roots meet to form a seemingly normal plexus in the trunk which

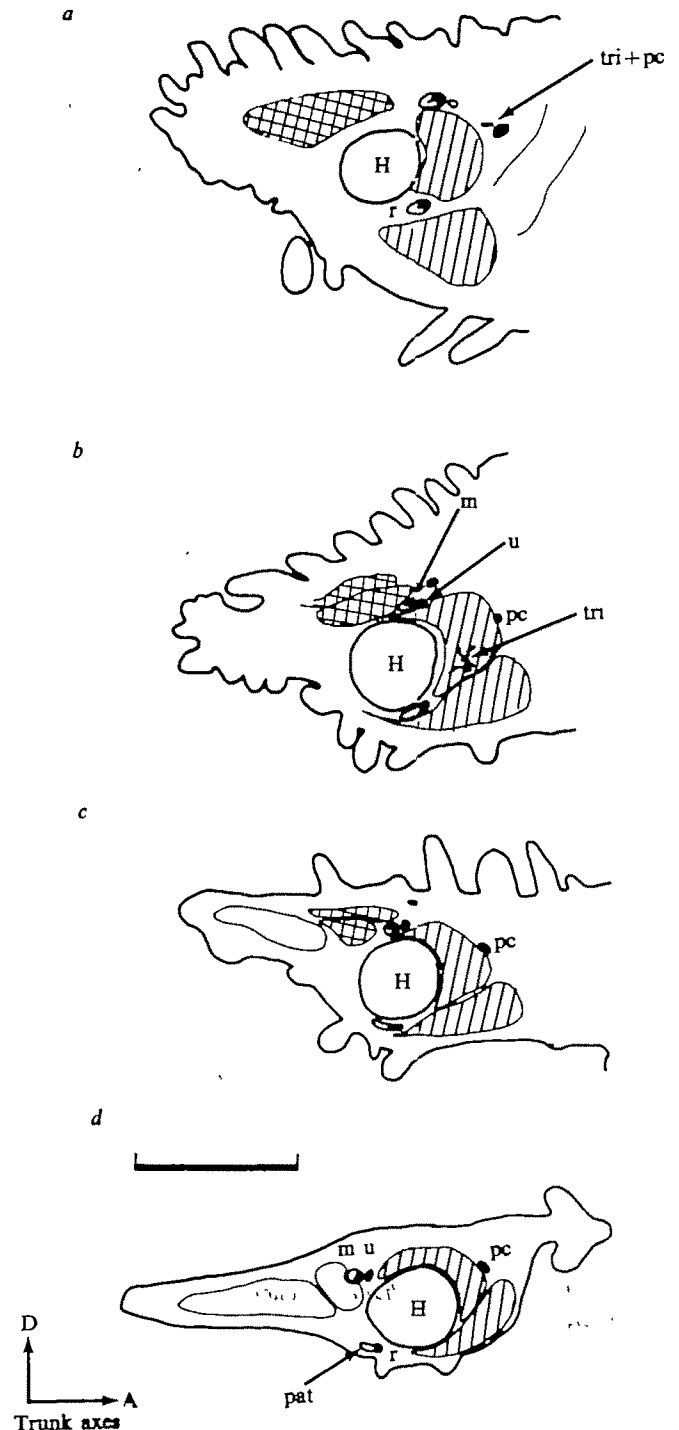


Fig. 3 Rotated wing, n14 filled. Camera lucida drawings of transverse section at four levels, all viewed from the elbow. Trunk axes are as shown, wing axes are the reverse, that is, anterior to the left and dorsal downwards. Filled axons are shown in black, they occupy the posterior sector of nerves in the graft. *a*, Triceps and cutaneous nerves with filled axons (arrowed). *b*, Triceps muscle contains filled axons (arrowed), biceps does not. Filled axons are also present in median and ulnar nerves (arrowed). *c*, Fascicle of patagial nerve begins to separate from radial nerve. *d*, Unfilled axons leave the radial nerve to form patagial nerve (arrowed). A photograph of this is shown in Fig. 4*c*. Abbreviations as for Fig. 2.

produces the normal main dorsal and ventral branches to the wing. On entering the rotated graft the dorsal branch apparently behaves as if it were the ventral branch forming the medio-ulnar nerve. The ventral branch behaves as if it were the dorsal branch forming the radial nerve. Thus, the anatomical arrangement of nerves within the graft is very similar to that found in the normal wing.

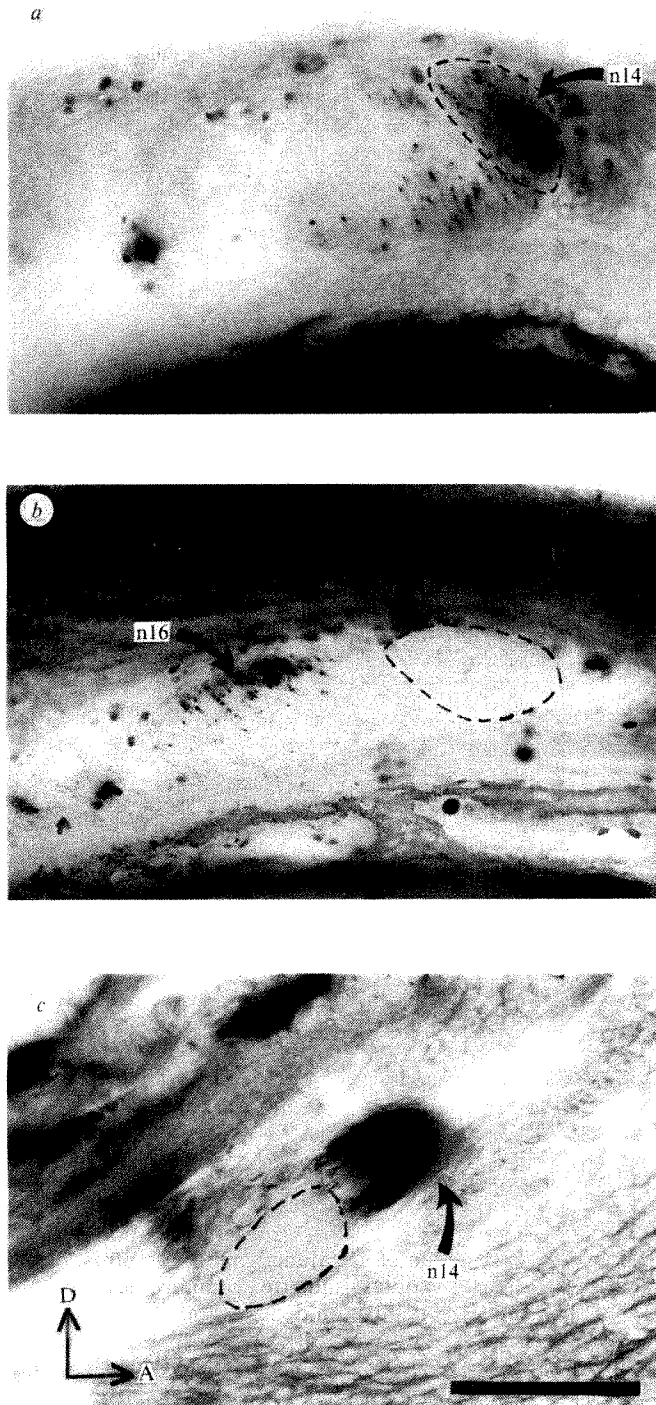


Fig. 4 Photomicrographs of transverse sections of radial nerve including the branch point of the patagiales nerve. The arrows at the bottom indicate trunk (host) axes for all photographs; D, dorsal; A, anterior. The sector surrounded by the dotted line forms the patagiales nerve. The arrows indicate filled axons. Calibration bar, 100 μ m. *a*, Radial nerve from section shown in Fig. 2c, n14 filled. *b*, Radial nerve from section taken from a similar level to Fig. 4a, in which n16 had been filled. *c*, Radial nerve from section shown in Fig. 3d. There are no filled fibres in the fascicle giving rise to the patagiales nerve although n14 was filled.

The anatomy and physiology of the rotated wings suggest that although the pattern of nerves is determined by the environment, the arrangement of axons within the nerves is non-selective. To test this directly we have traced the connections anatomically by filling the axons of each root in turn with cobalt chloride, which can subsequently be visualised after treatment with H_2S and silver intensification⁷⁻⁹. When we fill n14 in the normal wing, we find that its axons maintain their anterior position through the plexus and into the main dorsal and ventral nerves, whereas axons from n16 maintain a posterior position, and axons from n15 lie in the region between the two and overlap both. The distribution of axons within the nerves as they enter the wing is therefore related to their spinal root origin. We further find that the axons continue to run in parallel, maintaining their positions over long distances, certainly to below the elbow, so that the cross-sections of each nerve consist of sectors which have relatively homologous axonal origins (Fig. 2). When a branch leaves the main nerve from its anterior aspect it contains axons which have been running in the anterior sector (Fig. 2c, 4a). When a branch leaves the main nerve posteriorly it contains axons from the posterior sector (Fig. 4b). Thus, n14 axons enter branches to the alar web and the biceps, whereas n16 enters posterior branches such as the triceps and ulnar nerves.

In the rotated wings the axons of n16 enter the brachial plexus in the trunk in their normal posterior position with those of n14 in their normal anterior position. This arrangement is maintained through the plexus and into the graft (Fig. 3). Thus, the axons of n14 come to occupy posterior sectors of the wing dorsal and ventral nerves, and are then channelled into wing posterior branches, for example, triceps and ulnar nerves (Figs 3, 4c). Conversely, axons from n16 from their posterior sectors in the trunk nerves come to occupy anterior sectors of the wing nerves and travel down anterior branches to the alar web and biceps.

There is no accepted theory to explain how each segmental root comes to innervate its particular limb territory. Recently, ideas have polarised to two extremes: target specificity where axons actively seek out their 'appropriate' targets, or initial random outgrowth with 'inappropriate' axons being subsequently eliminated by cell death¹⁰⁻¹⁴. Our results suggest that the pattern of innervation results from the way axons travel together in parallel over relatively long distances and the manner in which the segmental roots combine together in the plexus. The fate of individual axons (once they have taken up a certain position in the plexus) is determined by the peripheral branching pattern, and this is clearly controlled by the environment rather than by the nervous system. We do not have any information on the spinal cord anatomy and the next stage of our investigation is to observe the motor neurone pools supplying such rotated grafts.

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Single glutamate-activated channels in locust muscle

EVIDENCE indicates that glutamate-activated membrane channels (glutamate channels) are responsible for the post-synaptic potential at insect¹ and crustacean² excitatory neuromuscular junctions. They may also be involved in the regulation of pre- and postsynaptic activity in the central nervous system of vertebrates^{3,4}. Analysis of the current noise produced by application of glutamate to excitatory synapses on locust muscle suggested that glutamate channels have a conductance of about 125 pS and a mean open time of about 2 ms (refs 5, 6). Direct measurements of the currents passing through individual acetylcholine-activated channels (ACh channels) in

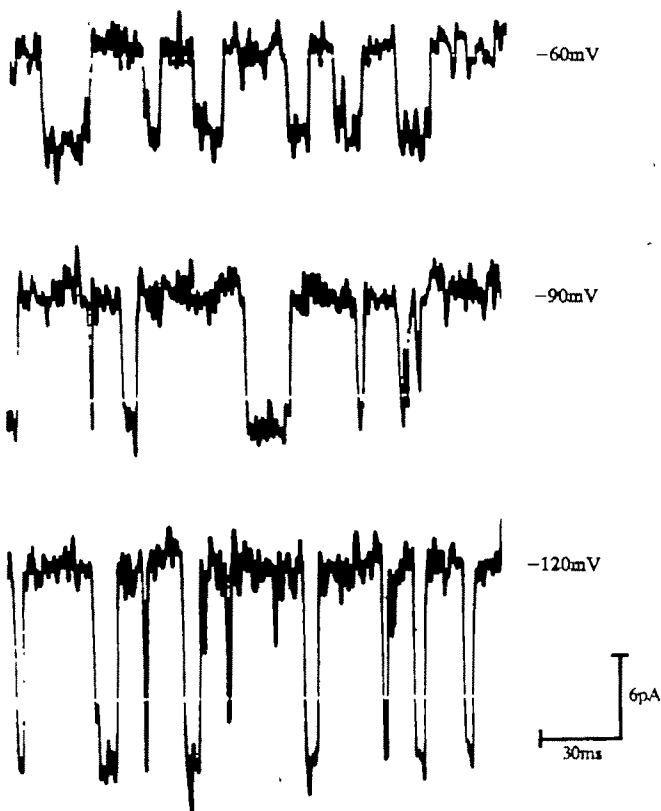


Fig. 1 The currents through single glutamate-activated channels in the metathoracic extensor tibiae muscle of a 6-d denervated locust (*Locusta migratoria*). The muscle cell was voltage clamped with standard techniques, and the membrane potential held at the values shown next to each trace. A patch electrode filled with physiological saline containing 100 μ M L-glutamate was pressed on to the surface of the cell, and measured the currents passing through the muscle membrane under the patch. The electrode had a 2- μ m fire-polished tip which had a free-solution resistance of about 2 M Ω . When pressed on to the cell, the resistance increased to more than 10 M Ω , electrically isolating the membrane patch with a voltage divider ratio (leak to series resistance) of more than 5. The downward events are currents passing from the electrode into the cell. The background noise is the Johnson noise expected for the combination of the series and leak resistances. The patch electrode was placed within a cell diameter of the intracellular voltage electrode. Currents measured from the patch electrode were amplified and stored on an analogue FM tape recorder. The illustrations were made by play-back of the data through an active 4-stage filter ($f_c = 450$ Hz) with Bessel characteristics. All three records were from the same patch. The temperature was 21 $^{\circ}$ C. Standard locust physiological saline was used for all experiments. The cell had been previously treated for 15 min with 1 μ M Con A in saline, then washed with normal saline for more than 30 min. No single channel responses could be measured when the glutamate was omitted from the patch electrode.

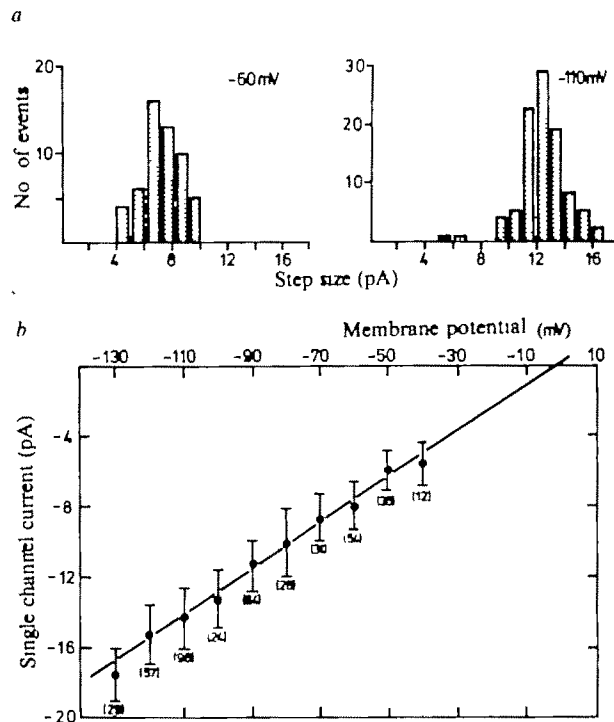
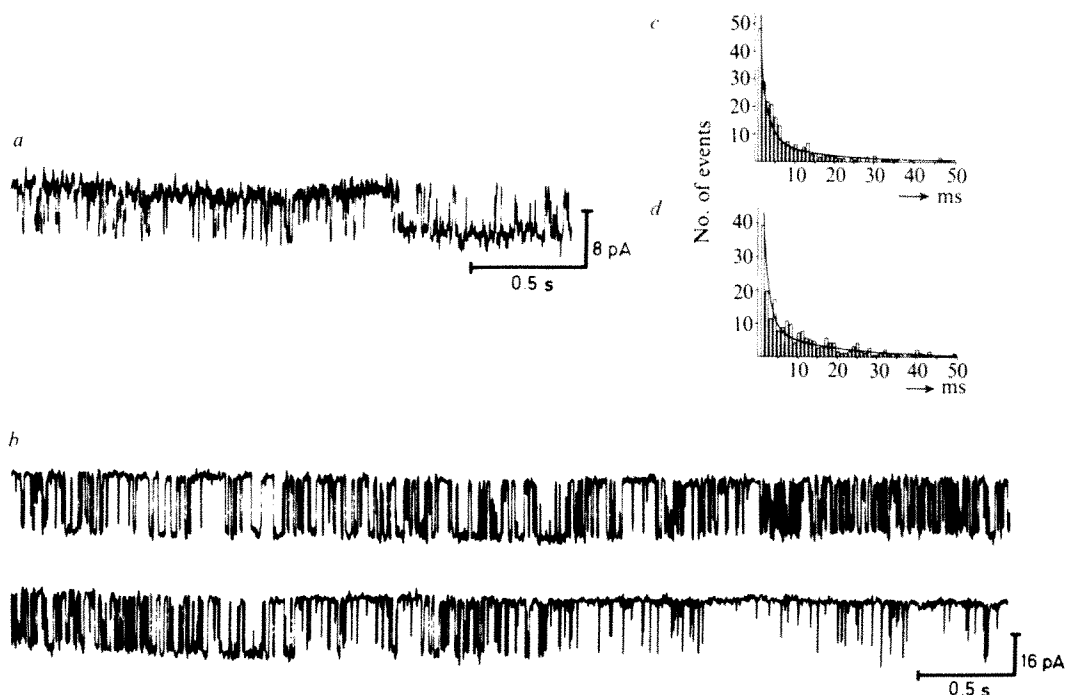


Fig. 2 Current-voltage characteristics of single glutamate channels. Patch recordings at various different membrane potentials were sampled with a PDP 11/34 computer, and channel amplitudes measured as the difference between the average value of the current 2 ms before and after individual current steps. *a*, Histograms of measured step sizes at two membrane potentials, -60 and -110 mV. Channel amplitudes measured by hand from strip chart recordings gave essentially the same distributions as those illustrated here. *b*, The mean of the measured step sizes and the standard deviations plotted as a function of the holding potential. Numbers in brackets are the number of individual events analysed at each potential. The solid line is the least-squares fit to the mean value at each holding potential. The slope of this line was 133 pS. The zero current intercept was -2.5 mV. The recording conditions were the same as described in Fig. 1.

vertebrate muscle membrane have recently been made. The single channel conductance (about 25 pS) and kinetics correspond well with those inferred from noise analysis^{7,8}. We report here the measurement of the currents passing through individual glutamate channels in locust muscle. The conductance of an open single channel is about 130 pS. The gating kinetics are complex, and evidence is given for sudden changes in an individual channel's distribution of open and closed times.

Single glutamate channels have been measured on metathoracic extensor tibiae and retractor unguis muscles in adult locusts (*Locusta migratoria*) by placing a patch electrode containing low concentrations of glutamate against the surface of the cell. However, due to a low channel population density and rapid desensitisation of extrajunctional D receptors, these records were difficult to obtain and often contained less than 10 individual events. The population density of extrajunctional D receptors was increased by using muscles denervated for at least 6 d (refs 9-11). Nevertheless, even with denervated muscle, desensitisation was often complete before the patch electrode could be properly sealed against the membrane. Therefore, most studies were made on extrajunctional receptors of chronically denervated muscle which was pretreated with concanavalin A (Con A). Brief exposure of the cells to this lectin irreversibly prevents the desensitisation of junctional glutamate and extrajunctional glutamate D receptors on locust muscle^{12,13}. Pretreatment of the muscles with 1 μ M Con A in physiological saline for 15 min, followed by a thorough rinse, enabled us to make patch recordings which exhibited a stable level of channel activity for up to 30 min or longer.

Fig. 3 Complex kinetics of individual channels. *a*, A sudden kinetic state change in the activity record of what seems to be one channel. This record was made at the resting potential of this cell, -45 mV, without voltage clamp. The cell was from a 6-d denervated animal, and had been Con A treated. The mean channel open time before the transition was 2.8 ± 0.3 ms. After the transition it was 22.5 ± 4 ms. The probability that these events were from the same population, but distributed by chance, was $P < 0.001$. The mean closed times were 16.6 ± 2.1 ms, and 3.0 ± 0.5 ms, corresponding to a $P < 0.001$. *b*, a 6-s record of a channel's activity from a cell voltage clamped at -120 mV. The two segments



are contiguous. A broad variety of kinetic states are represented in this record. Note especially the long segment with a high frequency of fast activity, and the change to the ground state at the end of the segment. Both *a* and *b* were made by replaying pre-filtered data at a 60 times slower tape speed and recording on a normal strip chart recorder. f_c was 350 Hz. The reduced amplitude of some events was due to filter attenuation. Note the difference between the calibration scales in *a* and *b*. *c*, Histogram of the channel open times from the data segment in *b*. Open times were measured from digitised data as the time difference between the first downward deflection of the current and the next following upward deflection. All pattern recognition and signal-to-noise judgments were made by eye. Only transitions greater than 2–3 times the noise amplitude were measured. The data were unfiltered. The histogram was fitted with two exponentials using a non-linear, least-squares fitting program. The best fit was obtained with $\tau_{\text{fast}} = 1.8$ ms, $\tau_{\text{slow}} = 12.3$ ms and an amplitude ratio of 7.0. The normalised χ^2 for this fit was 1.0. *d*, Histogram of channel closed times made concurrent with *c*. The best fit was obtained with $\tau_{\text{fast}} = 1.6$ ms, $\tau_{\text{slow}} = 16.3$ ms, and an amplitude ratio of 6.2. χ^2 was 1.2.

Figure 1 shows glutamate channels in a 6-d denervated muscle which had been treated with Con A. The downward events are the sudden increase and subsequent decrease of inward currents. These currents are due to the opening and closing of individual channels. The current which flows through the open channel is essentially constant and depends on the membrane potential. At -60 mV, the approximate resting potential of the cell, this current is about 8 pA. At -120 mV, the single channel current is almost twice this value.

Single channel amplitudes are uniform in most cases. Uncertainties of measurement of these amplitudes are caused by the limited frequency response of the system and by the background noise, leading to some spread in the measured values. Figure 2*a* shows histograms of the channel currents at two different membrane potentials. The distribution centres roughly around a peak value, as in a gaussian distribution. Figure 2*b* is a plot of single channel current levels against membrane potential.

The conductance of the open channel seems to be independent of the membrane potential within the range of our measurements, as might be expected of a simple resistor. The points in Fig. 2*b* are fitted well by a straight line, whose slope corresponds to the conductance of the channel. The least-squares fit to the data points yields a line which has a slope of 133 pS and which has a zero-current potential very close to 0 mV. The extrapolated zero-current potential corresponds well with that of the miniature excitatory junctional currents (m.e.j.cs) in this cell. The m.e.j.cs have been shown to reverse at 0 to +5 mV, as do the responses to iontophoretically applied glutamate¹⁴.

The kinetics of the channel's open–close reaction are not stationary in these conditions. This inconstancy is most clearly observed during recordings where the frequency of the simultaneous opening of two or more channels (double events) is very low or zero. When two independent channels are open simultaneously within the measurement area the current measured should be double the normal current. The frequency of double

events is predicted by a binomial distribution, and for the high levels of activity illustrated here, this frequency is substantial. As few double events are observed, it is most probable that only one channel is active within the patch area. Recordings made in these conditions show that the channels can sometimes shift from one kinetic 'state' to another. Figure 3*a* shows one such transition. Both the mean open time and the mean closed time have suddenly shifted. Neither is likely to have come from the same exponentially distributed interval population found before the transition (see legend to Fig. 3). This segment is a small part of a recording lasting several minutes which contains virtually no double events. Transitions such as those in Fig. 3*a* occur very frequently in our records, and have been observed at several different membrane potentials.

Several different kinetic states have been observed in long records of what seems to be the activity of only one channel. The most common state is that shown in the beginning of the trace in Fig. 3*a*. In more than 90% of our measurements the activity was similar to that illustrated, that is, opening events with short (< 5 ms) durations, separated by relatively longer (> 20 ms) closed intervals. Histograms have been made of channel open times in long segments of data which seem to be only in this 'ground' state, and these open times are roughly exponentially distributed. However, the range of time constant values for the best fit exponentials vary from 1 to 3 ms, in many cases in identical conditions. These values are similar to those measured by noise analysis of the synaptic receptor kinetics and by the decay of m.e.j.cs⁵.

During records in which a channel's activity is not in the ground state, both the open and closed times are multi-modal. Figure 3*b* is an example of this type of activity. As was the case in the record shown in Fig. 3*a*, the number of double events was very low for several minutes before and after the illustrated record. Both the frequency of events and the event duration change many times during this segment, with an apparent return to the ground state at the end of the record. Figure 3*c* and *d* are

histograms of the open and closed intervals during this burst. Both histograms could only be adequately fitted by curves consisting of at least two exponentials. The lines shown are the least-squares fit to the data by double exponential curves. The various time constants are given in the legend to Fig. 3. The data indicate that the kinetics of this channel are the result of a complex process. Models of this channel's activity must take this into account. Furthermore, this multi-modal activity does not predict the relatively prolonged kinetic state changes observed in the channel's activity. These state changes must also be accounted for in a complete model of glutamate channel kinetics. It is not yet possible to determine whether the presence of Con A is responsible for the appearance of these multiple states. In several records made without Con A treatment, the channels seemed to be in the ground state. However, each of these records was too short, due to rapid desensitisation, to demonstrate the absence of other states without Con A.

In conclusion, direct measurement of the currents which pass through single glutamate-activated membrane channels in the locust muscle demonstrates that these channels have a conductance of about 130 pS and multiple kinetic states of channel gating. This conductance is almost five times larger than that of the ACh channels at the vertebrate neuromuscular junction, and is among the largest yet found in biological membranes. The measured conductance agrees well with that inferred from previous noise analysis in this system⁶. Our measurements also directly demonstrate that the conductance of the open channel is independent of voltage between -130 and -40 mV, and that the extrapolated reversal potential is very close to 0 mV, as has been suspected for this channel. We also measure a kinetic state of this channel which is very common, and which has a distribution of channel open times similar to those found for synaptic channels. Other kinetic states also occur in individual channels, often with sudden inter-state transitions. It has not been possible to determine if these states are also a property of the normal synaptic glutamate channel, or if they are induced either by the presence of Con A or by denervation. In either case, the observation that a single channel can suddenly change its kinetic properties is new, and must be investigated further. The patch electrode technique is the only method with resolution sufficient to clarify this problem further.

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Effects of oxotremorine demonstrate presynaptic muscarinic and dopaminergic receptors on motor nerve terminals

THERE has been little conclusive evidence about the presynaptic effects of acetylcholine (ACh) and cholinomimetics on mammalian motor nerve terminals^{1,2} which may have functional implications in motor abnormalities^{3,4}. We now report the existence of dopaminergic muscarinic receptors on motor nerve terminals which participate in the local release of ACh from motor nerve terminals.

Details of the collection and assay of ACh released from isolated rat phrenic nerve into the bathing fluid have been given elsewhere⁵. Two samples (control and experimental) were collected from each preparation and assayed immediately (cross-over) on leech dorsal muscle, the bathing solution containing equivalent concentration(s) of the experimental drug(s). We also examined the effect of 0.5 μ M atropine on the action potentials of the phrenic nerve.

Oxotremorine sesquifumarate (Oxo-T)—a muscarinic agonist with no post-junctional effect on the diaphragmatic muscle⁶—induces transmitter release from phrenic nerve endings⁷ and thus serves as an excellent tool for studying the cholinergic presynaptic mechanisms at this site. The dose-dependent effect of Oxo-T in increasing ACh release and its prevention by preincubation with atropine (Table 1) demonstrate the presence of presynaptic muscarinic receptors on motor nerve terminals. Incubation with 0.5 μ M atropine alone

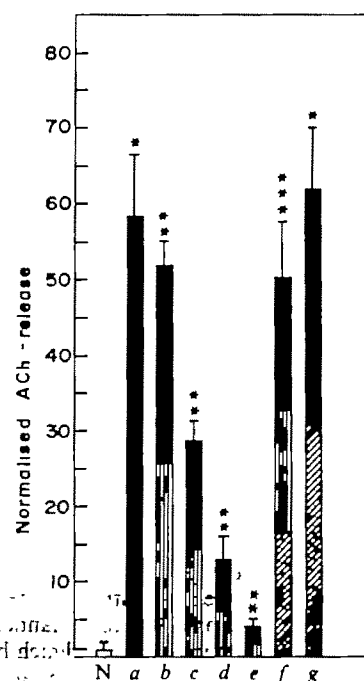


Fig. 1 Histogram showing: a, increase in ACh release caused by 10 μ M Oxo-T sesquifumarate; b, c, d and e, dose-dependent inhibition of a by dopamine hydrochloride (0.005, 0.02, 0.04 and 0.05 μ M, respectively); f, reversal of e in presence of 0.25 μ M pimozone; g, influence of 0.25 μ M pimozone on a. All values are normalised to the mean control ACh release during 15-min periods of electrical stimulation ($N = 4.05$ ng). Values represent means of at least six experiments $\pm$ s.e. One asterisk denotes significant increase from N ($P < 0.05$); two asterisks denote significant reduction from a ($P < 0.05$); three asterisks denote significant increase from e ($P < 0.05$). More than one symbol per bar indicates respective drug interactions. Black indicates Oxo-T; vertical hatching indicates dopamine, and cross hatching indicates pimozone.

Table 1 ACh released by different treatments

Treatment	ACh released (ng)
Control†	4.05 ± 0.73
Atropine sulphate, 0.5 µM	Not measurable (necessarily <1.25)
Oxo-T sesquifumarate, 2.5 µM†	135.14 ± 20.16*
Oxo-T sesquifumarate, 5 µM	208 ± 20.7*
Oxo-T sesquifumarate, 10 µM†	236 ± 33.12*
Atropine sulphate, 0.5 µM plus Oxo-T, 10 µM	Not measurable (necessarily <1.25)
Dopamine hydrochloride, 0.05 µM	4.0 ± 1.1
Dopamine hydrochloride, 5 µM	Not measurable (necessarily <1.25)
Pimozide, 0.25 µM	3.2 ± 0.42

Figures show ACh released, in the form of ACh chloride, into a 4-ml bath from rat phrenic nerve terminals in response to supramaximal stimulation (0.2 ms) at 1 Hz for 15 min in different experimental conditions. Values represent means of at least six experiments ± s.e. Concentrations of drugs are expressed in terms of molarity of the salts.

* Significant difference from control ($P < 0.005$).

† Data taken from ref. 3.

in control experiments inhibited the evoked ACh release to a level below the sensitivity range of our preparation (Table 1).

Dopamine hydrochloride by itself at 0.005–0.05 µM did not affect the normal evoked ACh release (Table 1), but it inhibited, as a function of its concentration, the increased release due to Oxo-T (Fig. 1). In the presence of a greater concentration of dopamine (5 µM), the normal evoked release of ACh was inhibited to a degree that could not be measured (Table 1).

The presence of dopamine receptors with an inhibitory influence on muscarinically (Oxo-T) mediated ACh release was demonstrated by reversal of the dopamine effect in the presence of 0.25 µM pimozide, a specific dopamine-receptor-blocking agent (Fig. 1). Incubation with pimozide alone, unlike atropine, did not affect the normal evoked and Oxo-T-induced release of ACh in control experiments (Table 1 and Fig. 1).

Our results demonstrate for the first time the presence of presynaptic dopaminergic muscarinic receptors on motor nerve endings. A function has been proposed for presynaptic muscarinic receptors at cholinergic sites in the brain⁵ and in the periphery⁶, apparently at variance with our findings. In such studies Oxo-T decreased ACh release and prevented the facilitatory effect of atropine. On the other hand, excess ACh release due to Oxo-T at both central and peripheral sites is well documented^{3,7–9}. Thus it is not clear whether the reported inhibition of ACh release by Oxo-T is mediated through a direct or an indirect mechanism. Indeed incubation with eserine inhibited ACh release from cerebral cortical slices^{10,11}.

The inhibition of normal evoked release of ACh by atropine, at a concentration that failed to affect phrenic nerve action potentials, indicated that the presynaptic muscarinic receptors participate in ACh release from motor nerve endings. Although a similar effect of atropine was reported earlier², its mechanism of action remained obscure. Our study raises the possibility that locally liberated ACh from motor nerve terminals activates the presynaptic muscarinic receptors, leading to further release. The inability of pimozide to increase normal evoked ACh release (Table 1) shows that the inhibitory dopaminergic sites on the motor nerve terminals do not normally influence transmitter release. This is expected because dopamine is not known to be liberated at this site. But in the light of our findings it seems plausible that systemic circulatory dopamine, more so in subjects receiving L-dopa therapy, must modulate ACh release from motor nerve endings, if not at cholinergic synapses in general. This presynaptic dopamine action may be of therapeutic significance because presynaptic cholinergic dominance at the neuromuscular junction and at the junction of motor axon collaterals and Renshaw cells in the spinal cord have been

clearly implicated^{4,12–14} in the 'Oxo-T-model'^{15,16} of parkinsonism. Indeed L-dopa pretreatment was found to protect Oxo-T-induced tremor to some extent^{17,18} and to inhibit the antidromically activated spike discharges of single Renshaw cells¹⁹. Our findings support the hypothesis of presynaptic modulation of ACh release by dopamine at cholinergic sites^{20,21}.

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Decreased content of immunoreactive enkephalin-like peptide in peripheral tissues of spontaneously hypertensive rats

THE dopaminergic modulation of neuronal transmission in coeliac ganglia of spontaneously hypertensive rats (SHR) has been shown to be less efficient than in control Wistar Kyoto rats (WKY). From these results it was inferred that the development of hypertension may be due to this defect. Recent reports^{2,3} suggest that polypeptides may function as neurotransmitters or neuromodulators in sympathetic ganglia. A heterogeneous group of immunoreactive Met⁵-enkephalin-like (ME) peptides have been found in these ganglia³, and are also present in adrenal glands, in both chromaffin cells and afferent axons (unpublished observations). Some of these peptides, when released from the afferent axons, may modulate opiate receptors located in adrenal medulla (unpublished observations). The vasodilatory action of morphine suggests that if the opiate agonists present in the chromaffin cells were to be secreted they might also cause vasodilation by acting on distant receptors. In view of their possible role in the regulation of sympathetic transmission and vascular tone, we have studied the content and chromatographic characteristics of the immunoreactive ME-like peptides in adrenal gland, sympathetic ganglia, salivary gland and hypothalamus of SHR and WKY rats. We report here

Table 1 The content of ME- and LE-like material in various tissues of WKY and SHR rats

Rat strain	Rat age (weeks)	Coeliac ganglion		Superior cervical ganglion	Salivary gland	Adrenal gland	Hypothalamus
		ME	LE	ME	ME	ME	ME
WKY	4	3.4±0.31 (7)	1.8±0.097 (7)	1.9±0.12 (7)	—	—	—
SHR	4	2.0±0.16* (8)	1.1±0.048* (8)	1.9±0.059 (8)	—	—	—
WKY	8	2.0±0.12 (15)	1.2±0.051 (10)	1.3±0.11 (5)	0.090±0.012 (5)	0.046±0.0081 (5)	3.7±0.14 (10)
SHR	8	0.95±0.028* (15)	0.68±0.030* (10)	0.78±0.20† (5)	0.045±0.010† (5)	0.020±0.004† (5)	3.3±0.17 (10)
WKY	12	1.7±0.14 (5)	—	1.4±0.081 (5)	—	—	—
SHR	12	0.93±0.082† (5)	—	0.93±0.18† (5)	—	—	—

Immediately after dissection, the ganglia were added to boiling 0.1 M CH_3COOH and heated for 3–5 min at 100 °C. The neutralised supernatant was then radioimmunoassayed for ME and LE. Adrenal, salivary glands and hypothalamus were treated by microwaves and extracted with 0.1 M CH_3COOH . Values are expressed as ng per mg protein. No difference was noted in the weights or the protein content of the various tissues from WKY or SHR.

* $P < 0.01$; † $P < 0.05$.

that the content of these peptides was lower in SHR than WKY rats in all tissues studied, except the hypothalamus. In sympathetic ganglia this decrease was due to a reduction in the ME content and not to that of the high molecular weight (MW) immunoreactive ME-like peptides.

Male 3-week-old SHR and WKY rats (Thaconic Farm) were reared in our animal facility in optimal conditions of illumination (12 h light/12 h dark), temperature (24 °C) and humidity. At various ages, an equal number of the SHR and WKY animals were killed by decapitation and exsanguinated. Table 1 shows that SHR already have a lower concentration of immunoreactive ME-like peptides in the coeliac ganglion at 4 weeks after birth, when the blood pressure of WKY and SHR rats is similar¹. Immunoreactive ME-like peptide content is also decreased (Table 1) at 12 weeks in the coeliac and superior cervical ganglia of SHR when their blood pressure is pathologically higher than that of WKY. Also, the coeliac content of Leu⁵-enkephalin-like (LE) polypeptides in SHR is smaller than that in WKY animals. The content of immunoreactive ME-like peptides in the adrenal and salivary glands was also significantly smaller in SHR than in WKY rats (Table 1). In contrast, immunoreactive ME-like peptide content in the hypothalamus was similar in 8-week-old SHR and WKY rats.

The various molecular forms of immunoreactive ME-like peptides present in sympathetic ganglia include a low MW polypeptide which has previously been shown to be immunologically and chromatographically indistinguishable from ME. We wondered whether in SHR animals there was a

reduced content of ME or of the immunoreactive ME-like high MW polypeptides. Therefore, we extracted the sympathetic ganglia with absolute alcohol which selectively solubilises a polypeptide which on thin layer chromatography behaves like ME, leaving the other high MW immunoreactive ME-like peptides undissolved. We found that a coeliac ganglion of a 12-week-old WKY rat contains about 0.27 ng ME, whereas the homologous ganglion of SHR rats contains only about 0.18 ng ME. A superior cervical ganglion of WKY and SHR contains 0.087 and 0.055 ng ME, respectively. The chromatographic profile of the immunoreactive ME-like peptides present in coeliac ganglia shows that the ganglia of SHR rats contain less of the low MW immunoreactive ME-like peptides but about an equal amount of the high MW immunoreactive material (Fig. 1). This abnormality is present in peripheral tissues but not in the hypothalamus (Table 1) or in the striatum (data not shown). The decrease of peripheral immunoreactive ME-like peptides may be due to a deficit in the enzyme that forms ME, an increase in the enzyme that catabolises ME or an increase in the rate of ME utilisation. Preliminary data from our laboratory^{3,4} indicate that the ratio of high MW immunoreactive ME-like peptides to ME in brain is much smaller than that in peripheral tissues, and we have also shown that the high MW immunoreactive ME-like peptide can release ME when subjected to controlled proteolysis (unpublished observations). Thus, it is possible that the mechanisms which synthesise ME in brain and in periphery differ and that the genetic defect in SHR rats which reduces the content of ME-like peptides in peripheral tissues may be an abnormality in ME biosynthesis. If this defect is an important cause of the hypertension in SHR animals, one could infer that ME-like peptides, like morphine, may lower the vascular tone. However, the nature, mechanism, site and pathogenetic significance of the defect in ganglionic ME-like peptides reported here remains to be investigated.

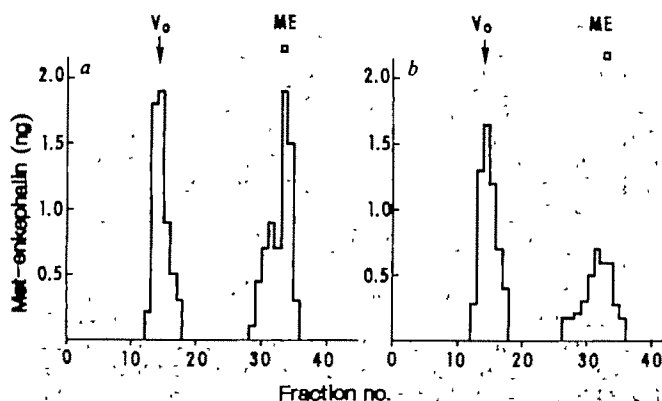


Fig. 1 Biogel P-2 column chromatography of sympathetic ganglia from WKY (a) and SHR (b) rats. Coeliac ganglia (30) and superior cervical ganglia (60) were extracted in 1 M CH_3COOH and heated for 5 min at 100 °C. The resulting supernatant was lyophilised, dissolved in 0.5 ml of 1 M CH_3COOH and chromatographed with Biogel P-2 column (0.9×60 cm) using 1 M CH_3COOH as the eluent. Aliquots of each fraction of the eluate were lyophilised and radioimmunoassayed with antiserum directed against ME. Two fractions appear, one of high MW, is eluted with the void volume (V_0), the other is eluted within the volume that is characteristic of ME. The exclusion limit of this column is 1,800 daltons.

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Histamine receptors on rabbit blastocyst and endometrial cell membranes

DESPITE much study, the role of histamine in ovum implantation remains unclear¹⁻⁵. Histamine action is mediated by H_1 and H_2 receptors^{6,7}, which are widely distributed on the cell membranes of mammalian tissues⁸⁻¹⁴. However, no information is available about the histamine receptors on blastocyst or endometrial cell membranes. Recent evidence indicates that a certain lymphocyte subpopulation carries histamine receptors⁹ and has an important regulatory function *in vitro*¹⁰⁻¹² and *in vivo*^{12,13} in mice⁹⁻¹¹, guinea pigs¹² and man¹³. Murine T cells, capable of regulating antibody secretion *in vitro*¹⁰, and precursors of T cells, capable of mediating cytotoxic effector cells¹¹, carry histamine receptors. The density of these receptors increases with cell maturation¹¹. Cell activation by histamine results in increased levels of intracellular cyclic AMP¹¹ and production of small molecular weight material, called histamine suppressor factor, capable of suppressing both cell proliferation and lymphokine production by sensitised lymphocytes following exposure to the antigen¹². Histamine suppression of delayed hypersensitivity in guinea pigs *in vivo* can be blocked completely or partially by H_2 and H_1 antagonists, respectively¹². Also, the administration of H_2 antagonists to man results in enhancement of delayed skin tests¹³. These observations indicate that lymphocytes carrying H_2 receptors acquire immunosuppressor potential on activation.

We demonstrate here that 6-day rabbit blastocyst and uterine endometrial cells carry H_2 and H_1 receptors, respectively. The possible significance of H_1 and H_2 receptors in ovum implantation and in suppressing *in situ* maternal capacity to reject the implanting embryo carrying alloantigens are discussed.

Six-day blastocysts from superovulated¹⁵ domestic rabbits were recovered by flushing the uteri with chilled medium RPMI 1640 supplemented with bovine serum albumin (BSA). The blastocysts were washed twice in the same medium, transferred to a small disposable sterile Petri dish, ruptured and gently teased with a needle. The preparation was passed through a 25-gauge needle to obtain single-cell suspensions. Endometrial cells from the same animals were obtained by scraping off the uterine endometria with a scalpel blade. The scrapings were transferred to a sterile plastic tube containing BSA-supplemented cold RPMI 1640 medium. The clumps of endometrial cells were first passed through a 19-gauge needle and then a few times through a 25-gauge needle to obtain single-cell preparations. The blastocyst and endometrial single-cell suspensions were centrifuged at 720g, washed and resuspended in phosphate-buffered saline (PBS) with 10% fetal calf serum. Cell numbers were then counted and adjusted to 0.5×10^5 cells ml^{-1} . With this technique, less than 1% of cells were found to be dead by the standard Trypan blue exclusion method.

Histamine receptors were visualised by incubating single-cell preparations in fluorescein isothiocyanate-BSA-histamine (FITC-BSA-H) conjugate for 30 min at 4°C. FITC-BSA-H was prepared by conjugating 50.0 mg FITC-BSA (fluorescein-protein ratio, 3:1) to 350.0 mg histamine dihydrochloride (Calbiochem) in the presence of 1.0 g of 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (ECDI; Story) at room temperature for 2 h followed by extensive dialysis at 4°C against 2 l of PBS for 48 h. In all the experiments, FITC-BSA-H was used at a dilution of 1:25 with PBS. Following incubation the cells were washed twice, mounted in PBS-glycerine mixture and examined under a fluorescence microscope using an epillumination system. Membrane stain was demonstrated as fluorescent speckles. The control cells, incubated in FITC-BSA in the absence of histamine, were negative. Moreover, red blood cells, as contaminants in the endometrial cell suspensions, did not

show any binding with FITC-BSA-H conjugate. The specificity of binding was determined by incubating cell suspensions in free histamine, chlorpheniramine (H_1 -receptor antagonist)¹⁶ and metiamide (H_2 -receptor antagonist)¹⁷ before incubation in FITC-BSA-H. Statistical analysis was according to Student's *t* test.

Figure 1a shows the results of histamine binding with blastocyst cell membranes: $37 \pm 11\%$ of blastocyst cells showed bright membrane fluorescence on staining with FITC-BSA-H. Histamine and metiamide, at both 10^{-4} M and 10^{-5} M concentrations, displaced the binding significantly ($P < 0.01$). Chlorpheniramine significantly displaced the binding only at 10^{-4} M ($P < 0.05$). Figure 1b shows the results of histamine receptor determinations on endometrial cell membranes: $21 \pm 9\%$ of endometrial cells showed binding with FITC-BSA-H conjugate and this binding was significantly suppressed by free histamine and chlorpheniramine at both 10^{-4} M ($P < 0.005$) and 10^{-5} M ($P < 0.05$). Metiamide, on the other hand, did not suppress the binding. None of the above agents at 10^{-6} M were able to alter the binding response of either blastocyst or endometrial cell membranes. The above results indicate that blastocyst cell membranes carry mainly H_2 receptors, whereas endometrial cells carry H_1 receptors.

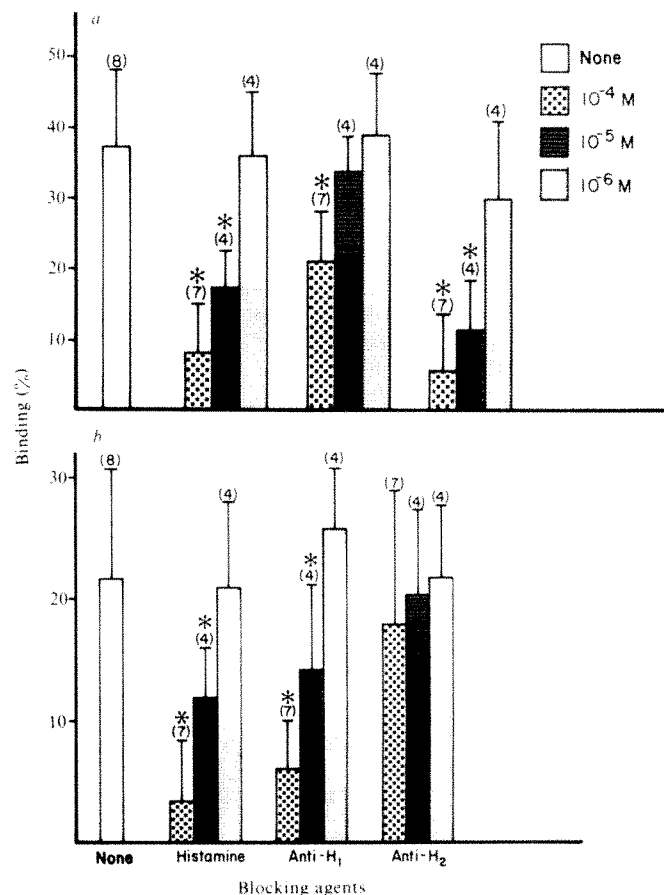


Fig. 1 The blocking effects of histamine or various histamine antagonists on the binding of blastocyst (a) and endometrial (b) cell suspensions to FITC-BSA-H. Results shown are means $\pm$ s.d. of several experiments. The number of experiments is shown in parentheses. Asterisks denote a significant difference from binding values in the absence of a blocking agent. Rabbit blastocyst or endometrial cell suspensions (0.5×10^5 in 1.0 ml of RPMI 1640) were incubated for 30 min at 4°C with 0.1 ml of various concentrations of the blocking agents, histamine, anti H_1 (chlorpheniramine) or anti- H_2 (metiamide). They were washed three times in chilled medium, stained by incubating them with FITC-BSA-H at 4°C for 30 min and then washed and mounted. In all experiments at least 200 cells per slide were counted.

The mammalian blastocyst is composed of two types of cell: the inner cell mass and trophoblast. Similarly, the endometrium is composed of epithelial, stromal and glandular cells. Our findings do not indicate the cell type in the blastocyst or endometrial that carries histamine receptors. The observation that histamine receptors could be detected on some of the blastocyst or endometrial cells is not unique; human blood lymphocytes are also heterogeneous and only $30 \pm 3.8\%$ of these cells can bind to histamine¹⁸.

Further work is necessary to elucidate the possible role of histamine receptors in blastocyst-endometrium metabolism, implantation and acceptance of the blastocyst allograft by the maternal tissues¹⁹. Despite many suggestions, it remains a challenge to reproductive biologists and immunobiologists to explain the escape of the embryo from the immunological responses of the mother¹⁹. In an immunologically competent system, histamine binding to H₁ receptors mediates a variety of pro-inflammatory events, such as increased permeability and vasodilatation, whereas histamine activation of H₂ receptors mediates several immunosuppressive effects through stimulation of cyclic AMP synthesis¹¹. A local inflammatory reaction, accompanied by accumulation of histamine in the uterus, occurs around the time of implantation²⁰. Implantation in the rabbit begins within 6½–7 d of pregnancy (day 1 = 24 h post coitum)²¹, and the cyclic-AMP/cyclic GMP ratio in the rabbit blastocyst changes before implantation²². Moreover, cyclic AMP has been shown to participate in blastocyst implantation in the mouse^{23,24}. The above bimodal function of histamine through H₁ and H₂ receptors is feasible, as systemic administration of either H₁ or H₂ receptor blockers in rats has no effect on preimplantation stages of pregnancy, whereas the combination of the two interferes with implantation²⁵. Furthermore, histamine involvement in inducing implantation in mice or rats during delayed implantation^{26,27}, and interruption of implantation in rabbits by inhibition of uterine histamine release have been reported recently²⁸. Also, 6-day rabbit blastocysts have been shown to suppress the mitogen response of autologous splenic lymphocytes *in vitro*²⁹. It remains to be seen whether this suppressor activity of blastocysts is mediated through activation of histamine receptors.

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Early pregnancy factor is immunosuppressive

IMMUNOSUPPRESSION during pregnancy has important consequences not only with respect to the recognition of the antigenically dissimilar embryo¹ but also in terms of susceptibility to viral or tumour assaults^{2,3}. We have recently described an early pregnancy factor (EPF) detectable within 24 h of mating and have postulated that this protein is responsible for depression of lymphocyte activity during pregnancy^{4–6}. Immunosuppressive properties have been attributed to other proteins detectable during pregnancy. These include pregnancy-associated macroglobulin^{7,8}, α -fetoprotein⁹ and human chorionic gonadotropin (HCG)^{10,11}. One characteristic these substances have in common, but in which they differ from EPF, is that they are not present, or not detectable, in the early stages of pregnancy. Recently the immunosuppressive activity of HCG has been disputed by several workers working with highly purified material^{12,13}. We have now shown that EPF inhibits the expression of an immune response *in vivo*.

After fertile matings, EPF has been detected on the lymphocytes and in the serum of mice, humans and sheep within 6–24 h and has been shown to be present for approximately the first two trimesters of pregnancy^{4–6}; EPF activity was not detected after sterile matings in any of the three species (refs 4, 6, B. Fitzgerald, personal communication). This substance was found in serum in two different forms, one of apparent molecular weight 200,000–240,000 detectable from 5 h after mating, and the other, ~50,000 MW detectable from 6 d after mating¹⁴.

The rosette inhibition test has been used as the assay method for the detection of EPF. This test was originally used to assess the immunosuppressive activity of anti-lymphocyte serum (ALS)^{15,16} and the results showed that the rosette inhibition titre (RIT) of each serum did not vary significantly using lymphocytes from different normal donors. If the lymphocytes were from pregnant donors, or had been incubated in serum from pregnant donors, the RIT of the ALS was significantly increased^{4–6}. This was a novel use of the rosette inhibition test and, on the basis of the ALS-sparing properties of EPF, we proposed that EPF is immunosuppressive. The use of an *in vivo* test, however, would clearly establish this point. We have now used the technique of adoptive transfer of contact sensitivity to trinitrochlorobenzene (TNCB) by lymphoid cells from sensitised mice^{17,18}. Lymph node cells, taken 4 d after sensitising mice with TNCB, were incubated with EPF preparations from pregnant sheep (Table 1), before injection of these cells into naive syngeneic mice. Skin tests for delayed-type hypersensitivity were immediately performed on the recipients¹⁹.

Serum or serum fractions having EPF activity (Table 1) suppressed the adoptive transfer of contact sensitivity (Table 2). When TNCB-sensitised lymph node cells were incubated with 24-h pregnant sheep serum (EPF I, Table 1), the ability of these

cells to transfer TNCB sensitivity was completely abolished (Table 2, experiment 1). Partial suppression of activity was observed when cells were preincubated with normal sheep serum, but significant transfer of activity was still achieved. Preincubation with the high MW fraction (EPF II, Table 1) also abolished transfer of TNCB sensitivity, whereas the use of the corresponding non-pregnant sheep serum fraction (control II) had no effect (Table 2, experiment 2). Two preparations of the low MW fraction prepared from 24-h pregnant sheep serum (EPF IIIa and EPF IIIb, Table 1) completely suppressed adoptive transfer, compared with normal serum controls. Although one of these controls (control IIIa) gave a slight decrease in reaction, there was still a significant transfer of sensitivity. Control IIIb gave a result not significantly different from that obtained with the cells incubated in medium alone (Table 2, experiment 3). A preparation of the low MW fraction obtained from the serum of 1-2-month pregnant sheep (EPF IV, Table 1) completely abolished adoptive transfer, whereas the corresponding fraction from normal serum (control IV) had no significant effect (Table 2, experiment 4).

In these experiments we have shown that EPF inhibits the expression of an immune response *in vivo*; the suppressive effect demonstrated was not species specific as sheep EPF inhibited the activity of mouse lymphocytes. Previously EPF activity had been detected using the rosette inhibition test and, although this test had proved convenient and very accurate, its role as a measure of immunological function was still debatable. The demonstration that EPF can completely suppress the adoptive transfer of contact sensitivity, well established as an immunological process, clearly indicates that EPF is immunosuppressive *in vivo*. The indirect technique of adoptive transfer was used, as preliminary experiments involving the injection of sheep EPF directly into mice, both before and after sensitisation, indicated

Table 1 Preparation of serum and serum fractions containing EPF

Source	EPF I Whole serum (24 h)	EPF II Serum fraction (24 h)	EPF III DEAE- Sephadex treated serum (24 h)	EPF IV Serum fraction (1-2 mth)
Approximate MW	240,000	240,000	50,000	50,000
Rosette inhibition titre	28	24	(a)24 (b)22	26

EPF I, pregnant sheep serum, taken 24 h after mating, was inactivated at 56°C for 30 min, and the rosette inhibition titre (RIT) determined by the method described previously¹⁰. Serum taken from a nonpregnant ewe was similarly treated (control I). EPF II, pregnant sheep serum, as above, was fractionated on an Ultrogel AcA34 (LKB) column¹⁴ and tested for EPF activity by the RIT. The fractions that contained the EPF activity in the region of MW 240,000 were pooled, dialysed against Hank's balanced salt solution (HBSS) and concentrated to 1/5th of the original serum volume. The RIT of the preparation was determined before use. Nonpregnant ewe serum was similarly processed (control II). EPF IIIa and EPF IIIb, serum was collected from two pregnant ewes, 24 h after mating. Thirty ml of each were partially purified with DEAE-Sephadex A50 (Pharmacia), which removed the macroglobulin and albumin fractions of the serum proteins, then dialysed against HBSS and concentrated to 1/5th of the original serum volume. Before use, the RIT of these preparations was estimated and they were fractionated to determine the MW of the EPF activity¹⁴. Serum from two nonpregnant ewes was similarly treated (control IIIa and IIIb). EPF IV, serum was collected from pregnant sheep 1-2 months after mating and applied to an Ultrogel column. The fractions, MW ~50,000, which had EPF activity, were pooled, dialysed against HBSS and concentrated to 1/5th of the original serum volume. Control IV was nonpregnant ewe serum run in parallel.

* The rosette inhibition titre (RIT) is expressed as the logarithm (base 2) of the reciprocal $\times 10^{-3}$ of the highest dilution of anti-lymphocyte serum (ALS) to inhibit the rosette formation to 75% or less than that of control test (cells in HBSS alone). RIT obtained using serum from non-pregnant sheep: mean = 9.8, s.e.m. = 0.3, n = 20.

Table 2 Suppression of adoptive transfer of contact sensitivity by EPF

Expt	Cells transferred [†]	Cells preincubated with [†]	No of recipient mice	Skin test [‡] 24 h ear swelling ± s.d. (cm $\times 10^{-3}$)	
1	None	-	6	4.3 ± 1.0	
	80 $\times 10^6$	-	5	9.4 ± 3.5	P < 0.001
	80 $\times 10^6$	Control I	5	6.1 ± 1.4	P < 0.001
	80 $\times 10^6$	EPF I	5	4.1 ± 2.3	P < 0.001
2	None	-	6	3.2 ± 1.3	
	160 $\times 10^6$	-	3	7.3 ± 2.8	P < 0.001
	160 $\times 10^6$	Control II	6	7.9 ± 2.6	P < 0.01
	160 $\times 10^6$	EPF II	6	4.5 ± 1.8	P < 0.02
3a	None	-	5	4.1 ± 1.7	
	100 $\times 10^6$	-	5	8.3 ± 2.0	P < 0.001
	100 $\times 10^6$	Control IIIa	5	6.6 ± 1.4	P < 0.001
	100 $\times 10^6$	EPF IIIa	5	4.1 ± 1.8	P < 0.001
3b	None	-	4	5.4 ± 1.6	
	130 $\times 10^6$	-	5	8.9 ± 3.4	P < 0.02
	130 $\times 10^6$	Control IIIb	5	9.6 ± 2.6	P < 0.01
	130 $\times 10^6$	EPF IIIb	5	5.8 ± 1.9	P < 0.01
4	None	-	6	4.3 ± 1.0	
	80 $\times 10^6$	-	5	9.4 ± 3.5	P < 0.001
	80 $\times 10^6$	Control IV	4	7.5 ± 2.4	P < 0.001
	80 $\times 10^6$	EPF IV	5	4.6 ± 1.6	P < 0.001

* Auxiliary and inguinal lymph node cells taken from CBA mice 4 d after skin painting with 7 mg TNCB in 0.1 ml acetone¹⁵ were washed three times in culture medium and preincubated at 50×10^6 cells ml⁻¹ in reaction mixture as indicated.

† Experiment 1: cells were incubated at 37°C for 60 min in Eagle's basal medium containing 10% fetal calf serum (Commonwealth Serum Labs) and 50% control I or EPF I (Table 1). Cells were washed twice in culture medium, suspended in saline and injected intraperitoneally (0.2 ml) into the recipients (CBA mice). Experiment 2: as above, but reaction mixture contained 20% of control II or EPF II (Table 1). Experiments 3a and 3b: as above, but reaction mixture contained 20% of control IIIa, EPF IIIa, control IIIb or EPF IIIb (Table 1). Experiment 4: as above, but reaction mixture contained 20% of control IV or EPF IV (Table 1).

‡ Within 1 h of cell transfer, both ears of control and recipient mice were measured using an engineer's micrometer (Mitutoyo) and painted with 10 μ l of a 1% solution of TNCB in acetone. After 24 h the ear thickness was again measured and ear swelling determined by subtraction.

that immunosuppression was partially obscured by the effect of heterologous protein.

Adoptive transfer of contact sensitivity and inhibition of rosette formation are both T-cell dependent^{16,17}. As EPF affects both of these reactions, its primary function in protecting the fetus may be directed towards a depression of T-cell activity in the maternal system. We may speculate that EPF might protect the mother from immunological attack by the fetus. EPF has been detected in the fetal tissues (H.M. *et al.*, unpublished observations), which suggests another possible role for EPF, namely in the regulation of the development of the fetal immune system. The mechanism of action of EPF is still far from clear; its action is obviously not antigen specific and not even species specific. Whether it is suppressive by simply coating lymphocyte surfaces and preventing antigen presentation, or by a more active method, is yet to be established.

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Myosin as a constituent of the *Drosophila* egg cortex

MYOSIN and actin have been found in many cell types, including the eggs of Amphibia¹, sea urchins², starfish³ and rats⁴. Their distribution correlates well with the distribution of microfilaments in structures such as the contractile ring, and these proteins are believed to have a major role in cell cleavage and plasmalemma elongation⁵. They have not previously been recorded in *Drosophila* eggs and embryos. The egg of *Drosophila* develops as a syncytium with about 6,000 nuclei present in a common cytoplasm⁶. Subsequently, nearly all the nuclei are simultaneously surrounded by plasmalemmas which grow down into the cortex of the egg. Because of this, and because of the existence of several mutants which specifically affect cellularisation^{7,8}, *Drosophila* eggs are a particularly appropriate system in which to study cell membrane formation. The simultaneous

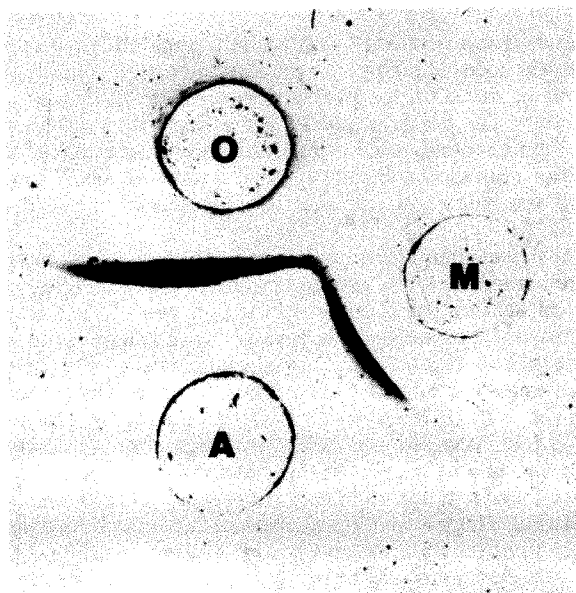


Fig. 1 Precipitin bands against 20 μ g fly flight muscle in 2% SDS and 60 mM Tris-HCl, pH 6.8 (M), and 80 μ g of an extract of locust oocytes (O). The extract was obtained by homogenising in 1.2 M KCl, 15 mM Na pyrophosphate and 15 mM Tris-HCl, pH 7. The 50% $(\text{NH}_4)_2\text{SO}_4$ cut was dissolved in 0.6 M KCl, 2 mM dithiothreitol (DTT), 2 mM ATP and 15 mM Tris-HCl and dialysed against 0.25 M KCl, 2 mM DTT, 2 mM ATP and 15 mM Tris-HCl, pH 7. The precipitate was dissolved in 2% SDS and 60 mM Tris-HCl, pH 6.8. Ouchterlony plates contained 2% Noble's agar, 0.5 M NaCl, 0.01 M phosphate buffer, pH 7.5, 0.1% SDS and 0.5% Triton and were left for 4 d at 4°C before washing for 2 d in 0.6 M KCl and 20 mM Na pyrophosphate, pH 7, 1 d in 0.8% NaCl, followed by drying down and staining. 'A' marks the antiserum well.

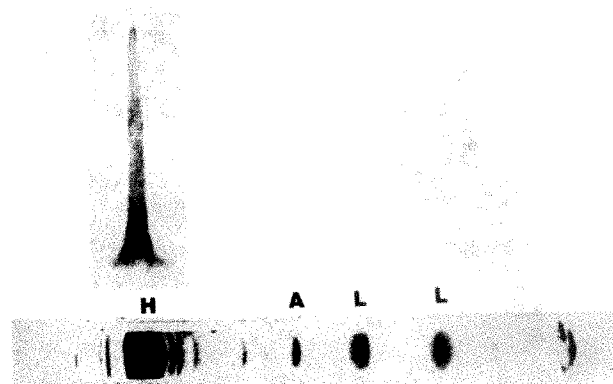


Fig. 2 Crossed immunoelectrophoresis to demonstrate antibodies against myosin. *Sarcophaga* flight muscle myosin (14 μ g) was electrophoresed on a tube gel with SDS by the method of Weber and Osborn¹⁴. Electrophoresis in the second dimension was by the method of Converse and Papermaster¹⁵. A strip (1 mm thick) from the SDS gel was electrophoresed into 1% agarose; the layer of agarose (0.5 cm wide) next to the SDS gel contained 0.5% Tween and the rest of the agarose contained 2% IgG from antiserum. The stained SDS gel is heavily loaded to show minor bands. H indicates the heavy chain of myosin, L the light chains, and A contaminating actin.

cellularisation of the whole of the egg cortex would seem to require the existence of large numbers of microfilaments but these have not yet been visualised in the electron microscope⁹. We report here the finding of a continuous band of myosin immediately below the egg plasmalemma which could well be associated with massed microfilament bundles. This is laid down by the time of fertilisation and remains continuous until the arrival of the nuclei in the cortex. The structure of the band and its relationship to early developmental events are described.

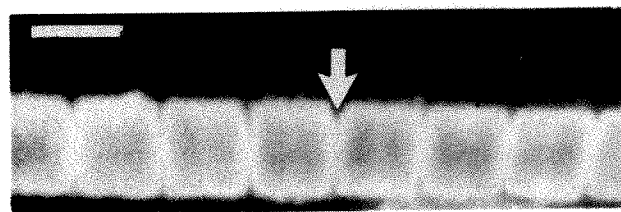


Fig. 3 Freshly dissected *Drosophila* flight muscle myofibril decorated with anti-myosin at a final concentration of 2 mg ml⁻¹. Decoration technique as for embryo sections. Arrow indicates Z line. Scale bar, 3 μ m.

Antibodies were made against *Sarcophaga* (blowfly) flight muscle myosin. Myosin was prepared by the method of Bullard and Reedy¹⁰, and further purified by running on 5% SDS-acrylamide slab gels. The band containing the heavy chain of myosin was identified, cut out, homogenised with four volumes of phosphate-buffered saline (PBS) plus 0.5 ml Freund's adjuvant, and injected into white New Zealand rabbits. The total volume was about 3 ml. A total of 1 mg of myosin heavy chain was injected in three equal fortnightly injections. The rabbit was bled 1 week after the last injection. IgG was purified from the antiserum following the method of Campbell *et al.*¹¹. This antiserum gave a single band when tested in Ouchterlony plates (Fig. 1). It also reacted with an extract of locust oocytes containing a putative myosin as a major component. The fusion of the precipitin arcs formed with muscle myosin and oocyte extract shows that the antigens in these preparations had common antigenic sites. This situation is like that of the starfish, where antiserum against egg myosin reacts with tube foot muscle myosin¹². The specificity of the antiserum was further tested in crossed immunoelectrophoresis. A large rocket was obtained

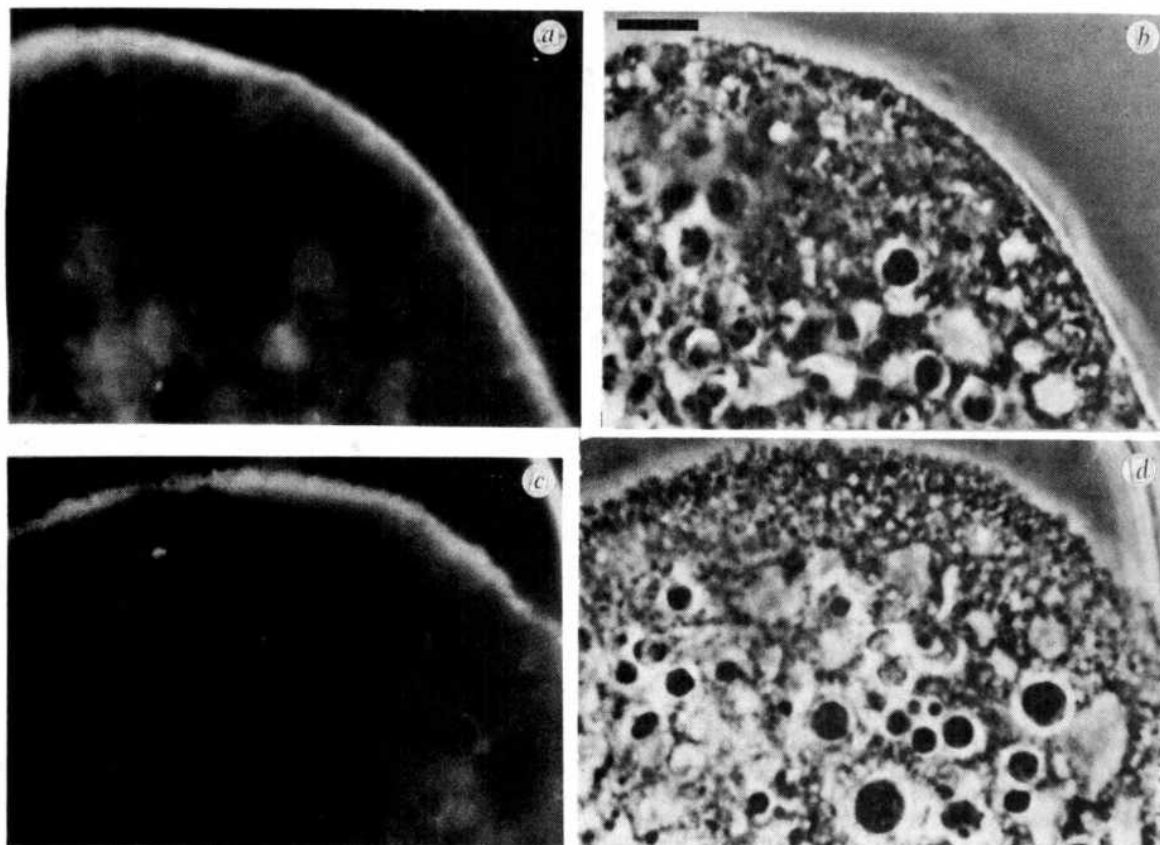


Fig. 4 Sections of 1 nucleus stage *Drosophila* embryos decorated with anti-myosin IgG. In both cases the same section is seen under epifluorescence (a, c) and phase contrast (b, d). a And b show the sidewall running obliquely into the anterior tip region, c and d show the posterior pole. In c and d the brightly fluorescent structure at upper right is the autofluorescent vitelline membrane. Scale bar, 5 μm .

opposite the myosin heavy chain (Fig. 2). Our antiserum binds to A-bands of *Drosophila* flight muscle myofibrils giving patterns similar to those described by Garrett for *Lethocerus* flight muscle myofibrils¹³ (Fig. 3).

Sections of embryos were prepared by a modification of Sainte-Marie's method¹⁶. All procedures except for embedding were carried out at 4 °C. Fixing was done for 1 h using a 6 : 16 : 22 mixture of formaldehyde : ethanol : distilled water. The embryos were carefully punctured to allow adequate penetration of the fixative. They were then dehydrated by passing through each of the following solutions for 30 min: 70% ethanol, 90% ethanol, absolute ethanol and isopropanol (two changes). Clearing was accomplished using ligroin (BDH) for 1 h. The embryos were embedded in paraplast and sectioned at 5 μm .

The technique very effectively preserves delicate structural features such as the cleavage furrows. Sections were treated with either anti-myosin IgG or IgG from the same rabbit before immunisation, using a final concentration of 2 mg ml⁻¹ in buffered saline in both cases. They were incubated at 37 °C for 1 h and then washed for 15 min with PBS, pH 9.5. Results were complicated by nonspecific fluorescence for some pre-immune sera. This was reduced by using a pH 9.5 wash as recommended by Bennett *et al.*¹⁷. Fluorescein isothiocyanate-conjugated sheep anti-rabbit IgG (Wellcome) was added as the second part of the sandwich at a concentration of 0.8 mg ml⁻¹ in PBS and incubated and washed as before. Sections were mounted in a 9 : 1 mixture of glycerol : 1 M Trizma base, pH 9. Microscopy was carried out with a Zeiss standard R microscope equipped with an epifluorescence attachment and a Planapo lens ($\times 63$ NA 1.4).

The results show the existence of a continuous band of fluorescence immediately below the cell membrane, extending around the whole egg (Fig. 4). Controls show no such band, only background fluorescence. Below the band more diffuse material extends deeper into the cortex. Analysis of photographs from 18 embryos (1 nucleus to 32 nuclei stages), indicated a thickness of

1.00 μm (s.d. ± 0.15 μm). This figure is apparently constant all along the sidewall. Towards the posterior pole (Fig. 4c, d) it gradually thickens to give a maximum of 1.59 μm (s.d. ± 0.15 μm). The posterior pole is associated with a considerable development of microvilli, which also show fluorescence. At the anterior pole the band thickens to 1.5 μm (s.d. ± 0.11 μm) but this is associated with a smaller development of microvilli (Fig. 4a, b).

The myosin band seems to be unlike the whorl-like masses of microfilaments in the amphibian cortex¹, but similar to the cortical distribution of myosin in rat oocytes⁴. What may the functions of the band be? We have evidence that once the nuclei arrive in the cortex this band of material breaks up and parts of it becomes associated with each nucleus. It subsequently becomes involved in the formation of cell membranes around the nuclei (R. W., R. Magrath and B. B., in preparation). It therefore seems reasonable to propose that one function of the epicortical myosin band is as a store of materials required in cell membrane formation. There is no simple explanation for the thickenings of the band at the anterior and posterior poles. At the posterior pole, the thickening may be partly concerned with the formation of a double layer of cells, the pole cells and the underlying somatic blastoderm cells. However, this does not explain the increased thickness at the anterior pole.

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The topography of the purple membrane

THE purple membrane of *Halobacterium halobium* functions as a light-driven proton pump^{1,2}. It is about 45 Å thick³ and contains a single polypeptide chain comprising about 240 amino acids⁴. Retinal is covalently attached to a specific lysine residue⁴ and has been implicated in the absorption of incident light energy². The retinal lies at an angle of about 20° to the plane of

the bilayer⁵. Seven protein rods running through the membrane, probably α -helices, have been detected by electron diffraction^{6,7}. Circular dichroism studies indicate a 75% helical content which is consistent with a seven-helix model⁸. A structure containing seven such α -helices requires about 210 amino acids to cross a membrane 45 Å thick seven times, thus implying that most of the polypeptide chain is buried in the lipid bilayer. Digestion of the membrane with proteolytic enzymes supports this view and only three parts of the polypeptide chain are apparently exposed. Here, we show by sequence analysis of digestion products that a section corresponding to the N-terminal seven residues is removed by pronase and proteinase K.

In common with pepsin and papain⁹, pronase and proteinase K also split at several internal sites¹⁰ in the region of residues 71–76 in the sequence of the polypeptide chain (Fig. 1), thereby removing a small segment. A third digestion site for these enzymes is in the region of the C-terminus. Trypsin, which does not split the chain internally, liberates into solution a 21-residue peptide from the C-terminus (residues –1 to –21 in Fig. 1) but does not split the bond between residues –23 and –24. Thus, 21 residues at the C-terminus are exposed to the aqueous environment.

We have determined the amino acid sequence of the N-terminal 83 residues and C-terminal 39 residues containing these three exposed regions (Fig. 1). We note four differences between our results and those published elsewhere¹⁰. These are listed in the legend to Fig. 1. The N-terminal segment is very hydrophobic, and taken in conjunction with the results of the protease digestions of the membrane above, suggests that a section of 63 amino acids—residues 8–70—is buried in the lipid bilayer. The remaining problem is to decide how this buried segment crosses the membrane and whether a structure consistent with the seven-helix model can be proposed. The most

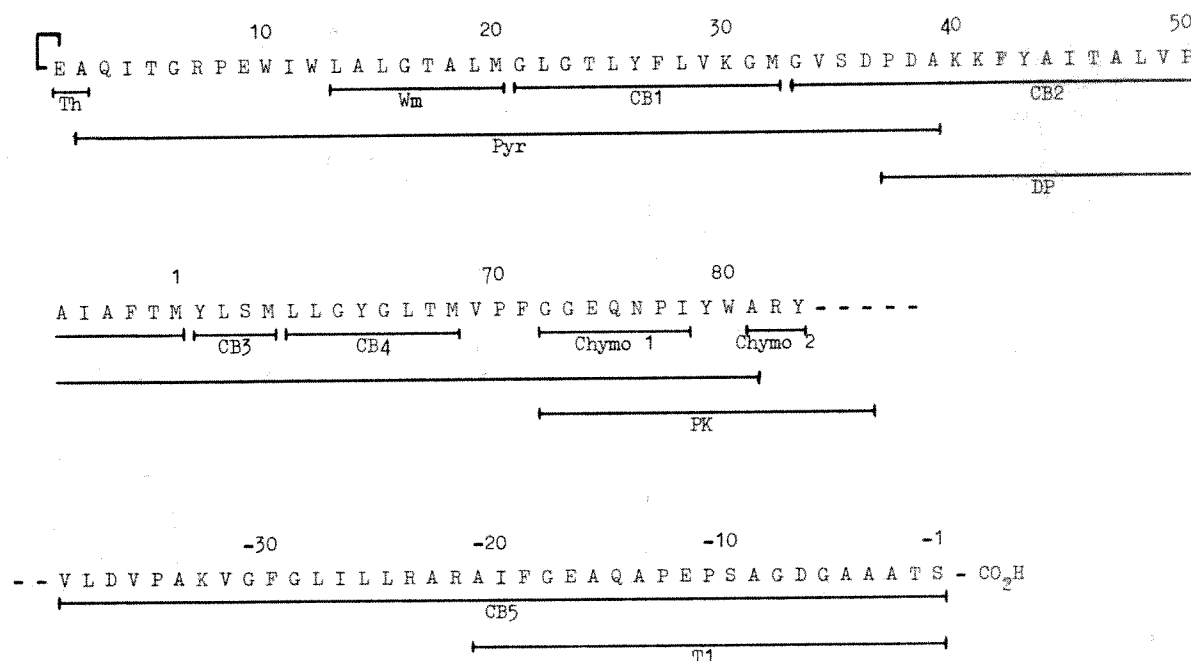


Fig. 1 The amino acid sequence of the N-terminal and C-terminal regions of bacteriorhodopsin. Peptides were derived from digests of the protein with trypsin (T), thermolysin (Th), chymotrypsin (Chymo), cyanogen bromide in 80% formic acid (CB), cyanogen bromide in 88% formic acid and heptafluorobutyric acid (Wm)¹¹. Enzyme digests were carried out on succinylated protein in 0.5% ammonium bicarbonate, and chemical cleavages on delipidated protein. In addition, the linkage between residues 37 and 38 was cleaved specifically by incubation of delipidated protein in 70% formic acid at 37 °C for 48 h. The pyroglutamyl moiety was removed in 70% yield from succinyl protein by incubation for 30 h at 37 °C with pyroglutamyl amino peptidase (Boehringer) with a substrate: enzyme ratio of 50:1. The peptide PK was obtained from a digest of the membrane with proteinase K (4 h, 37 °C). T1 was liberated from the membrane by tryptic digestion. Peptide Th was isolated according to Bruton and Hartley¹². Wm, CB3, CB4, Chymo 1 and Chymo 2 by paper electrophoresis and chromatography. CB1, CB2, CB5, DP and PK by gel filtration of the digests in 70% formic acid on Biogel P-30. The sequences of Pyr, DP, PK, CB1, CB2 and CB5 were obtained with the aid of a spinning cup sequencer¹³ and CB1, CB2, CB3 and CB4 with a solid phase sequencer after coupling to amino-propyl glass¹⁴. Th was sequenced by mass spectrometry, Wm, Chymo 1, Chymo 2 and T1 by the dansyl-Edman procedure¹⁵. Ovchinnikov *et al.*¹⁶ found threonine at position 47, serine at –35, serine at –23 and glutamic acid at –14.

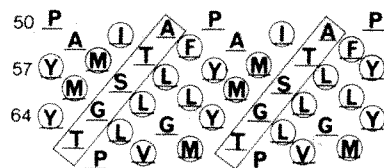


Fig. 2 Amino acid sequence of residues 50–70 plotted on a helix surface with 3.5 residues per turn. Sequence reads downwards right to left as if projected on to a sheet of paper wrapped twice round the helix axis and then unrolled. Each residue appears twice. Hydrophobic residues are circled. The box represents a relatively hydrophilic groove on the helix surface. This is consistent with a helical structure¹⁸.

probable interpretation is that the polypeptide crosses the membrane twice in two helices. Prolines at 8 and 37 are consistent with the notion that residues 9–34 (25 amino acids) form a helix which would span a distance of approximately $25 \times 1.5 \text{ \AA} = 37.5 \text{ \AA}$. This section is terminated by the sequence -S-D-P-D (residues 35–38) which could readily form a β -turn. Residues 39–70 would then cross the membrane a second time, emerging from it at about residue 70. This segment can be considered in two parts: residues 39–49, containing the retinal binding site, and the section between proline residues 50–70. It is likely that this latter section also forms an α -helix. This view is supported by plotting this sequence on a helix surface (Fig. 2). This shows a groove composed of amino acids with less hydrophobic side chains, a feature characteristic of an α -helix¹⁸.

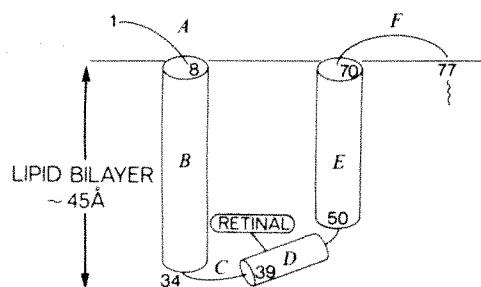


Fig. 3 Proposed topography of N-terminal region of the purple membrane protein. Sections A (residues 1–7) and F (71–76) are accessible to proteolysis. B (residues 8–34) is probably an α -helix, C (residues 34–38) is a β -bend. D (residues 39–49) contains the retinal binding site, E (residues 50–70) may be an α -helix. The angle between the axes of D and E is not considered here. Proline residues occur at positions 8, 37, 50, 70 and 77.

The second helix (residues 50–70, E in Fig. 3) would be about 30 Å long and thus too short to cross the membrane. Hence, residues 39–50 containing the retinal binding site (D in Fig. 3) must partly cross the membrane. The secondary structure of this segment is rather uncertain but it too may be partly α -helical. These features are summarised in Fig. 3. Note that this model places all charged residues near the periphery of the membrane and that none are buried in the interior.

Our model places the N-terminus and loop F on the same side of the membrane. The retinal binding site is near to the opposite side. This is in contrast to earlier models which place loop F on the opposite side from the N-terminus and the retinal lysine variously midway between the two sides of the membrane or near the same side as the N-terminus surface^{9,17}.

Moreover, it has been suggested that the C-terminus is orientated towards the inside of the bacterial cell, and hence in a structure of seven helices it would be outside¹⁰. If this is so the retinal lysine will be near the inside surface of the membrane and loop F will be on the outside.

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Note added in proof: Recently, Gerber *et al.*¹⁹, using a somewhat different methodology, have determined sequences covering essentially the same regions of the polypeptide chain of bacteriorhodopsin as described here.

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Globin gene deletion in HPFH, $\delta^0\beta^0$ thalassaemia and Hb Lepore disease

THE thalassaemias are a group of inherited disorders characterised by the defective production of either α (α thalassaemias) or non- α (β and $\delta^0\beta^0$ thalassaemias) globin chains of haemoglobins (Hb)¹. In β thalassaemias the decreased synthesis of β -globin chains is only partially compensated by the increased production of γ chains, which probably reflects² the massive hypertrophy of the erythron with selective survival of the clones of adult haemoglobin F-producing cells (F cells^{3,4}). The situation is very different in other genetic disorders of the non- α gene cluster, known as $\delta^0\beta^0$ thalassaemias and Negro type of hereditary persistence of fetal haemoglobin (HPFH). In these two forms there is a genuine increase of γ -chain production, as shown by the high level of HbF found in heterozygotes. Although a clearcut distinction from both the clinical and haematological point of view cannot be traced between these two forms, the HPFH differs from the $\delta^0\beta^0$ thalassaemia in having a higher degree of γ -chain synthesis and a more homogeneous distribution of HbF within red cells. Recently, it has been possible to carry out gene analysis on DNA prepared from β^0 , $\delta^0\beta^0$ thalassaemic and HPFH patients. The β -globin gene is present in β^0 thalassaemias^{5–9}, but in $\delta^0\beta^0$ thalassaemias and HPFH a major deletion, possibly involving both δ and β genes, has been demonstrated by hybridisation studies^{8,10–12}. To characterise the molecular defect in these genetic disorders more precisely, we have hybridised DNA from homozygotes with HPFH, $\delta^0\beta^0$ thalassaemia and Hb Lepore disease (in which non- α -chains are a $\delta\beta$ fusion product¹³). For this we used a pure full-size cDNA _{β} probe and specific 5' end and 3' end cDNA fragments (we designate as 5' end cDNA the portion corresponding to the 5' end of the mRNA; the same for the 3' end). Our results, reported here, show that in contrast to HPFH, where a complete δ and β gene deletion occurs, in $\delta^0\beta^0$ thalassaemia a 5'-end fragment of the δ gene is present.

Figure 1 shows the titration of β -globin sequences in DNA from a normal human, a case of Hb Lepore, a southern Italian patient with newly discovered $\delta^\circ\beta^\circ$ thalassaemia and two patients with HPFH, Negro type (all homozygotes). The cDNA $_\beta$ probe used was a full-size β band eluted from an agarose gel (see legend to Fig. 1), further purified to over 98% (not shown) by hybridisation with mRNA followed by hydroxyapatite (HAP) chromatography⁵. With HPFH DNA no hybridisation could be obtained (except at high cDNA-DNA ratios, when minor contaminating sequences are expected to hybridise^{14,15,22}), in agreement with Orkin *et al.*²³; in contrast, $\delta^\circ\beta^\circ$ and Lepore DNA showed a plateau level corresponding to approximately 25–30 and 30–40%, respectively (over the background HPFH DNA), of the hybridisation obtained with normal DNA. These data suggest that part of the $\delta\beta$ sequences are retained in $\delta^\circ\beta^\circ$ DNA.

To locate the residual fragment we used 5'-end and 3'-end cDNA probes. A 142-nucleotide long 5'-end fragment (nucleotides 81–222 (ref. 21)) of the β -globin sequence was obtained by digesting the full-size β -globin band with *Hae*III and eluting the heaviest fragment (H1 (ref. 21)) from a preparative polyacrylamide gel. This fragment was further purified from an identical size (141 nucleotides) α sequence²⁴ by hybridisation

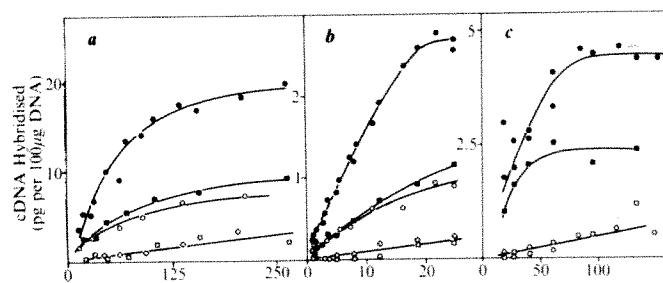


Fig. 1 *a*, Hybridisation of full-size cDNA $_\beta$ to normal, Lepore, $\delta^\circ\beta^\circ$ and HPFH DNA in cDNA excess^{5,14,15}. cDNA was copied with avian myeloblastosis virus reverse transcriptase in the presence of actinomycin D from globin mRNA from an HbH disease (α thalassaemia) patient, as described previously¹², except that ³²P-dCTP (Amersham, 400 Ci mmol⁻¹) was 300 μ M, and incubation was carried out at 41 °C for 45 min. The cDNA was then separated from unreacted nucleotides by G50 chromatography, incubated with 0.3 M NaOH at 37 °C for 15 min to break the RNA, neutralised and ethanol precipitated with 40 μ g ml⁻¹ carrier *Escherichia coli* tRNA, pelleted, resuspended in 10 μ l of 1/10 E buffer¹⁶ containing 0.04% bromophenol blue, and electrophoresed at 120 V on a 2% agarose slab gel (15 cm $\times$ 15 cm $\times$ 0.2 cm) in E buffer, until the dye was 1 cm from the bottom of the gel. The gel was then autoradiographed using a Kodak Royal X-0 Mat X-ray film and the heaviest radioactive band (full-size cDNA $_\beta$ (ref. 20)) eluted electrophoretically from slices cut from the gel using the developed film as a reference. All DNAs were prepared by a HAP procedure¹⁷ and fragmented to 200–300 nucleotides by boiling for 20 min in 0.3 M NaOH (ref. 18). Normal and Lepore DNA were from the spleens of an anaemic non-thalassaemic patient and a Lepore Boston disease homozygote, respectively. HPFH DNA 1 was from white blood cells from the recently described homozygote found in Ghana¹⁹ and HPFH DNA 2 was from cultured fibroblasts from the Baltimore homozygote¹². $\delta^\circ\beta^\circ$ thalassaemic DNA was from white cells from a newly diagnosed southern Italian patient. DNAs (10–50 μ g) were mixed with increasing amounts of the full-size cDNA $_\beta$ probe in 0.12 M Na phosphate buffer, pH 6.8, 1 mM EDTA, at a DNA concentration of 5 mg ml⁻¹. The aliquots were sealed in silicon-coated glass capillaries, boiled for 10 min and incubated at 70 °C for 4,400 min. At the end of the incubation, the samples were assayed for S₁ nuclease-resistant radioactivity as described elsewhere⁵. The amount of cDNA $_\beta$ hybridised is converted to pg cDNA $_\beta$, and corrected for the background S₁ nuclease resistance (0.6%). Control experiments with cDNA $_{\alpha\gamma}$ show that all these DNAs hybridise identically. Normal, $\bullet$; Lepore, $\blacksquare$; $\delta^\circ\beta^\circ$ thalassaemia, $\circ$; HPFH 1, $\square$; HPFH 2, $\diamond$. Hybridisation of 5' cDNA $_\beta$ fragment (*b*) and 3' cDNA $_\beta$ fragment (*c*) to normal, Lepore, $\delta^\circ\beta^\circ$, HPFH 1 and HPFH 2 DNA. Full size cDNA $_\beta$ (ref. 20) eluted from the agarose gel (see above) was ethanol precipitated using 20 μ g ml⁻¹ Dextran (Sigma) as a carrier. After pelleting, the cDNA was resuspended and digested with 100 units μ g⁻¹ of *Hae*III restriction endonuclease in 10 mM Tris HCl, pH 7.6, 10 mM MgCl₂, 1 mM dithiothreitol for 4 h at 37 °C. The digested sample was directly loaded on a 7.5% polyacrylamide gel in E buffer and the separated bands located and recovered as described above. All the probes used for the hybridisation were further purified from contaminating α sequences by annealing to excess $\alpha\gamma$ mRNA and HAP chromatography⁵. DNAs were mixed with increasing amounts of *Hae*III H 1 (ref. 21) cDNA $_\beta$ fragment (*b*), or 3' end cDNA $_\beta$ probe (*c*), and hybridised as above for 5,000 min. A background S₁ nuclease resistance of 0.4% was subtracted.

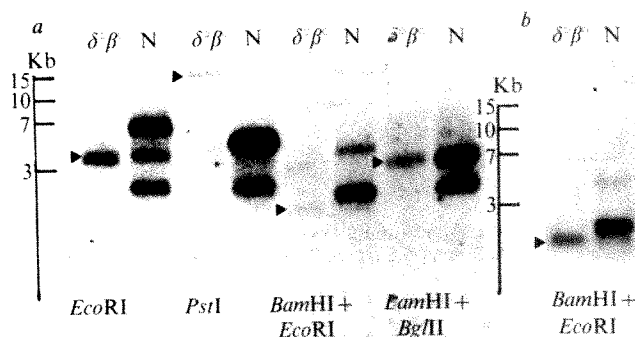
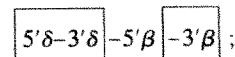


Fig. 2 Cleavage pattern of δ and β globin genes with different restriction enzymes. High molecular weight DNA was prepared according to Britten *et al.*³⁰ from normal human placenta or from purified white cell nuclei³¹ of the $\delta^\circ\beta^\circ$ homozygous patient. DNA (30 μ g) was digested with the indicated restriction enzymes, denatured with alkali^{32,33} and electrophoresed in 0.8% agarose gel in E buffer¹⁶. DNA transfer on to nitrocellulose filters and hybridisation at 65 °C in 2 $\times$ SSC was according to Jeffreys and Flavell^{32,33} using nick-translated³² pHBG1 plasmid as a radioactive probe (specific activity 50–300 $\times$ 10⁶ c.p.m. μ g⁻¹). Filter washing was carried out in 0.1 $\times$ SSC (*a*) at 65 °C for 6 h with vigorous shaking. This washing procedure seems to be more stringent than that used by Flavell *et al.*^{29,32,33} at the same salt concentration, as shown by the faintness of δ bands in 0.1 $\times$ SSC (*a*) and the absence of γ bands even at 0.3 $\times$ SSC (*b*).

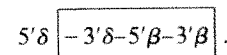
with mRNA $_{\alpha\gamma}$ and HAP fractionation⁵. Figure 1*b* shows that this 5'-end fragment does not hybridise to HPFH DNA (as expected), whereas it does hybridise to a comparable extent to both Lepore and $\delta^\circ\beta^\circ$ DNA (30% and 40%, respectively, of the normal). As the non- α chain of this case of Hb Lepore is a Boston type (in which a change-over from the δ -like sequence to the β -like sequence occurs between amino acids 87 and 116 (refs 25, 26)), this result demonstrates that this portion of the cDNA $_\beta$ can cross-hybridise to the corresponding δ sequence.

A 3'-end probe was obtained by eluting from an agarose gel a partial transcript (of approximately 200–250 nucleotides) obtained by reverse transcription of α -thalassaemic mRNA. In agreement with Efstradiatis (see ref. 27, and results not shown), we find that these fragments are incomplete oligo(dT)-containing products initiated at the 3'-poly(A) tail of the template and terminated at defined internal points. In contrast to the results obtained with the 5'-end probe, this fragment (further purified as above by HAP chromatography) does not hybridise with $\delta^\circ\beta^\circ$ DNA; the same was obtained with HPFH DNA, whereas the Lepore DNA hybridised to approximately 40–50% of normal (Fig. 2*c*), in agreement with the expected absence of the 3' end of the δ gene (which in these conditions seems to cross-hybridise efficiently to the probe used).

Two different schemes can fit our data; first, that the 5' end of the β -globin gene is retained in $\delta^\circ\beta^\circ$ thalassaemia, whereas the whole of the δ gene and the 3' end of the β -globin gene are missing:



alternatively, only the 5' δ -globin fragment would be present:



To distinguish between these two possibilities, we carried out mapping experiments using as a probe nick-translated radioactive pHBG1 plasmid containing human β -globin sequences²⁸.

As shown in Fig. 2, on digestion with *Eco*RI, $\delta^\circ\beta^\circ$ DNA shows, instead of the three main bands of 6.5, 4 and 2.3 kilobases present in normal DNA²⁹, a single band running slightly faster than the 4-kilobase β fragment; this result agrees with that reported by Mears *et al.* in a different patient³⁴. Double digestion with *Bam*HI and *Bgl*II results in a single 3.3-kilobase fragment in the $\delta^\circ\beta^\circ$ DNA; in normal DNA this fragment can originate both from the 3' end β gene and from the 5'-end δ gene. Thus, two alternative schemes can be proposed: the first

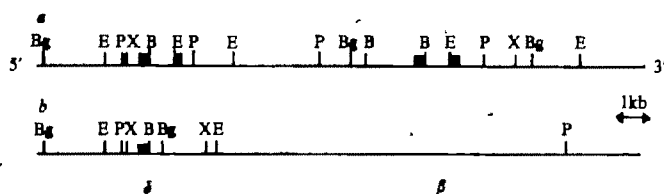


Fig. 3 Physical maps of δ and β DNA region in normal (a) and thalassaemic (b) DNA. Normal map is from Flavell *et al.*³⁰, $\delta^{\circ}\beta^{\circ}$ map was deduced from the digests reported in Table 1. B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; P, *Pst*I; X, *Xba*I. The position of the *Pst*I site 5' to the δ gene in $\delta^{\circ}\beta^{\circ}$ patient is based mainly on its location in normal DNA and indirectly deduced from the persistence of the flanking *Eco*RI and *Xba*I sites.

implies the deletion of the β -globin gene and possibly part of the δ gene; the second requires deletion of the δ gene and the 5' end of the β -globin gene. The demonstration of the normal 1.3-kilobase *Bam*HI + *Eco*RI fragment (Fig. 2 and Table 1) and the 0.7-kilobase *Bam*HI + *Xba*I fragment (Table 1) in the $\delta^{\circ}\beta^{\circ}$ DNA confirms the validity of the first scheme. The experiment shown in Fig. 2 was carried out in highly stringent conditions (see Fig. 2 legend), a fact that explains the faintness of the δ bands. As expected, the use of less stringent washing conditions (Fig. 2b) results in a striking increase in the intensity of these bands (nevertheless, these conditions are stringent enough to prevent the cross-hybridisation of γ genes to the β probe, as confirmed by control experiments with HPFH DNA, not shown).

From the liquid hybridisation and restriction mapping experiments, we conclude that: (1) a 5'-end fragment is retained in $\delta^{\circ}\beta^{\circ}$ thalassaemia (Fig. 1b) which can be identified as the 5' end of the δ gene (Fig. 2 and Table 1); (2) no δ or β 3'-end fragments are retained in $\delta^{\circ}\beta^{\circ}$ DNA, as shown by liquid hybridisation methods (Fig. 1c), by the absence of the normal 4-kilobase fragment in the *Eco*RI + *Bam*HI double digest, and by the disappearance of the 2.3-kilobase δ fragment expected if the *Eco*RI site present in the δ gene-coding region were retained; (3) from the data in Table 1 we have constructed the physical map (Fig. 3) of the $\delta\beta$ -globin region in this $\delta^{\circ}\beta^{\circ}$ thalassaemic DNA. It is clear that the deletion removes a large DNA fragment beyond the δ intragenic *Bam*HI site, extending past the first *Eco*RI site, on the 3' side of the β -globin gene region: in fact, the relative positions of the *Pst*I, *Xba*I, *Bgl*II and *Eco*RI sites in this region do not correspond to the normal ones.

Genetic data based on the analysis of the non- α chain of Hb Kenya (a $\gamma\beta$ fusion product^{35,36}) and Hb Lepore ($\delta\beta$ fusion product) indicate that the most likely arrangement of the non- α genes on the chromosome is $\gamma^{\circ}\gamma^{\circ}\delta^{\circ}\beta^{\circ}$. Because Hb Kenya mutation is associated with high levels of HbF in adult life, whereas usually the Hb Lepore is not, a 'deletion hypothesis' (refs 37, 38) has been proposed to explain the molecular basis of HPFH. According to this model, the deletion in HPFH involves an area of the $\gamma\delta\beta$ -gene complex (probably located to the left of the δ gene; Y area of Huisman *et al.*³⁷) which is crucial to the post-natal suppression of γ -chain production; removal of this DNA segment allows the combined expression of γ genes, although not to the extent that they function in fetal life.

Our results only partially agree with this model, as a significant de-repression of γ genes occurs even in the presence of an intact 5'-end region of the δ gene. Thus, the structural δ -gene sequences and/or the insert might be part of the postu-

lated regulatory area controlling γ -gene expression. On the other hand, a larger deletion, involving an additional region including the 5' end of the δ gene, results in the HPFH picture. Establishment of the exact DNA region involved in determining the difference between the HPFH and $\delta^{\circ}\beta^{\circ}$ thalassaemia picture, will require mapping studies of non-structural gene areas at the 5' end of the δ gene in additional patients (see Orkin *et al.*²³). So far, our data are consistent with the hypothesis of Huisman *et al.*³⁷ that a continuum of molecular lesions might exist between two extremes: the HPFH syndrome with maximum de-repression of γ -chain synthesis and deletions extending from the β -globin gene to the γ gene; and the $\delta^{\circ}\beta^{\circ}$ thalassaemia with minor de-repression of the γ -globin genes and a more limited gene deletion.

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Table 1 Size of β -like globin fragments in $\delta^{\circ}\beta^{\circ}$ human thalassaemic DNA cleaved by various restriction enzymes

<i>Pst</i> I	<i>Bgl</i> II	<i>Eco</i> RI	<i>Xba</i> I	<i>Bam</i> HI + <i>Xba</i> I	<i>Bam</i> HI + <i>Eco</i> RI	<i>Eco</i> RI + <i>Xba</i> I	<i>Bam</i> HI + <i>Bgl</i> II
15	4	3.9	2.8	0.7	1.3	2.8	3.3

Size (in kilobases) was estimated by comparison with β -like DNA fragments generated by cleavage of normal DNA with *Eco*RI (6.5, 4, 1.3 kilobases), *Pst*I (5, 2.3 kilobases), *Bam*HI + *Bgl*II (3.3, 1.8 kilobases) and *Bam*HI + *Eco*RI (4, 1.8, 1.3 kilobases) and run in parallel lanes (for size, see ref. 29).

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Common regulatory mechanism of expression and conjugative ability of a tetracycline resistance plasmid in *Bacteroides fragilis*

RESISTANCE to various antibiotics is common in the anaerobic gut bacterium *Bacteroides fragilis*. A transferable plasmid coding for resistance to antibiotics of the lincosamide/macrolide/synergistin groups has been found^{1,2} and transfer is most probably a conjugative process. This supports the observation of the recent emergence of resistance to antibiotics in these groups and its rapid spread in certain areas (ref. 3 and our unpublished data). However, in the case of tetracycline resistance the limited genetic and physical data were at variance with the epidemiological data. Although tetracycline resistance is common in *B. fragilis*⁴ (at present 60% of strains isolated in the USA⁵ and 30% of those isolated in France (our unpublished results) are resistant) intraspecies transfer of tetracycline resistance could not be demonstrated⁶. We report here that transfer of tetracycline resistance between *B. fragilis* strains is easily achieved if the donor bacteria are grown in the presence of tetracycline before mating. This suggests that tetracycline resistance is borne on a transferable plasmid whose conjugative transfer requires activation of the tetracycline resistance gene. This is supported by our finding that the transfer of tetracycline resistance is either inducible or constitutive, depending on whether tetracycline resistance itself is respectively inducible by tetracycline or constitutive.

At first we failed to transfer Tc resistance from *B. fragilis* strain 92 to a sensitive plasmid-free *B. fragilis* strain (638 rfm); using the same recipient we occasionally succeeded in transferring Tc resistance from a *B. distasonis* donor strain (BEN) at very low frequency¹. Nevertheless, with both donor strains, erythromycin-clindamycin (Em-Cl) resistance was easily shown to be transferable. The plasmid character of resistance to the

macrolides, the lincosamines, and to the synergistins was obvious because they were curable *en bloc* and were easy to characterise in strain 92 and transipients as DNA molecules of molecular weight between 27,000 (ref. 2) and 28,000 (our unpublished results). In similar experiments we failed to eliminate the Tc resistance¹ but the extrachromosomal localisation of Tc resistance was shown in an Em-Cl cured derivative of strain 92 and in Tc transipients (plasmid of 17,000 molecular weight).

It seemed possible that the rapid spread of Tc resistance in *B. fragilis* could be due to a plasmid whose transfer requires certain conditions present in its usual human or animal habitat (the intestine), but not in the normal experimental situation. Among the possible factors 'necessary' for this transfer, a good candidate was tetracycline itself which is widely used in therapy and in animal foods.

In strain 92, tetracycline resistance is inducible by tetracycline if the bacteria are exposed to the antibiotic for 1 h or more (Fig. 1); this strain is said to be Tc^R. The mechanism of plasmid-mediated Tc resistance is not known in *B. fragilis*. But we can reasonably assume that it is under negative regulatory control, as is inducible Tc resistance in facultative anaerobic bacteria⁷.

When strain 92 was cultivated for several generations in the presence of subinhibitory concentrations of tetracycline and then immediately mated with the recipient, Tc transipients were obtained. Results of a typical experiment are given in Table 1, and similar results were obtained in other serial subculture experiments. The number of Tc transipients recovered increased during these initial subcultures to 10⁵-10⁷ ml⁻¹ (Table 1, column 3), and at the same time the number of Em-Cl transipients was relatively stable, or decreased by spontaneous curing of the Em-Cl plasmid. The ratios of Tc^R transipients to Em^R transipients are indicated in Table 1 as an approximate measure of Tc transfer efficiency.

When the donor strain was in contact with tetracycline for 15 generations or less, its ability to transfer Tc resistance was strictly inducible (Tra_i), as it disappeared when the Tc-induced cultures were serially subcultured in the absence of tetracycline for >120 generations (de-induction). The Tc resistance of these de-induced cultures remained inducible (Tc^R), as in the original strain. In contrast to these results, data from Table 1 show that after more generations (>20) of the donor strain in the presence of tetracycline, its ability to transfer Tc resistance was maintained after de-induction; the transfer ability appeared to be constitutive (Tra_c). Concomitantly, the Tc resistance itself turned out to be constitutive (Tc^R). After a greater number of

Table 1 Inducibility against constitutivity of Tc transferability and Tc resistance in *B. fragilis* strain 92

Donor strain*	Tetracycline concentration used before mating (μg ml ⁻¹)	Induced conjugative ability (No. transipients per ml of suspension)†			Inducible versus constitutive Tc ^R transfer‡	Inducibility versus constitutivity of Tc resistance§
		Tc	Em	Tc/Em		
92	0	0	3 × 10 ⁵	<3 × 10 ⁻⁴	—	i
92-Tc ₅	10	2 × 10 ⁵	2 × 10 ⁵	1	i	i
92-Tc ₁₀	10	3 × 10 ⁵	1 × 10 ⁵	3	i	i
92-Tc ₁₅	40	4.5 × 10 ⁷	5 × 10 ³	~10 ⁴	i	i
92-Tc ₂₀	40	+	+	—	i and c	i and c
92-Tc ₂₅	40	+	+	—	c	c

* The donor strain was inoculated at 4 × 10⁷ bacteria ml⁻¹ in TYG broth¹ in the presence of variable concentrations of tetracycline. Subcultures were similarly made daily from the higher non-inhibitory Tc concentration. In these conditions each subculture is equivalent to five generations. After 5, 10... generations in the presence of Tc, strain 92 is designated as 92-Tc₅, 92-Tc₁₀ and so on.

† Conjugative ability was tested after each subculture in the presence of Tc, by mating the donor with *B. fragilis* 638 rfm according to ref. 1; 0.05 ml of each strain were mixed onto TYG plates of 5 cm diameter. After an overnight incubation the culture was scraped, suspended in 2 ml of TYG and various dilutions of the suspension spread onto agar plates supplemented with rifampicin (Rfm) at 25 μg ml⁻¹ and either tetracycline (Tc) at 2 μg ml⁻¹ or erythromycin (Em) at 10 μg ml⁻¹. The numbers of transipients per ml of this suspension are indicated in column 3. Isolation and characterisation of transipients, and conditions of growth were as in ref. 1.

‡ After each subculture in the presence of Tc, the culture was de-induced by growth without Tc for at least 120 generations and tested for its transferability as before.

§ After de-induction, the cultures were tested for inducibility (i) versus constitutivity (c) of Tc resistance using the conditions in Fig. 1, except that the induction time was routinely 18.

serial subcultures (between 40 and 100 generations) in the presence of tetracycline, identical results were obtained, but the minimal inhibitory concentrations (MIC) of Tc for the donor were significantly higher ($100 \mu\text{g ml}^{-1}$) than initially ($16 \mu\text{g ml}^{-1}$), in contrast to identical values during the initial serial subcultures (MIC Tc, $20\text{--}40 \mu\text{g ml}^{-1}$).

In the experiment described in Table 1, the de-induced donor 92-Tc₂₀ which was at first phenotypically Tra₊ turned out to be a mixture of Tc^R and Tc^S clones; when both clones were rechecked for ability to transfer Tc resistance, they were found to be Tra₊ and Tra₋ respectively.

The number of generations in the presence of tetracycline required to obtain constitutive Tc resistance and Tc transfer varied from one serial subculture experiment to another, but the phenomenon was experimentally reproducible between the 10th and 25th generations.

The fact that Tc transfer appeared even after a few generations (5–25) in the presence of tetracycline without significant modification of the MIC of the donor seems to suggest a modification of the conjugative ability itself, rather than quicker or more efficient Tc^R expression in transipients. Two findings support this view. (1) In the mating of strain 92 (without any previous contact with Tc) and of the recipient strain 638 rfm, no Tc transipients were obtained even when the Tc concentration was lowered in the selective plates to $0.2 \mu\text{g ml}^{-1}$ instead of $2 \mu\text{g ml}^{-1}$ as used in all experiments previously reported (MIC Tc of the recipient strain, $0.064 \mu\text{g ml}^{-1}$). (2) In a similar mating using as donor a Tra₊ Tc^R strain obtained by 20 serial subcultures at a sub-inhibitory concentration of tetracycline (MIC Tc, $100 \mu\text{g ml}^{-1}$) the same number of transipients was obtained by using for the selection different concentrations of tetracycline (Table 2).

We verified that the Tc plasmid transfer was specifically induced by tetracycline or minocycline, but not by erythromycin. The Em-CI plasmid is not required for Tc plasmid transfer, because identical results were obtained with strain 92-1 which is a derivative, cured of its Em-CI plasmid¹.

Physical analysis of Tc transipients and of a Tra₊ Tc^R derivative from strain 92 shows that these strains harbour a plasmid of molecular weight 17,000 as does the wild type strain; consequently plasmid amplification can be ruled out as a possible mechanism for the newly acquired properties.

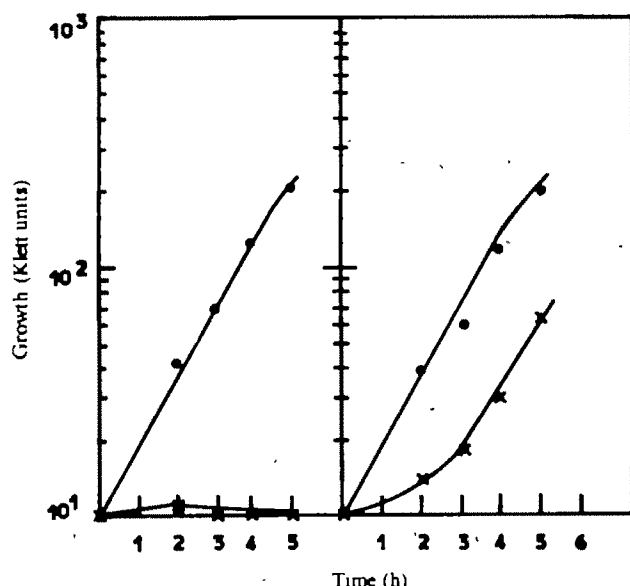


Fig. 1 Induction of tetracycline resistance in *B. fragilis* strain 92. Cultures inoculated with early stationary cells (10^8 cells ml^{-1}) were grown in TYG broth¹ for 1 h without tetracycline (Tc) (a) or with $0.5 \mu\text{g ml}^{-1}$ of Tc (b). At this time (T_0) they were inoculated at 5×10^6 cells ml^{-1} in TYG broth without Tc (O) or with Tc at $5 \mu\text{g ml}^{-1}$ (x). Cultures were incubated at 34°C in anaerobic jars and the optical densities were measured at hourly intervals (Klett photocolormeter, filter no. 54).

Table 2 Mating of strain 92-Tc₁₀₀ and strain 638 rfm*

	Tc concentration in the selective plates ($\mu\text{g ml}^{-1}$)†		
	2	20	40
No. of transipients‡	3.2×10^3	3.0×10^3	2.1×10^3

* The donor strain had a MIC for Tc of $100 \mu\text{g ml}^{-1}$. Before mating it was de-induced by 150 generations in TYG broth in the absence of tetracycline. Other experimental conditions were as in Table 1.

† Selection was made onto TYG agar medium supplemented with rifampicin at $25 \mu\text{g ml}^{-1}$ and tetracycline at the indicated concentration.

‡ The number of transipients is expressed per ml of mating suspension (see Table 1)

Similar results were obtained with other clinical isolates of *B. fragilis*; in these strains, Tc resistance and transfer ability were shown to be inducible by tetracycline in the same experimental conditions. Similarly, when strain BEN of *B. distasonis*¹, also Tc^R, was used as a donor, a considerable increase ($>10^3$) in Tc^R transipients was regularly obtained when the strain was subcultured in the presence of tetracycline before mating. The phenotype Tra₊ can be attributed to this strain in spite of its very low Tc^R conjugative ability without Tc induction¹. In strain BEN it is probable that an Em-CI plasmid is co-transferred by Tc plasmid¹. The induction of transferability of Tc plasmid led to a parallel increase of co-transfer. Spontaneous Tra₊ Tc^R mutants were also obtained from strain BEN.

These results show that growth of a few generations of the Tc^R donor strain in the presence of tetracycline reversibly and specifically induces Tc conjugative ability. This suggests induction (or de-repression) of the conjugative ability of the Tc plasmid by tetracycline. At the same time tetracycline is likely to act as the inducer or de-repressor of a product required for expression of Tc resistance. This result is comparable to two reports from studies of metabolic plasmids: Nakasawa and Yokota⁸ showed stimulation of TOL plasmid transfer by *m*-toluate in *Pseudomonas putida*, and Petit *et al.*⁹ demonstrated a stimulatory effect of nopaline or octopine on Ti plasmid transfer in *Agrobacterium tumefaciens*. Our findings with an antibiotic resistance plasmid confirm the prediction of Petit *et al.*⁹ that RTF factors will exist with a conjugative ability inducible by the antibiotic whose resistance they are coding. The induction of plasmid transfer by induction of a gene carried on it appears therefore to be a widespread phenomenon in bacteria. Moreover, with *B. fragilis* strain 92, a greater number of generations grown in the presence of Tc led to Tc^R derivatives stably constitutive for transfer (Tra₊) (data not shown). These derivatives are probably spontaneous mutants selected by the antibiotic, whose mutation confers constitutivity for both Tc resistance and Tc^R conjugative ability. The mutation could be in a regulatory gene controlling both properties.

These results lead us to postulate for the Tc plasmids of *B. fragilis* a common regulatory mechanism for resistance and for conjugative ability. These results may help to explain the emergence of Tc^R strains in the intestinal flora during long-term tetracycline therapy. They emphasise the extreme danger of using tetracycline in animal foods or in therapy, unless strictly indicated.

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Cytochrome c_2 sequence variation among the recognised species of purple nonsulphur photosynthetic bacteria

IN higher organisms, the nature and amount of amino acid sequence difference between homologous proteins from different species correlates well with what is known about the phylogeny of the organisms from morphological and palaeontological evidence¹⁻³. We are interested in the application of amino acid sequence information to the study of bacterial phylogeny, a subject that is not amenable to investigation by the classical methods because of the paucity of morphological features and the lack of a palaeontological record. We have, therefore, determined the amino acid sequences of the principal soluble cytochromes c of representatives of each recognised species of the Rhodospirillaceae, the nonsulphur purple photosynthetic bacteria. In contrast to higher organisms, there was no correlation between a species' cytochrome c amino acid sequence and its phylogenetic position.

The Rhodospirillaceae are the best studied group of phototrophic bacteria^{4,5}, and they have been divided into three genera containing a total of 12 species⁶ (Table 1). The properties of most of these species were found by the study of numerous separate isolates in pure culture. The different species (Table 1) vary widely in their physiology and morphology, and most seem to be definite taxonomic entities. Selected strains of each species have been grown in large quantities and contain high concentrations of a variety of photosynthetic pigments and electron transport proteins⁷, of which the relevant ones are shown in Table 1.

Rhodospirillum rubrum is the type species of the family which produces the well known cytochrome c_2 . Both the amino acid sequence⁸ and the three dimensional structure⁹ are known, and considerable similarity to mitochondrial cytochrome c has been recognised in both sequence and folding. Cytochromes c_2 function in photosynthetic electron transport^{10,11}. They may also function in some modes of non-photosynthetic metabolism, but this is not proved.

We have examined the major, soluble, low-spin c -type cytochromes from 11 other species of Rhodospirillaceae. Most of them produce a protein (or proteins) that resemble cytochrome c_2 and mitochondrial cytochromes c in sequence (Fig. 1). However, two of the species, *Rhodospirillum tenue* and *Rhodopseudomonas gelatinosa*, do not apparently produce any protein of this class but instead form a cytochrome¹² that resembles the small cytochrome c -551 of the denitrifying pseudomonads. The cytochromes c_2 from five of the species (Fig. 1*c-i*) have amino acid sequences resembling those of mitochondrial cytochromes c at least as closely as they do those of *R. rubrum* cytochrome c_2 . These sequences can be aligned with those of the mitochondrial proteins with good matching throughout¹³ (Fig. 1). The remaining four species (Fig. 1*j-o*) produce proteins that resemble *R. rubrum* cytochrome c_2 in that they have appreciably longer sequences than the others (116-124 residues), and to get good matching with the smaller cytochromes c_2 or with mitochondrial cytochromes c require at least three- and eight-residue insertions plus a single-residue deletion. Each of these four species produces sequences containing distinctive insertions and deletions. The cytochrome c -550 from the denitrifying bacterium *Paracoccus denitrificans*^{14,15} also belongs to this sequence group, and shows particular affinity to the *Rhodopseudomonas capsulata* sequence.

We have examined the proteins from two independent strains of *Rps capsulata* and *Rhodopseudomonas palustris*. The former proteins differ from each other in only two positions, while the latter differ in 12 positions. These differences are much smaller than those between any two different species except *Rhodospirillum molischianum* and *Rhodospirillum fulvum*.

*R. molischianum*¹⁶ and *R. fulvum* both produce two distinct soluble cytochromes c_2 . Despite earlier reports¹⁶, both isocytocromes are very similar in size. The sequences of both the *iso*-1-cytochrome c_2 and the *iso*-2-cytochrome c_2 of *R. fulvum* differ from the corresponding proteins of *R. molischianum* in 12 positions. *Rhodospirillum molischianum* and *R. fulvum* are taxonomically similar⁶, the main distinctions between them being cell width *p*-aminobenzoate requirement and ability to utilise benzoate.

The *iso*-1-cytochrome c_2 of both *R. molischianum* and *R. fulvum* lack the 'evolutionarily invariant' tryptophan residue at position 59 (Fig. 1), common to all natural mitochondrial cytochromes c so far examined and to the other cytochromes c_2 shown in Fig. 1. An unstable mutant form of yeast cytochrome c has been produced in which this tryptophan residue has been replaced¹⁷.

The *iso*-2-cytochromes c_2 of both *R. molischianum* and *R. fulvum* seem to contain a mixture of phenylalanine and tyrosine

Table 1 Properties of members of the family Rhodospirillaceae⁶ including occurrence of electron transport proteins⁷

Species	Strain	ATCC no.	Electron transport proteins					Mode of cell division	Type of photosynthetic membrane	Cell form
			Large c_2	Small c_2	c -551	c'	HiPIP			
<i>Rhodospirillum rubrum</i>	S1 (=1.1.1)	11170	+			+		Binary fission	Vesicular	Spiral
<i>R. tenue</i>	3761				+	+	+	Binary fission	Tubular	Thin spiral
<i>R. fulvum</i>	1360	15798		+		+		Binary fission	Lamellar	Short spiral
<i>R. molischianum</i>	S	14031		+		+		Binary fission	Lamellar	Spiral
<i>R. photometricum</i>	SP113		+			+		Binary fission	Lamellar	Large spiral
<i>Rhodopseudomonas</i>	2.1.6	17001	+					Bud	Lamellar	Rod
<i>palustris</i>	2.1.37	17007	+			+		Bud	Lamellar	Rod
<i>Rps viridis</i>	NTHC 133			+				Bud	Lamellar	Rod
<i>Rps acidophila</i>	7050	25092		+				Bud	Lamellar	Large rod
<i>Rps gelatinosa</i>	2.2.1	17011			+	+	+	Binary fission	Tubular	Small rod
<i>Rps capsulata</i>	2.3.1	11166	+			+		Binary fission	Vesicular	Rod
	St. Louis	23782	+			+		Binary fission	Vesicular	Rod
<i>Rps sphaeroides</i>	2.4.1	17023	+			+		Binary fission	Vesicular	Sphere
<i>Rhodomicrobium vannielli</i>	3.1.1	17100		+			+	Bud	Lamellar	Stalked oval

Two distinct members of this class of protein present.

Mitochondrial cytochrome c		10	20	30	40	50	60	70	80	90	100	
a	<i>Equus caballus</i> ²³	AGDVEKGKKIFVQKCAQCHTVEKGG	-----	KKHTGPNLHGLFRKTRGAPGFT	YTDANKN	---	KGITWKEETLMEYLENPKKYI	-----	PG	-----	TKM	IFAGIKKTEREDLIAYLKATNE
b	<i>Euglena gracilis</i> ^{24,25}	AGDAERGKKLFESRAAQCHSAQKG	-----	VNSTGSPSLGVGYRTSGSVGYA	YSNANKN	---	AAIVWEETLHKFLENPKKYV	-----	PG	-----	TKM	AFAGIKAKKDRQDIAYMKTLKD
Cytochrome c ₂												
c	<i>Rm. vanielii</i> ¹³	AGDPVKGQVF-KQCKICHQVPTA	-----	KNGVGPQNDFVQKAGARPGFN	YSDAMKN	---	SGLTWDEATLDKYLENPKAVV	-----	PG	-----	TKM	VFVGLKNPQDRADVIAYLKQLSGK
d	<i>Rps. viridis</i> ¹³	QDAASGEQVF-KQCLVCHSIGPGA	-----	KNKVGPVLNGLFGRNGTIEGFS	YSDANKN	---	SGITWTEEVFREYIRDPKAKI	-----	PG	-----	TKM	IFAGIKDEQKVSGLIAYLKQFNADGSKK
e	<i>R. acidophila</i>	AGDPDAGQKF-LKCAACHKIGPGA	-----	KNGVGPPLNGVANRKAQGAEGA	YSDANKN	---	SGLTWDEATFKEYITAPQKKV	-----	PG	-----	TKM	TFPGLPNEADRDNIWAYLSQFADGSK
f	<i>R. molischianum iso-1</i>	ADAPP--PAF-NQCKACHSIDAG	-----	KNGVGPPLSGAYGRKVLAPNYK	YSPAHLA	---	SGMTIDAMLTLYLANPKETI	-----	PG	-----	NKMGAFGLKKNPDAVAIVAYLKTVK	
g	<i>R. fulvum iso-1</i>	ADAP--TAF-NQCKACHSIEAG	-----	KNGVGPPLSGAYGRKVLAPNYK	YSPAHLA	---	SGMTIDEAMLTNYLANPKATI	-----	PG	-----	NKMGAFGLKKNPDDVAVIAYLKTVK	
h	<i>R. molischianum iso-2</i>	ADAP--AGF-TLCKACHSVEAG	-----	KNGVGPPLAGVYGRKAGTISGFK	FSDPHIK	---	SGLTWDEPTLTXYLADPKTVI	-----	PG	-----	NKM	VFAGLKNPDDVAVIAYLKTLK
i	<i>R. fulvum iso-2</i>	ADAP--PAF-GMCKACHSVEAG	-----	KNGVGPPLAGVYGRKAGTLAGFK	FSDPHAK	---	SGLTWDEPTLTXYLADPKGVI	-----	PG	-----	NKM	VFAGLKNPDAVAIVAYLKSL
j	<i>R. rubrum</i> ⁸	EGDAAAGEKV-SKKCLACTFDQGG	-----	ANKVGNPLFGVFENTAANKDNYA	YSESYTEMKAKGLTWTEANLAAYKDPKAFVLEKSGDPK	---	AKSKM	---	TFK	---	LTKDDEIENVIAYLKTLK	
k	<i>R. photometricum</i>	AGDAAVGEKIAKAKCTACHDLNKG	-----	PIKVGPPFLGVFGRTTGTGAGYS	YSPGYVTMGQKHTWDDNALKAYLLDPKGYVQAKSGDPK	---	ANSKM	---	IFR	---	LEKDDVANVIAYLHTMK	
l	<i>Rps. palustris</i> 2.1.6	QDAAKEAVF-KQCMCTHRAD	-----	KNMVGPALGGVGRKAGTAAGFT	YSLPLNHNHSGEAGLVWTDNIN	---	IAYLPDPNAYLKKFLTDKGQADKATGSTM	---	TFK	---	LANQQRKDVAAYLATLK	
m	<i>Rps. palustris</i> 2.1.37	QDAAKEAVF-KQCMCTHRAD	-----	KNMVGPALGGVGRKAGTAAGFT	YSLPLNHNHSGEAGLVWTDNIN	---	IAYLPDPNAYLKKFLTDKGADQAVGVTKM	---	TFK	---	LANEQQRKDVAAYLATLK	
n	<i>Rps. sphaeroides</i>	QEGDPEAGAKAF-NQCTCHVVDGSGTTIAGRNAGTGNLYGVVGR	TAGTQADFQGGGKAGKAGLWDEEH	FVQYVQDPTFLKEYTGDAK	-----	AKGKM	---	TFK	---	LKKEADAHNIWAYLQVAVRP		
o	<i>Rps. capsulata</i> 2.3.1	GDAAKEGEF-NKCKTCHSIAPDGT	IV-KGAKTGNLYGVVGR	TAGTYPEFK	YKDSIVALGASGFVAWTEED	---	ATYKDPGAFLEKTDGKK	---	AKSGM	---	AFK-LAK	---

Fig. 1 Amino acid sequences of Rhodospirillaceae cytochromes *c*₂ aligned with selected mitochondrial cytochrome *c* sequences. Residues are numbered from the horse (*a*) sequence. Underlined residues are referred to in this caption. In sequence *b* residue 86 is *ε*-*N*-trimethyl-lysine. In sequence *e* the evidence for the amide ascriptions among residues 87–90 is weak. In sequences *h* and *i* position 67 contains phenylalanine (about 30%) as well as the predominant tyrosine. In sequence *i* only the compositions of regions 32–36 and 93–97 are known. In sequence *k* there is conflicting evidence, some of which suggests that there is an additional glycine residue between residues 56 and 57. Sequence *o* is for *Rps capsulata* strain 2.3.1; in strain St Louis, residue 75 + 4 is leucine (not threonine) and residue 78 is threonine (not serine). Experimental evidence will be published elsewhere. The one-letter notation used is that recommended by the IUPAC-IUB Commission on biochemical nomenclature. A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; M, methionine; N, asparagine, P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

at the position that corresponds to the highly conserved tyrosine-67 of mitochondrial cytochromes *c*. Such polymorphism is well established in higher organisms, where it can occur through population differences¹⁸, heterozygosity, or multiple genes^{19,20}. Only the last of these explanations can apply to the prokaryotes in the present instance. It is remarkable that this heterogeneity is present at the same site in the proteins of two organisms that have otherwise diverged appreciably.

Our results show that the deduction of phylogenetic information from the sequence of homologous bacterial proteins is not straightforward. Studies of the sequences of cytochromes *c*-551 from different *Pseudomonas* species^{21,22} have shown interspecific differences to be much larger than intraspecific differences. Sequence differences between similar species for these cytochromes *c*-551 are as large as those between mitochondrial cytochromes *c* of mammals and insects, although both classes of protein probably have similar redox functions. Our results for the Rhodospirillaceae emphasise this sequence variability more strongly. The predominant low-spin cytochromes *c* from these organisms fall into three distinct sequence classes whose distribution (Table 1) do not correlate with the genera into which the family has been divided on a morphological basis^{5,6}. Even within one of these sequence classes, the cytochrome *c* is more variable between different species of the bacterial family than it is across the whole range of eukaryotic mitochondria. It could be argued that this sequence heterogeneity in the Rhodospirillaceae is a consequence of the extreme age of the family, as organisms with a similar photosynthetic capability could have flourished in the Precambrian epoch before the appearance of oxygenic photosynthesis. Nevertheless, this theory does not predict the lack of correlation of sequence type with morphology nor the occurrence of the similarities observed between the cytochromes of species of Rhodospirillaceae and those of organisms with very different metabolic patterns, for example, *Rps capsulata* and *P. denitrificans*¹⁵, *Rhodopseudomonas viridis* and mitochondria¹³ (Fig. 1), or *R. tenue* and *Pseudomonas aeruginosa*¹².

Our observations are consistent with the hypothesis that bacterial evolution proceeds both by the assimilation of genes for single functions or for whole metabolic pathways from other organisms as well as by the gradual processes of mutation and selection. We have no information about the frequency of successful gene assimilation or about the timescale for protein evolution and speciation in bacteria. We have no evidence at all

to suggest that either a gene product (such as a cytochrome *c*) or an assemblage of genes (such as *Rhodospirillum rubrum*) should be stable through geological time.

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Anomalies in amino acid sequences of small cytochromes *c* and cytochromes *c'* from two species of purple photosynthetic bacteria

MOST species of the Rhodospirillaceae¹, the purple nonsulphur photosynthetic bacteria, produce large quantities of cytochrome *c*₂ (ref. 2), a protein which is similar in sequence to mitochondrial cytochrome *c*³. Many species also produce the high-spin haem protein cytochrome *c'* (ref. 2). Cytochromes *c'* have been identified in *Rhodospseudomonas gelatinosa* and in *Rhodospirillum tenue*, but in these organisms the predominant low-spin cytochromes *c* do not have properties similar to those found for cytochromes *c*₂ (ref. 2). We have now determined the amino acid sequences of the cytochromes *c'* and of the predominant low-spin cytochromes *c* from the two organisms (Fig. 1).

Comparison of these sequences with those of analogous cytochromes reveals some anomalies (Table 1). The sequences of the two low-spin cytochromes, *Rps gelatinosa* cytochrome *c*-551 and *R. tenue* cytochrome *c*-553 (Fig. 1a) markedly resemble the cytochromes *c*-551 of denitrifying pseudomonads⁴ and of *Azotobacter vinelandii*⁵. For alignment with the *Pseudomonas* and *Azotobacter* sequences, the *Rps gelatinosa* cytochrome requires the postulation of a three-residue insertion immediately after the methionine residue which is presumed to be the sixth iron ligand, while alignment of the *R. tenue* cytochrome requires the postulation of a five-residue insertion shortly before this methionine residue (Fig. 1a). The *R. tenue* cytochrome *c*-553 is particularly similar in sequence to the cytochrome *c*-551 of *Pseudomonas aeruginosa* (Fig. 1a), even in regions that are variable among the *Pseudomonas* species of known sequence^{4,5}.

The sequences of the cytochromes *c'* (Fig. 1b) can be aligned with each other if a one- and a three-residue deletion are postulated in the *Rps gelatinosa* sequence, and a single one-residue deletion is postulated in the *R. tenue* sequence. The sequences show 31% identity overall when so aligned, with poor matching between residues 16 and 50 (Fig. 1b). This compares with 37% identity for the cytochromes 'c-551' (Fig. 1a) and the

27% determined in another study⁶ for their high potential iron sulphur proteins (HiPIP) (Fig. 1c). The sequences of three other cytochromes *c'* are already known, those of an *Alcaligenes* species⁷, *Rhodospirillum rubrum*⁸ and *Chromatium vinosum*⁹. If a two-residue insertion is postulated, the *Rps gelatinosa* cytochrome *c'* matches well with the *Alcaligenes* protein (48% identity) and if a one-residue insertion is postulated, the *R. tenue* cytochrome *c'* matches well with the *C. vinosum* protein (47% identity). In both these cases, good matching extends throughout the sequences (Fig. 1b). The sequence of the cytochrome *c'* from *R. rubrum* is known⁸ and those from *R. molischianum*, *R. fulvum*, *Rps capsulata* and *Rps palustris* are being investigated. None of these proteins resemble the *R. tenue* protein as closely as that protein resembles the *C. vinosum* cytochrome *c'*; neither do any of them resemble the *Rps gelatinosa* protein as closely as that protein resembles the *Alcaligenes* cytochrome *c'* (R.P.A., T.E.M., R. G. Bartsch and M.D.K., unpublished results). The cytochromes 'c-551' and the cytochromes *c'* of *Rps gelatinosa* and *R. tenue* all seem to be anomalously closer in sequence to proteins from organisms of other families, including non-photosynthetic ones, than they are to proteins of other members of the Rhodospirillaceae. *Rps gelatinosa* and the strain of *Alcaligenes* are very different both physiologically and in electron transport protein complement¹⁰. The pattern of similarity observed for the cytochromes *c'* does not apply to the HiPIPs, as the *R. tenue* HiPIP is more different to that from *C. vinosum* than to any of the others of known sequence⁷. *C. vinosum* has been reported to contain a cytochrome *c*-551 (ref. 11), but the amount present is small and nothing is yet known about its structure. Pfennig¹² has recently discussed the relationship between the Chromatiaceae and the Rhodospirillaceae. A reassessment of the evidence of sulphur metabolism, and a consideration of the genus *Ectothiorhodospira* has lessened the distinction between these families; some similarity between their electron transport proteins can now be expected.

To detect evolutionary anomalies, it has been proposed (for example, by Wilson *et al.*¹³) that the amino acid sequences of several different gene products from a set of organisms should be compared, so that evolutionary trees might be constructed and tested for congruence. Although the comparison of three sets of proteins from three different species does not provide sufficient data on which to base firm conclusions, phylogenetic trees constructed from the results in Table 1 could never be interpreted as congruent, nor would they have any predictive value.

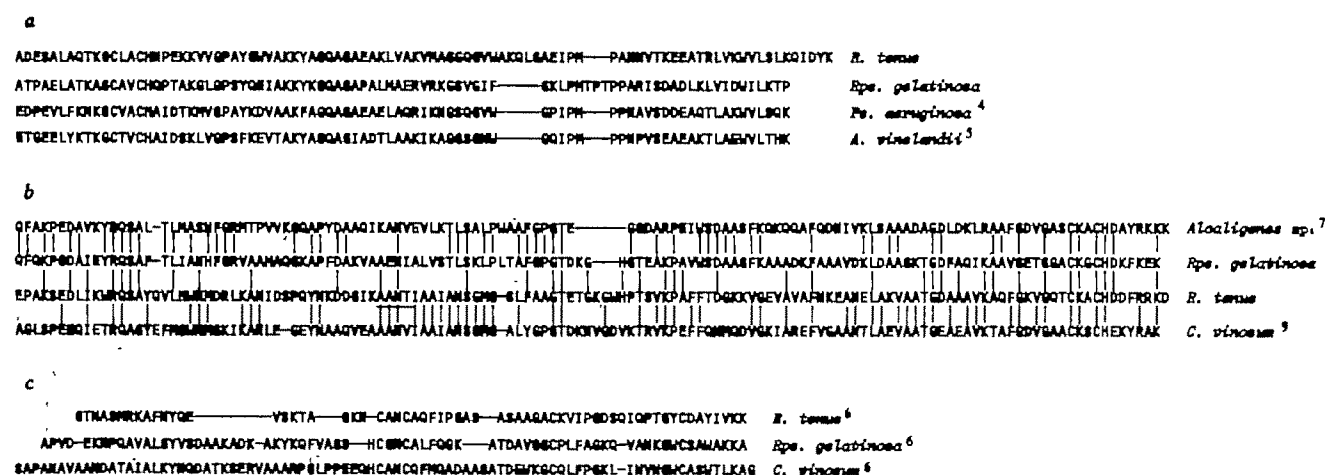


Fig. 1 Amino acid sequences of a, cytochromes 'c-551'; b, cytochromes *c'*; c, high potential iron sulphur proteins (HiPIP)⁷ from *Rhodospseudomonas gelatinosa* strain 2.2.1 (= ATCC 17011) and from *Rhodospirillum tenue* strain no. 3761. The sequences are shown aligned with other proteins of the same class and known sequence. In b, vertical lines mark identical residues in adjoining sequences. The evidence for the sequence of the underlined residues in *R. tenue* cytochrome *c'* (AANT) is weak. Experimental evidence for the sequences will be published elsewhere. The one-letter notation used is that recommended by the IUPAC-IUB Commission on biochemical nomenclature. A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

Table 1 Similarity matrices for amino acid sequences of cytochromes 'c-551', cytochromes c' and high potential iron sulphur proteins (HiPIP)⁶

Cytochromes 'c-551'			Cytochromes c'		
<i>Rhodospirillum tenue</i>			<i>Rhodospirillum tenue</i>		
37	<i>Rhodopseudomonas gelatinosa</i>		31	<i>Rhodopseudomonas</i>	
56	39	<i>Pseudomonas aeruginosa</i>		<i>gelatinosa</i>	
45	38	60 <i>Azotobacter</i>	47	28	<i>Chromatium vinosum</i>
		<i>vinelandii</i>	29	48	24 <i>Alcaligenes</i> sp.
HiPIP ⁶					
<i>Rhodospirillum tenue</i>					
27	<i>Rhodopseudomonas gelatinosa</i>				
21	37	<i>Chromatium vinosum</i>			

Values shown are matches per 100 residues when the sequences are aligned as in Fig. 1.

Noncongruence of evolutionary trees for *Desulfovibrio* and *Clostridium* ferredoxin and rubredoxin has already been observed¹⁴.

Noncongruence of protein evolutionary trees could be caused by several mechanisms. In higher organisms several anomalies have been explained by the realisation that 'paralogous' proteins had been compared¹⁵. Noncongruence could also be caused if the rate of change of the proteins in separate branches were very different. If functional requirements severely limit the possibility of further change in a protein, the possibility of evolutionary convergence would be increased and would cause further distortion. Nevertheless the simplest (if most difficult to prove) explanation for all the anomalies described is that the proteins concerned have reached some of the organisms by processes of interspecific gene transfer.

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Processing of small subunit precursor of ribulose biphosphate carboxylase and its assembly into whole enzyme are stromal events

CHLOROPLAST development requires the activities of both nuclear and chloroplast genetic systems, but the precise contribution of each and the mechanism by which they interact are not understood. A prime goal is to determine the site of synthesis of each chloroplast polypeptide. Studies with isolated subcellular systems, as well as inhibitor experiments with intact cells, have established that many chloroplast polypeptides are made by cytoplasmic ribosomes¹. Such polypeptides include subunits of both thylakoid and stromal multi-subunit proteins. These observations raise two questions: how do these polypeptides traverse the chloroplast envelope, and where are they assembled into whole functional proteins? The small subunit of the chloroplast enzyme ribulose biphosphate carboxylase is made in the cytoplasm as a higher molecular weight polypeptide precursor². We report here that the cleavage of this precursor and the assembly of the small subunit into whole ribulose biphosphate carboxylase molecules takes place in the chloroplast stroma.

Isolated pea chloroplasts synthesise as the major soluble product the large subunit of ribulose biphosphate carboxylase/oxygenase, the key enzyme in photosynthesis and photorespiration³. The carboxylase enzyme (RBPCase) is an oligomer of 16 subunits. Besides eight large catalytic subunits, the whole enzyme contains eight small subunits (molecular weight 14,000) of unknown function, which are encoded in the nuclear genome⁴. We have shown that the small subunit is synthesised as a precursor of higher molecular weight (20,000) when poly(A)-containing RNA from pea leaf cytoplasmic ribosomes is translated by a heterologous protein-synthesising system^{2,5}. This precursor enters intact isolated chloroplasts with processing to the final size. This uptake and processing does not require concomitant protein synthesis, and thus differs from the signal mechanism advanced to explain the transport of polypeptides across the endoplasmic reticulum⁶. We have suggested an envelope carrier mechanism in which the small subunit precursor is released from cytoplasmic ribosomes and interacts through some aspects of its tertiary structure with a specific binding site in the chloroplast envelope^{2,3}. Unlike known signal sequences, the extra amino acid sequence present in the precursor was postulated to be charged, and its removal by an envelope protease was suggested to trigger a conformational change leading to the transport of the small subunit across the envelope. However, attempts to detect processing activity in preparations of pea chloroplast envelopes purified by the method of Joy and Ellis⁷ have been unsuccessful; instead we find the processing activity to be stromal⁸.

Small subunit precursor (P20) was synthesised by programming a wheat-germ protein-synthesising system with poly (A)-containing RNA from polysomes isolated from etiolated shoots of *Pisum sativum* greened for 48 h (ref. 2). After incubation for 60 min with ³⁵S-methionine the wheat-germ extract was centrifuged at 180,000g for 60 min to remove ribosomes and ribosome-bound labelled polypeptides. The supernatant solution contained P20 as the most heavily labelled polypeptide. Aliquots of this solution were incubated with fractions prepared from intact chloroplasts. Table 1 shows that the processing activity is present in both intact chloroplasts purified on silica-sol gradients, and in stromal extracts prepared from intact chloroplasts. In contrast to previous results², the activity in stromal extracts is not sedimented by centrifugation at 180,000g for 3 h; this treatment sediments both chloroplast ribosomes and whole RBPCase molecules. This soluble stromal nature of the processing activity has been observed in at least 10 separate

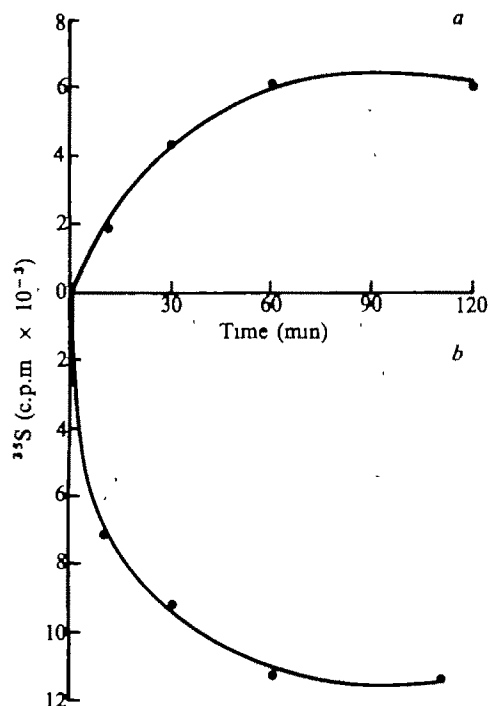


Fig. 1 Time course of processing of P20 to small subunit. Wheat-germ products and 3,200-g stromal supernatant fractions were prepared as in Table 1. Samples (10 μ l) of wheat-germ supernatant were incubated with 40- μ l samples of stroma for various times at 27 °C, and then boiled for 2 min in polyacrylamide gel loading buffer². Each incubation mixture contained the equivalent of 5.7 μ g chlorophyll and TCA-insoluble radioactivity equal to 2.5×10^5 c.p.m. Autoradiography and the quantification of the processing activity were as described in Table 1. The results presented are those obtained by counting the radioactivity in the P20 and small subunit bands of the polyacrylamide gel. *a*, Increase in radioactivity in small subunit over that present at zero time; *b*, decrease in radioactivity in P20 below that present at zero time.

experiments carried out independently by two workers. The failure of previous experiments to show that the processing activity is soluble may indicate a loose association of the processing activity with the inner membrane of the chloroplast envelope. The small subunit precursor from *Chlamydomonas* is also processed to the small subunit by a soluble enzyme⁹, but in that case it was not possible to show that the activity was located in the stroma because intact chloroplasts cannot be isolated from this alga.

Loss of label from P20 is not invariably associated with the appearance of label in the small subunit, as we have previously assumed. Membrane preparations often cause a decrease in P20 label, but this loss is never accompanied by an increase in small subunit label (Table 1) or other polypeptide. No other polypeptide synthesised by the wheat-germ system shows a decrease in label after incubation with any chloroplast fraction, suggesting that nonspecific proteolysis is absent. The time course of processing by stromal extracts shows that more label is lost from P20 than appears in the small subunit (Fig. 1). This observation establishes that the sequence removed during processing contains at least one methionine residue. If this methionine residue is at the N terminus, its removal by an N-terminal peptidase would account for the activity seen in the membrane preparations. Such N-terminal peptidase activity may also occur in the stromal fractions, but this is impossible to establish until specific inhibitors of processing are available.

The soluble nature of the P20 processing activity raises the question as to how it functions in the transport of the precursor through the chloroplast envelope. One possibility is that the precursor interacts with the envelope in such a way that the

sequence to be removed becomes accessible from the stromal side. Removal of the sequence may be the event which triggers the release of small subunit into the stromal compartment. A critical test of this idea would be to determine whether removal of the extra sequence by purified processing enzyme affects the ability of intact chloroplasts to take up the molecule.

An additional or even alternative role for processing may be in the assembly of whole RBPCase molecules. Analysis on

Table 1 Subcellular location of small subunit precursor processing activity

Fraction tested	% Decrease in label in small subunit precursor (P20)	% Increase in label in small subunit
Buffer	0	0
Washed chloroplasts	41	75
Purified intact chloroplasts	31	50
Lysed chloroplasts	33	45
Stroma 3,200g 5 min	30	58
Stroma 180,000g 1 h	41	47
Stroma 180,000g 3 h	40	62
Thylakoids	21	0
Envelopes	35	0

Poly(A)-containing RNA was prepared from pea leaf polysomes and translated in a wheat-germ protein-synthesising system as before², except that the final concentrations of KCl and MgCl₂ were 90 mM and 2 mM, respectively. After incubation, the wheat-germ extract was centrifuged at 180,000g for 60 min at 4 °C to sediment ribosomes and ribosome-bound polypeptides. This treatment sediments about 60% of the trichloroacetic acid (TCA)-insoluble radioactivity, leaving the small subunit precursor in the supernatant as the most heavily labelled polypeptide. During the preparation of the wheat-germ products chloroplasts were isolated from 10-d-old pea leaves as described previously³. These chloroplasts were washed once in isolation medium, and resuspended in intact plastid buffer prepared by mixing equal volumes of 10 mM Tris-HCl, pH 7.6 and 50 mM HEPES-KOH, 220 mM KCl, 6 mM MgCl₂, 20 mM dithiothreitol (DTT), pH 7.6. This preparation is referred to as washed chloroplasts, and consists of about 60% intact chloroplasts as judged by phase-contrast microscopy. Lysed chloroplasts were prepared by resuspending a pellet of washed chloroplasts in 10 mM Tris-HCl, pH 7.6, leaving it on ice for 5 min, and then adding an equal volume of 50 mM HEPES-KOH, 220 mM KCl, 6 mM MgCl₂, 20 mM DTT, pH 7.6. A sample of lysed chloroplasts was centrifuged at 3,200g for 5 min to obtain a stromal supernatant and a thylakoid pellet. The latter was washed once, and resuspended in an equal volume of intact plastid buffer. Samples of stroma were centrifuged further at 180,000g for 1 and 3 h at 4 °C while other preparations remained on ice. Intact chloroplasts were purified on gradients of Ludox AM silica sol¹² from a preparation of washed chloroplasts. Purified envelopes were prepared from washed chloroplasts by the method of Joy and Ellis⁷. For the measurement of processing activity, samples (10 μ l) of wheat-germ supernatant containing TCA-insoluble radioactivity equal to 2×10^5 c.p.m. were incubated with 40 μ l of chloroplast preparation, or fractions therefrom, for 60 min at 27 °C. Chlorophyll was measured by the procedure of Arnon¹³; each incubation contained the equivalent of 6 μ g chlorophyll, except that the purified chloroplast envelope incubation mixtures contained envelopes equivalent to 990 μ g chlorophyll. After incubation, samples were prepared for polyacrylamide gel electrophoresis and analysed as before². Gels were autoradiographed with Kodirex X-ray film for 100 h. Processing activity was measured quantitatively by two methods. Autoradiographs were scanned in a Joyce-Loebl densitometer, and the peak heights of the P20 and small subunit bands were measured for incubated and non-incubated samples. The change in peak height of each band due to the incubation was calculated as a percentage. In the other method the dried polyacrylamide gels were rehydrated, and the P20 and small subunit bands were excised. The gel pieces were dissolved in 0.2 ml H₂O₂ (100 volumes) at 50 °C overnight, and the radioactivity in each was counted in 4 ml Triton-toluene scintillant (0.4% (w/v) PPO, 0.005% (w/v) POPOP, 33% (v/v) Triton X-100 in toluene). The results presented were obtained by the first method and agree closely with those obtained by the second method. The percentage increase in small subunit label is not correlated with the percentage decrease in P20 label; this is because both the P20 and small subunit regions of the gel are contaminated with other products of the wheat-germ system in both the presence and absence of processing.

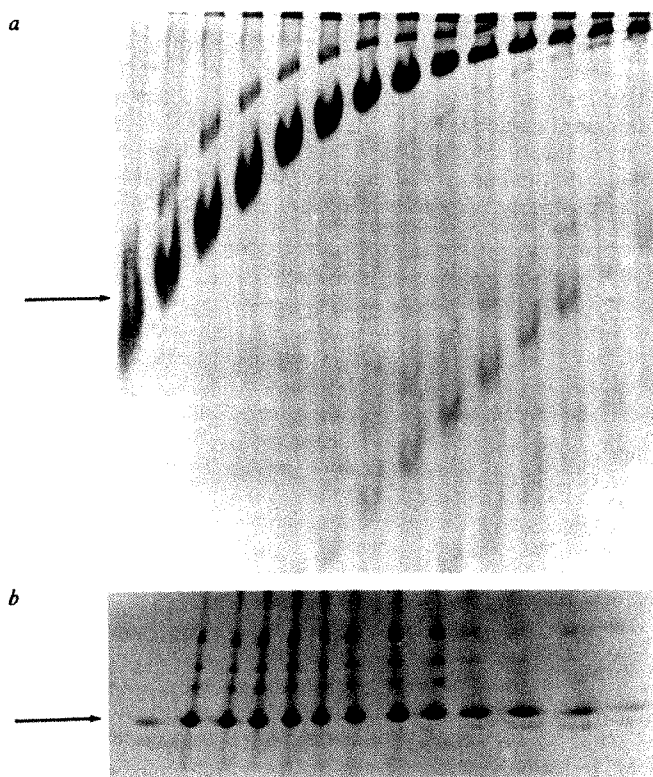


Fig. 2 Assembly of small subunit into whole RBPCase molecules. *a*, Separation of whole RBPCase molecules by transverse gradient non-denaturing gel electrophoresis. Wheat-germ products and a 3,200g stromal supernatant were prepared as in Table 1. Wheat-germ supernatant (50 μ l) containing 1.25×10^6 c.p.m. of TCA-insoluble radioactivity was incubated with 200 μ l of stromal extract, equivalent to 28.8 μ g chlorophyll, for 60 min at 27 °C. A control incubation mixture consisted of 50 μ l wheat-germ supernatant plus 200 μ l intact plastid buffer. After incubation, samples were brought to 300 μ l with a solution of sucrose and 2-mercaptoethanol with final concentrations of 10% (w/v) and 1.66% (v/v) respectively, for analysis by non-denaturing polyacrylamide gel electrophoresis. The gel system is based on that of Blair and Ellis³. A slab gel (17 $\times$ 17 cm) was cast with a linear gradient of 5–10% (w/v) acrylamide and 0.1–0.2% (w/v) bisacrylamide, stabilised with 0.2% (w/v) linear polyacrylamide. Once polymerised, the gel was turned through 90°, and a 5% (w/v) acrylamide, 0.1% (w/v) bisacrylamide slot former was cast along one edge of the gradient gel. The gel was pre-electrophoresed for 3.5 h at 30 mA. Samples of incubation mixture containing 2×10^5 c.p.m. TCA-insoluble radioactivity were loaded onto all the slots. Electrophoresis was for 18 h at 27 mA after which the gel was stained with Coomassie brilliant blue R and destained². The photograph shows the stained gel. RBPCase is the most heavily stained band, and is marked with an arrow at the 5% end of the gel. Other, minor bands, change their mobility relative to RBPCase according to the gel concentration. Autoradiography for 100 h shows radioactivity co-migrating with the stained bands of RBPCase, but not at corresponding positions in tracks lacking stromal extract (not shown). *b*, Identification of labelled small subunit in whole RBPCase molecules. The transverse gradient non-denaturing polyacrylamide gel shown in (*a*) was rehydrated, and each RBPCase region was excised. Gel pieces were equilibrated twice with 5 ml of 62 mM Tris-HCl, 2% (w/v) SDS, 0.5% (v/v) 2-mercaptoethanol pH 6.8 for 15 min each, and then affixed to the top of an SDS slab gel with 1% (w/v) Agarose, 0.2% (w/v) SDS, 62 mM Tris-HCl, pH 6.8. Electrophoresis was as previously described². The photograph shows an autoradiograph of the SDS gel in the small subunit region; the arrow marks the small subunit marker. The autoradiograph shows that radioactivity co-migrating with small subunit is present in RBPCase molecules excised from across the entire gradient of the non-denaturing gel. Other radioactive polypeptides migrate above and below the small subunit. However, these polypeptides vary in distribution across the gradient, indicating that they do not co-migrate with RBPCase at all gel concentrations. Control incubations lacking stromal extract contain no radioactivity in the small subunit region (not shown).

non-denaturing gels shows that the large subunit of RBPCase synthesised by isolated pea chloroplasts does not enter pre-existing RBPCase molecules³, but accumulates as an aggregate¹. This observation has been interpreted as indicating that isolated pea chloroplasts lack a pool of small subunits³. It follows that supplying P20 to pea chloroplast fractions may result in the assembly of whole RBPCase molecules. When wheat-germ incubation mixture containing labelled P20 is incubated with a stromal extract, labelled small subunit co-migrates with whole RBPCase molecules over a wide range of polyacrylamide gel concentrations when electrophoresed in non-denaturing conditions (Fig. 2). Co-migration in such conditions is a good test for identity¹⁰. Control experiments where stromal extracts were omitted from the incubation show no label migrating with whole RBPCase molecules. These observations establish that the assembly of small subunit into RBPCase occurs in the chloroplast stroma as previously proposed², and not in the cytoplasmic compartment. Chua and Schmidt¹¹ have reported the processing of P20 and assembly of small subunit into RBPCase in intact pea and spinach chloroplasts, but the stromal location of the processing and assembly process was not established. Whether processing is an integral part of the assembly mechanism, or merely provides small subunit for that mechanism, can be resolved when the processing activity has been purified.

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Sites of inhibition of *in vitro* DNA synthesis in carcinogen- and UV-treated Φ X174 DNA

THE availability of DNA molecules of known sequence and rapid methods for the determination of the sequence of particular DNA fragments^{1–3} make it possible to investigate the molecular action of mutagens and carcinogens more closely^{4,5}. For example, there is considerable evidence that some types of lesion in DNA such as UV light-induced pyrimidine dimers and various chemical adducts are blocks to DNA synthesis both *in vivo*^{6–9} and *in vitro*^{10,11}. The available data support the hypothesis that the block occurs at the level of the lesion on the template strand^{10,11}. We have used DNA containing such lesions on a template of known sequence for *in vitro* DNA synthesis by DNA polymerase 1 to investigate the site of the block at the level of the nucleotide sequence. This method allows us to

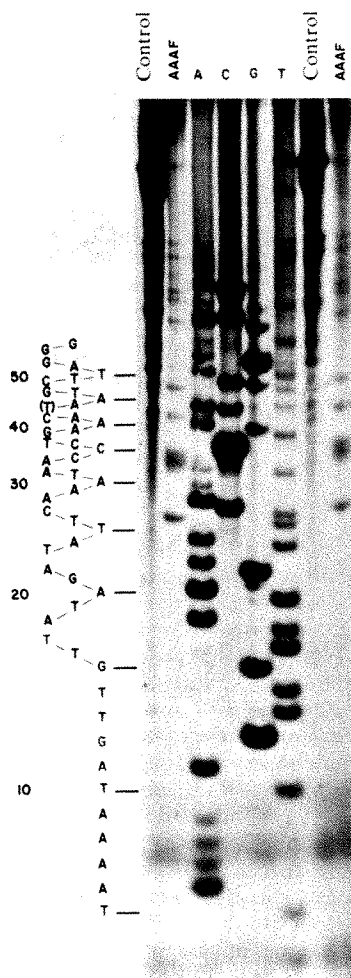


Fig. 1 Polyacrylamide gel analysis of the product synthesized by *polI* on a DNA template reacted with AAAF. Single-stranded phage Φ X174 DNA ($400 \mu\text{g ml}^{-1}$) was reacted with ^{14}C -AAAF ($50 \mu\text{g ml}^{-1}$) for 4 h at 30°C , extracted 4 times with amyl alcohol, precipitated with ethanol and resuspended in 10 mM Tris-HCl pH 7.5, 0.25 mM EDTA. The extent of reaction, determined from the ratio of ^{14}C c.p.m. to A_{260} was ~ 265 AAAF adducts per Φ X DNA molecule. Double-stranded RF Φ X DNA was digested with *HaeII* endonuclease (BRL) and individual restriction fragments were purified on agarose gels. Restriction fragment 2 (ref. 12) was heated at 100°C for 5 min and annealed to either AAAF-treated or unreacted DNA at 65°C for 90 min (molar ratio 1.2:1). Polymerase reactions were carried out in a volume of $20 \mu\text{l}$ and contained 50 mM Tris-HCl, pH 8.0, 8 mM MgCl_2 , 5 mM dithiothreitol, dATP, dGTP and dCTP, $50 \mu\text{M}$ each, $1\text{--}2 \mu\text{Ci}$ [$\alpha\text{-}^{32}\text{P}$]TTP (about 300 Ci mmol^{-1}), $0.1\text{--}0.2 \mu\text{g}$ DNA and 0.7 units DNA polymerase I (Klenow fragment, Boehringer). Incubation was at room temperature for 15 min and then $2 \mu\text{l}$ 0.5 mM TTP was added, incubation continued for a further 15 min and the reaction was stopped by heating at 65°C for 10 min. One unit of *HaeII* (BRL) was then added and incubated at 37°C for 60 min. For the sequence standards di-deoxyribonucleoside triphosphates were used as described by Sanger *et al.*³. The products were denatured and analysed on a 20% polyacrylamide gel². Lanes Control and AAAF are the products synthesized on untreated and AAAF-reacted templates respectively. Lanes A, C, G and T are the sequence standards produced with the di-deoxy chain terminating nucleotides. Numbering is from the position of the *HaeII* cleavage site.

determine both the site at which lesions capable of blocking synthesis by DNA polymerase occur and exactly where synthesis stops in relation to the lesion.

Φ X174 DNA was used in these experiments as it has already been completely sequenced^{12,13}. The Klenow fragment of *Escherichia coli* *polI* was used as the polymerase for most of our

experiments. The template consisted of single-stranded phage DNA to which individual restriction fragments, obtained by digestion of the double-stranded RF DNA, were annealed. We used fragments produced by the enzyme *HaeII* which cuts Φ X174 RF DNA at eight sites¹². These templates were used for the di-deoxynucleotide chain terminating method of Sanger and Coulson to provide a sequence standard. We found minor discrepancies with the preliminary sequence published by Sanger *et al.*¹²; however, in practically every case these were found to be in complete agreement with the revised sequence¹³. Single-stranded Φ X174 DNA was either UV-irradiated or reacted with *N*-acetoxy-2-acetyl aminofluorene (AAAF) or other carcinogens. The reacted template was then annealed with the priming restriction fragment and the polymerisation reaction carried out as for the sequencing reactions except that the di-deoxynucleotides were omitted.

In the reaction with AAAF-treated DNA a number of bands are seen which do not appear when untreated DNA is used (Fig. 1). These bands in the three cases in which identification is

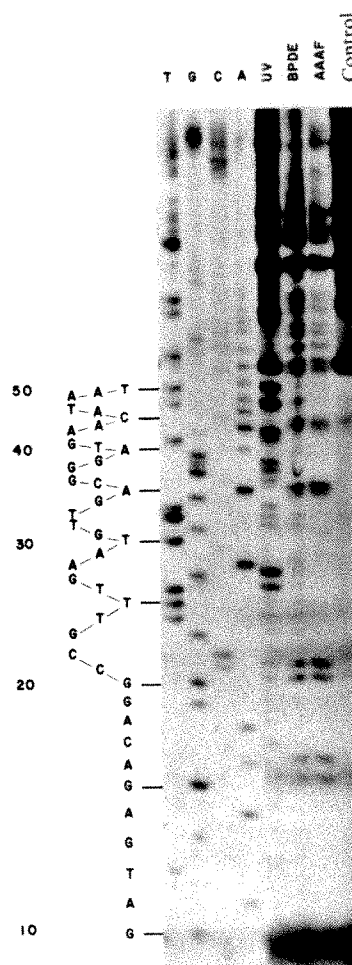


Fig. 2 Polyacrylamide gel analysis of the products synthesized by *polI* on DNA templates reacted with AAAF or anti-BPDE or irradiated with UV light. For the anti-EPDE template $46 \mu\text{g}$ Φ X DNA was incubated at 37°C with 30 nmol ^{14}C -anti-BPDE in 0.5 ml . At 60-min intervals a further three additions of ^{14}C -anti-BPDE were made and incubation continued for 5 h. The DNA was extracted four times with amyl alcohol and precipitated with ethanol. About 140 anti-BPDE adducts were added per Φ X DNA molecule. The DNA for the UV template was irradiated with 1000 J m^{-2} of UV at a DNA concentration of $100 \mu\text{g ml}^{-1}$. The primer used in this experiment was *HaeII* fragment 5. Reaction conditions were the same as for Fig. 1. Lanes Control, AAAF, BPDE and UV are the products synthesized by *polI* on untreated, AAAF or anti-BPDE-reacted and UV-irradiated DNA templates respectively. Lanes A, C, G and T are the sequence standards.

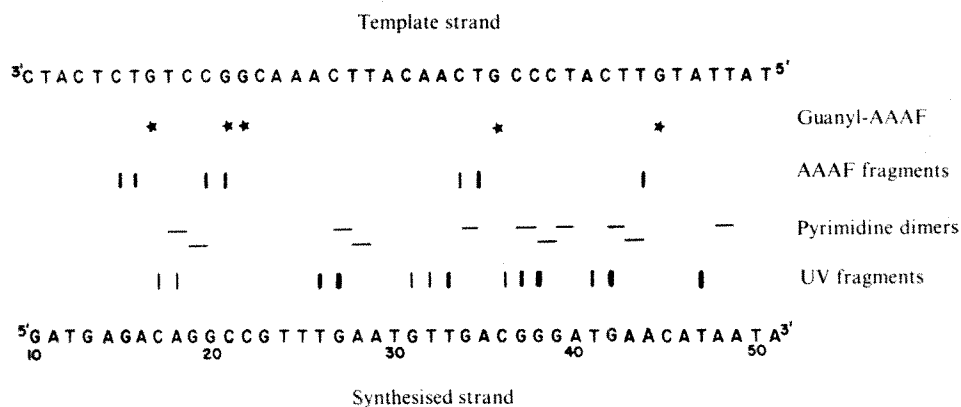


Fig. 3 The position of chain termination in relation to the sites of lesions in the DNA: diagrammatic representation of data shown in Fig. 2. Potential sites for the formation of *N*-acetyl-*N*-(guan-8-yl)-2-aminofluorene (guanines) and of pyrimidine dimers (adjacent pairs of pyrimidines) are shown along side of the position of bands obtained with synthesis by pol1 on AAAF-reacted or UV-irradiated DNA templates.

possible occur one base before that of a cytosine residue in the sequence standards, that is, synthesis has stopped at the base before a guanine in the DNA template. AAAF has been shown to react predominantly with guanine in DNA to form *N*-acetyl-*N*-(guan-8-yl)-2-aminofluorene^{14,15}. Our results both confirm the known major site of reaction of AAAF in DNA and demonstrate that this lesion is a block to DNA synthesis. Another conclusion that can be drawn from these experiments is that there seems to be no preferential reaction of AAAF with particular guanines in the section of tested DNA, at least in the conditions used here; that is, single-stranded DNA reacted *in vitro*. Bands can be detected corresponding to all the guanines in the region of the template for which the sequence can be resolved, and there is no obvious predominance of any of them. This may simply reflect the very high number of adducts in the DNA (about 1 per 5 guanines) although with a lower number of adducts (about 1 per 30 guanines) no bands at all were detected. There is no indication of lesions blocking synthesis at any position other than guanine. This conclusion was confirmed by an experiment using a different priming fragment (Fig. 2) which included templates either treated with ($\pm$)7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro benzo(a)pyrene (anti-BPDE) or irradiated with UV light. The pattern of bands obtained in this experiment is shown in Fig. 3. The bands produced with the AAAF-treated template are again consistent with those expected if synthesis terminated one nucleotide before an *N*-acetyl-*N*-(guan-8-yl)-2-aminofluorene residue. A completely different pattern of bands is seen with the UV-irradiated template. Synthesis on such a template terminates one nucleotide before any pair of pyrimidine bases. All three types of pyrimidine dimer, cytosine-cytosine, cytosine-thymine and thymine-thymine, are blocks to DNA synthesis although there is some indication that the bands resulting from thymine-thymine dimers are more prominent, probably due to their faster rate of formation during irradiation¹⁶ and thus more frequent occurrence in the template.

The results with anti-BPDE are more difficult to interpret. Prominent bands occur at the same positions as found with AAAF treatment although many other bands can also be seen. This would suggest that guanine is a major site of reaction for anti-BPDE¹⁷ although reaction also occurs at other bases, notably adenine.

As synthesis stops at the base preceding a guanine with AAAF-treated template DNA and at the base before the first of the two pyrimidine bases with UV-treated template DNA, it is clear that termination occurs without a nucleotide present at the site(s) opposite the lesion. It is possible that DNA polymerase 1 does add nucleotides in this position but that they are rapidly excised by the 3'-5' exonuclease activity of the enzyme due to lack of base pairing¹¹; however, our observation (data not shown) that a DNA polymerase α isolated from human lymphoblastoid cells¹⁸ and having no 3'-5' exonuclease activity gives the same band pattern with UV-irradiated template DNA

as does pol1 suggests that synthesis terminates due to the inability of the enzyme to incorporate nucleotides opposite a non-pairing lesion. We occasionally found additional slightly lighter bands one or even two bases before the main termination bands, for example, in Figs 2 and 3 at positions 15 and 34 with AAAF and at positions 31 and 32 with UV. These may result from degradation by either the 3'-5' exonuclease or residual 5'-3' exonuclease that contaminates some commercial batches of pol1 (Klenow). Degradation by the 5'-3' exonuclease is considered unlikely as treatment at 65 °C before addition of the restriction enzyme completely eliminated spurious bands on the sequence standards caused by this activity but did not affect the extra bands on the AAAF-reacted or UV-irradiated templates.

These experiments are essentially qualitative and do not show that DNA polymerase stops at every lesion; one cannot determine by this method the amount of 'read through'. However, we expect that the method will be useful to distinguish between lesions that are blocks to DNA synthesis and those that are not as well as elucidating the behaviour of different DNA polymerases on encountering a lesion and possible ways in which DNA synthesis may be able to bypass lesions¹⁹.

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Metastable crystals of β -cyclodextrin complexes and the membrane diffusion model

WE have previously suggested¹ that dimers of β -cyclodextrin (β -CD) may mimic membrane diffusion transport phenomena. This proposal was based on the orientation of guest molecules in the crystalline 3:2 *p*-iodophenol complex with β -CD, with the assumption that complex formation with guest molecules capable of hydrogen bond formation occurs through an initial interaction with one of the hydroxyl groups of β -CD. The 7:14 distribution of hydroxyl groups on the primary and secondary sides of the β -CD torus, respectively, was interpreted as favouring formation of a complex with the hydrogen bonding moiety on the secondary side of the torus. The fact that the guest molecules in the above complex were orientated in the opposite direction was taken as an indication that exchange of guest molecules between a dimeric complex and the solution had occurred. We report here that β -CD inclusion complexes with various guest molecules produce metastable crystals (designated CII) when growth is achieved by slow cooling, and we have determined the crystal structure of an *n*-propanol complex of

this phase. We find that comparison of the structure of this metastable phase and that of the thermodynamically more stable phase¹, CI, provides information relevant to the question of the presence of β -CD dimers in solution; this information is relevant to the suggestion that such dimers mimic bilayer membrane diffusion transport.

CII crystals of the *n*-propanol complex were grown from a 60:40 (v/v) *n*-propanol:water solution of β -CD by slow cooling. Typically, a solution near its boiling point was contained in a beaker sealed with a wax sheet topped with aluminium foil; the beaker was placed in a Dewar flask containing a water bath also near its boiling point. The flask was covered with thermal insulating material and allowed to stand for 1.5–2 d, during which the system cooled to a temperature describable as warm to tepid. Crystals with well formed faces and uniform morphology were routinely obtained in this manner. After several hours at room temperature, these crystals had obviously begun to dissolve and crystals of type CI appeared; their number increased with time as those of type CII decreased until the latter phase had disappeared.

Both crystalline phases were found to display P1 space-group symmetry but to have different unit cell volumes: $V_{CI} = 3,636 \text{ \AA}^3$ and $V_{CII} = 3,346 \text{ \AA}^3$ (both at $\sim 120 \text{ K}$); the lattice parameters for CII are $a = 15.461(2)$, $b = 15.575(3)$, $c = 15.316(3) \text{ \AA}$, $\alpha = 103.98(2)^\circ$, $\beta = 100.92(2)^\circ$ and $\gamma = 104.23(2)^\circ$. The initial crystal structure was solved by the use of

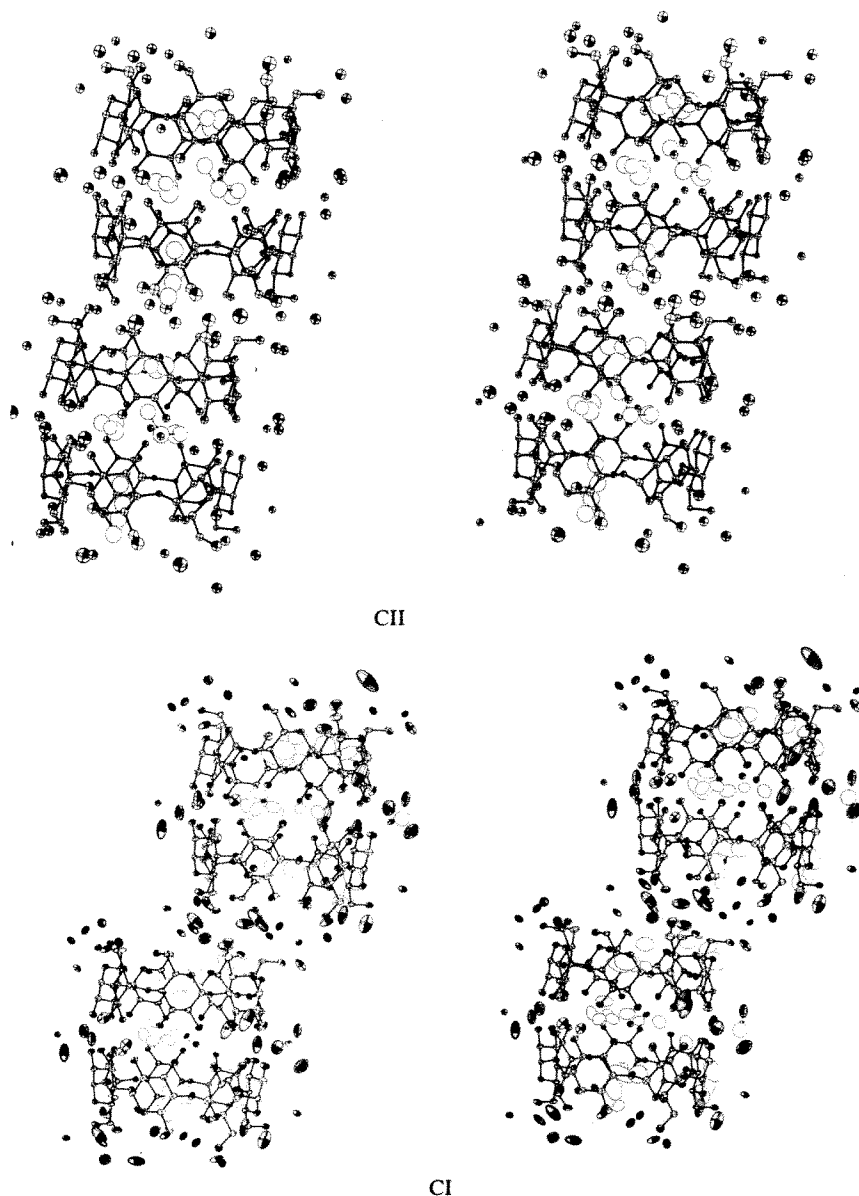


Fig. 1 Stereoscopic projections² of the crystal structure of *n*-propanol complexes of β -CD in thermodynamically stable phase CI and metastable phase CII. To illustrate the packing differences, two β -CD:*n*-propanol dimers have been drawn. The oxygen atoms of their environment (water molecules or adjacent β -CD molecule hydroxyl oxygen atoms) have been drawn. The disorder in the guest molecules is indicated by the size and shape of their thermal parameter representations. Those of phase CI are indicative of anisotropic thermal parameters whereas those of CII correspond to isotropic temperature factors.

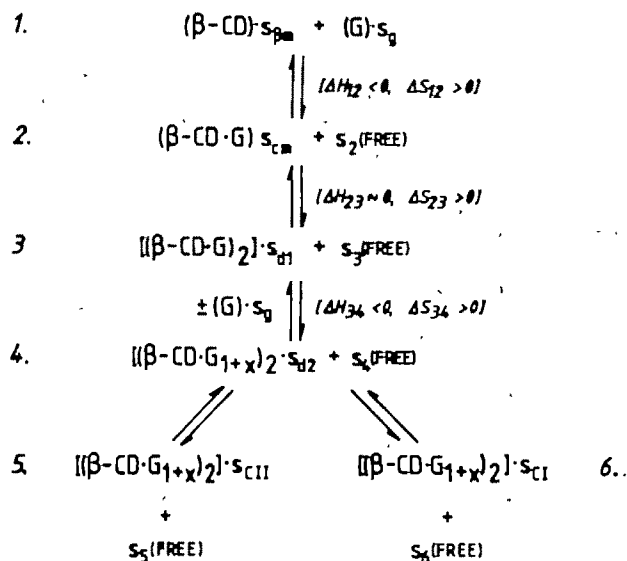


Fig. 2 A schematic representation of the principal processes leading to crystals of phases CI (thermodynamically stable) and CII (metastable) as the result of slow cooling of an aqueous solution. The symbols are: β -CD, β -cyclodextrin; G, guest molecules suitable for formation of inclusion complex β -CD·G, which may be of different stoichiometry in the monomeric and dimeric forms; s, solvation molecules (subscripted to indicate possible differences in solvation for the various species indicated). Trends in thermodynamic properties, ΔH , ΔS , and ΔG , are indicated for changes between numbered states.

non-crystallographic symmetry and the known structure of the β -CD dimer from the crystal structure analyses of phase CI, in a manner that will be described in more detail elsewhere. Refinement of the structural model of CII with isotropic temperature factors and a high resolution data set ($2\theta_{\max} = 60^\circ$, MoK $_{\alpha}$ radiation) has converged at $R = 12.0\%$ for 15,000 contributing data. At this stage of the refinement the unit cell composition is $C_{96}H_{172}O_{74} \cdot 18-19H_2O$. Refinement of the structural model for the CI *n*-propanol complex with anisotropic temperature factors is nearing completion. We now believe that the complex contains four disordered *n*-propanol guest molecules, giving a unit cell composition of $C_{96}H_{174}O_{74} \cdot 24H_2O$. The present R value is 8.0% for $\sim 19,300$ contributing reflections ($2\theta_{\max} = 60^\circ$).

Stereoscopic projections² displaying two β -CD dimers and oxygen atoms of their environment are presented for the *n*-propanol- β -CD complexes of phases CI and CII in Fig. 1. It is immediately apparent from the figure that the crystal packing is very different in the two phases. The metastable phase has a channel structure that seems to be similar to the packing reported for other β -CD complexes grown from aqueous solution by slow cooling^{3,4}, whereas phase CI displays a brickwork packing network. Another significant difference between the structures of the two phases is the presence of a water layer between the β -CD dimers in CI and its absence from the metastable phase. We find these structural differences and the metastable property of phase CII intriguing because of the inferences they have for the structure and properties of β -CD complexes in concentrated solutions.

The temperature-solubility gradient displayed by the β -CD:*n*-propanol:water system was used to grow crystals of phase CII. The fact that these crystals are metastable implies that they result from a kinetically rather than thermodynamically controlled process. Figure 2 summarises what we believe to be the principal processes giving rise to phase CII and, subsequently, to phase CI.

Complex formation to give β -CD·G, step 12, is expected⁵ to increase the entropy of the system as the result of release of molecules of solvation from both host and guest and to decrease

the enthalpy by freeing high energy (ref. 5) water molecules from the β -CD torus for subsequent hydrogen bond formation. We suggest that solution molecular dynamics are important in step 23. Water molecules have a considerably higher ($\sim 10\times$) mean velocity than the larger β -CD monomers. At temperatures at which thermal dissociation of hydrogen bonds is prevalent, this velocity difference can be important in determining which molecules form stable interactions. Two properties of β -CD·G monomers favour their association: their similar molecular velocities and the favourable distribution of secondary hydroxyl groups for the formation of multiple interdimer hydrogen bonds; dimerisation is also thermodynamically favourable (see Fig. 2). The inclusion of additional guest molecules in the extended torus of the dimer, step 34, is thermodynamically favourable but not crucial to the remainder of the process. We include it here to account for the four *n*-propanol molecules in these structures. Step 45, crystallisation of phase CII, is the kinetically controlled step in the process and we believe it reflects the solution molecular dynamics described for step 23. This proposal is clearly consistent with the nucleation of phase CII at temperatures well above room temperature.

The metastable character of phase CII crystals thus indicates that another component in the equilibrium becomes important at lower temperature. We suggest that this component is the building in solution of a stable hydration shell over the primary faces of the dimer (a component of step 46). This step may be accompanied by exchange of guest molecules with the solution and may also result in a change in 'stoichiometry' of the dimer. Orientation of hydrophilic substituents of the included molecules towards the primary side of the β -CD torus can be expected to stabilise the hydration layer further by promoting the formation of hydrogen bonds. Crystallisation of the more highly hydrated dimer gives rise to an equilibrium system and the thermodynamically stable phase CI.

We interpret the presence of the β -CD·G dimers in phase CII, and the observation that crystals of this phase dissolve at room temperature to give crystals of a second phase also consisting of β -CD·G dimers, as evidence that the predominant species in concentrated aqueous solutions is the dimer; a recent solution study supports this interpretation⁶.

The orientation of the guest molecules in the two structures also differs but it is inappropriate to take this as evidence for exchange of guest molecules with solution, as the *n*-propanol molecules are sufficiently small to allow their reorientation within the torus. The disorder in the *n*-propanol molecules in phase CI makes it unreliable to assign C and O atoms of the guest molecules in the middle of the dimer. However, it is possible that those in the ends of the dimer are hydrogen bonded to hydrate water molecules. There seems to be a short chain of hydrogen bonds involving the guest molecules and one hydrate molecule in phase CII, but we prefer to wait for the results of further refinement before seeking to analyse it further.

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matters arising

Injection events in ocean history

THE hypothesis that sudden intermittent injections of brackish or hypersaline water from large, temporarily isolated basins into the world ocean may have so altered the existing density stratification that mass extinctions (particularly of the open ocean plankton) resulted^{1,2}, is fraught with possibilities. The mid-Cretaceous anoxic event and the Messinian evaporite event may have workable models, but we are concerned that the models for the Cretaceous-Tertiary boundary and Eocene-Oligocene event have major flaws in that both models require an isolated, fresh to brackish water Arctic Ocean. The best (and perhaps only) available evidence for Maastrichtian time indicates that the open ocean plankton of the Arctic Ocean were neither endemic nor brackish or fresh water. Deep-sea sediment core FL-437 recovered in 1969 from the Alpha Cordillera of the central Arctic Ocean³, contains a rich phytoplankton biota described originally to show specific affinities with late Cretaceous marine silicoflagellates of Russia and California⁴, and subsequently of the south Pacific and Indian Oceans⁵⁻⁷. A study of the abundant diatom flora substantiates these affinities with known normal marine species (J. A. Kitchell and D. L. Clark in preparation). The complex is similar to the middle-late Maastrichtian complexes of California and the Koryak Range of Siberia⁸. Consequently, if a brackish water injection event occurred in the latest Cretaceous, it certainly did not originate from an Arctic basin encompassing the Alpha Cordillera.

The Eocene termination similarly requires an isolated Arctic basin. Unfortunately, neither Eocene nor Oligocene deep-sea sediment has been recovered from the central Arctic, and this model cannot be tested.

However, recent tectonic interpretations suggest that the time of rotation of the Amerasia Basin was pre-late Cretaceous^{9,10}. Detailed tectonic studies in the Bering Strait region support a late Cretaceous compressional event related to opening of the North Atlantic^{11,12}. The post-Miocene benthic foraminifera and ostracode fauna of the Alpha Cordillera

exhibit both endemism and Atlantic affinities¹³. Hence, the palaeontologic data derived from the central Arctic to date, support a transition from Pacific circulation patterns to Atlantic circulation, in conjunction with the time of opening of the North Atlantic. The observed extinctions of plankton species in the North Atlantic during this time interval may consequently represent the results of competitive interactions.

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THIERSTEIN REPLIES—There is little doubt that the Arctic Ocean was filled with marine waters at times during the late Cretaceous. In addition to the evidence cited by Clark and Kitchell of reworked late Cretaceous phytoplankton found in Plio-Pleistocene piston-cores^{1,2}, records of Maastrichtian marine microplankton succeeded by dominantly terrestrial palynomorphs are known from several basins in northern Alaska and the Canadian Arctic^{3,4}. The transition from marine to terrestrial deposition in these marginal basins, however, may be time-transgressive and could be due to local tectonic effects^{4,5}. Tectonic activity in conjunction with a worldwide regression would provide the ideal setting for a temporary isolation and subsequent flushing of the Arctic Ocean through, for instance, the McClure graben near the end

of the Mesozoic (refs 6, 7 and H. R. Balkwill, personal communication). Considering the short time estimated necessary for a freshening of the Arctic Ocean⁸ and the comparatively long duration of several million years of phytoplankton zones in the late Cretaceous^{9,10}, the evidence presently available from the Arctic does not preclude a temporary freshening and subsequent flushing of that basin at the very end of the Maastrichtian and Eocene.

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Shelf temperatures and reef growth on the south-east Florida coast

LIGHTY *ET AL.* describe a drowned Holocene *Acropora palmata* reef off the south-east Florida coast. In attempting to explain the lack of growth of this structure over the past ~7,000 yr, the authors list papers which they claim support low temperature as the principal limiting factor ("... pronounced temperature fluctuations and unusually cold bottom waters in the shallow waters off south-east Florida are effectively preventing active reef growth north of Miami."). However, none of their references apply to this point. The paper by Taylor and Stewart², for example, deals with upwelling off the north-east and central Florida coast—not

at all in the vicinity of the barrier reef. Similarly, there is no evidence that upwelling significantly alters water temperatures on the south-eastern shelf, although it may do so on the Miami Slope and Terrace³⁻⁵. While Vaughan's data^{6,7} indicate that Fowey Rocks (northern Florida Keys) occasionally becomes cooler than the rest of the Florida Reef Tract, there are indications that this is an atmospheric effect due to an open connection with easily-chilled, shallow bay waters⁸. Vaughan did not measure the temperature north of Fowey Rocks, but assumed the existence of a sharp decrease in winter shelf temperatures north of Miami.

In a more direct investigation of these reefs⁹ 27 species of scleractinians were found and it was noted that "Reduced temperatures ... inhibit coral growth further north, but appear to be insignificant in the area studied." Specifically, this meant that in an 18 month period (1969-70), the minimum bottom temperatures taken *in situ* with minimum-maximum thermometers at depths of up to 44m did not fall below 20°C. Similarly, thermistor arrays with sensors on the reef at Boca Raton and Pompano Beach, Florida, recorded no less than 22.2°C during the winters of 1968, 1969 and 1970^{10,11}. In addition, the unpublished XBT data for February 1973 referred to by Lee and Mooers³ shows the same temperature minimum at reefal depths from Fowey Rocks to Fort Lauderdale (T. Lee, personal communication). [The low datum of 17.04°C at 32 m given in the same paper seems to be a typographical error, judging from the published thermistor temperatures at the same station (T. Lee, personal communication).] These temperatures are not unexpected on such a narrow (3-5 km) continental shelf where tide and wind induced fluctuations are small compared with the influence of the warm Florida Current¹².

While it is clear that some limitation is being placed on coral growth here, there are no data which indicate that temperatures at 15-30 m off south-east Florida are much different from those at the same depth farther south, where it is generally considered that reef growth is appreciable. It would be instructive to determine accretion rates on the deep reefs of the Florida Keys where *Acropora palmata* is as absent as it is on the reefs described by Lighty *et al.*

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LIGHTY ET AL. REPLY—In our report¹, we attributed the demise of the shelf-edge *Acropora palmata* (Lamarck) barrier reef off south-east Florida to periods of extensive turbidity and to pronounced decreases in water temperatures during the first flooding of large, relatively flat areas of the shelf by rising seas approximately 7,000 yr ago. We did not suggest, as Goldberg implies, that low shallow-water temperatures were the principal cause of the reef's death. Rather, these cold temperatures were one of at least two unfavourable conditions that apparently existed about 7,000 yr ago, and that today control the distribution of modern *A. palmata* reefs in south Florida².

Goldberg's comments suggest that he has misinterpreted our discussion³ of the role of cold water temperatures in limiting reef growth during modern as well as early Holocene time on the entire south-east Florida shelf north of Miami. Goldberg has directed his attention to present-day temperatures at the shelf edge whereas we were referring to pre-existing temperatures at the shelf edge. The low water temperatures that may have contributed to the death of the shelf-edge reef would have resulted from atmospheric cooling of the large expanses of shallow water that formed when the sea initially flooded the continental shelf. Similar conditions currently exist in large shallow-water areas of south Florida, as shown by minimum water temperatures of 18° or less recorded for 23 winter months between 1954 and 1968 in northern Biscayne Bay near Miami³, and for eight winter months between 1968 and 1971 at more than 40 localities in southern Biscayne Bay⁴.

Furthermore, north of Miami present-day atmospheric cooling of shallow shelf water may be preventing *A. palmata* reef growth on the many submerged ridges in nearshore areas of the south-east Florida shelf^{5,6}. Although water depths are shallow enough for the development of *A. palmata* reefs, none were found during an extensive survey of these inner shelf ridges⁶. Along the shelf edge, however, water temperatures may be more favourable for reef growth, but the present water depths of 15-30 m over the relict barrier reef are well below depths in which an active *A. palmata* reef can exist. The hermatypic coral species found by Goldberg⁷ on the surface of the relict barrier reef were a deep reef fauna which did not

include rapid reef-forming species such as *A. palmata* and *Porites porites* (Pallas). In addition, *Acropora cervicornis* (Lamarck) and *Montastrea annularis* (Ellis and Solander) occurred in very low abundance⁷. Early Holocene radiocarbon dates for samples from close to the surface of the relict barrier reef¹ indicate that the deeper water epifaunal community has contributed no appreciable relief to this shelf-edge structure.

The temperature data to which Goldberg refers are not relevant to our discussion, nor are they as extensive as he suggests. The low values cited for "an 18 month period" do not appear in the report to which he refers. Measurements were made by thermistor probes at only one site, off Boca Raton (ref. 8, p 107), with three probes suspended vertically near the shelf edge at depths of approximately 7.5, 16.5, and 29 m, and not "with sensors on the reef." Temperature profiles along transects of the outer reef were measured only on selected dates by a portable salinometer (ref. 8, p 107, 110).

Therefore, it is evident that only minimal data exist for present-day water temperatures at the shelf edge in this area north of Miami, where depth is the most limiting factor in preventing reef growth. Temperature data are also lacking for the shallow inner shelf areas, where substrates are suitable for reef growth but lack any modern accumulation. Finally, although we cannot determine pre-existing low shallow-water temperatures at the shelf edge, we suggest¹ that they existed and that they were at least partly responsible for the death of the relict barrier reef off south-east Florida.

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reviews

Survival value of migration

J. R. Krebs

The Evolutionary Ecology of Animal Migration. By R. R. Baker. Pp. 1024. (Hodder and Stoughton: London, 1978.) £35.

MIGRATION is a subject which crops up at every major ethological and ecological conference. Orientation and navigation mechanisms of migratory birds, seasonal migrations of vast herds of African ungulates, and periodic emigrations of microtine rodents are among many migration phenomena which never fail to fascinate zoologists. It is surprising in view of the pervasive interest in migration in one form or another that Baker's book is the only modern attempt to deal with the subject in a comprehensive manner. The book covers a taxonomic range from earthworms to man. It boldly attempts to present a general theory of the survival value of migration, and integrate it with what is known about physiological mechanisms.

It is an awe-inspiring work; impressive in its size (over 1000 pages long, even the figure captions sometimes spread over three pages), the illustrations are excellent, and the detail with which many examples are described is remarkable. At the same time one cannot help feeling that the size and thus inaccessibility of the book will discourage many people from giving it the attention it deserves. Baker could have presented the essence of his argument in a much shorter book and reached a much bigger audience.

Baker starts with a 30 page discussion of the definition of migration. Ornithologists use the term specifically to refer to regular, seasonal, long distance, return movements. These are characteristic, for example, of the many birds breeding in temperate zones and wintering in warmer areas. Insects rather rarely make return journeys (at least within a single individual's lifetime) so that entomologists do not characterise migration by the criterion of return to a starting point. In fact, Baker argues, all the separate features which can be used to characterise migration such as distance, return and periodicity vary along a continuum. The upshot is that migration is defined by Baker in the most general way possible: "the act of moving from one spatial unit to another". This allows Baker to include

both an aphid flitting a few centimetres from one leaf to the next, and an Arctic Tern making a 16,000 km pole to pole trip under the rubric of migration. Although there are obvious disadvantages to such a wide definition (a branch falling off a tree "migrates" in Baker's terms), Baker argues that there may be general rules which apply, as he puts it, "to the black bean aphid, the blue whale and the barnacle".

Baker's general cost-benefit model of migration is developed in the next 50 pages of the book. The essence of the argument is that it is in theory possible to calculate for an individual at any moment in time the expected fitness resulting from staying put, and the expected fitness resulting from a decision to move to a new place. When the expected gain for moving is higher than that for staying, migration will be favoured. Baker derives the basic model of an optimal migration threshold, and then goes on to discuss various ramifications. These include stochastic variation in habitat quality, the effects of competition within a habitat on migration thresholds, and exploratory migration as a method of acquiring information about expected pay-offs. Most of the theoretical arguments are qualitative, and one sometimes feels that a rigorous derivation would make the point more convincing. An example is the statement on p72 that "unpredictable fluctuations in E (the expected fitness consequent upon migration) ... are more likely to lead to a ... distribution of staytimes ... corresponding to the distribution of frequency of different values of E ".

Baker's model of migration is essentially the same as the well known "marginal value theorem" published several years ago by E. L. Charnov, G. A. Parker, and others. Baker's application of the idea to migration in general is perhaps somewhat novel, but to some extent the edge is taken off the theoretical section of the book by the fact that the ideas have already been developed by others.

The remaining 900 pages of the book are entitled "evaluation of the model: examples and analysis". The organisation is mainly taxonomic. As there are virtually no data which can be used to test the model in a rigorous way,

by far and away the bulk of the "evaluation" is in terms of qualitative evidence and *post hoc* interpretation. One quantitative test of the model which Baker describes is the outstanding work of G. A. Parker on dungflies, and this occupies only two or three pages. I feel it could have been given more extensive coverage as it is so central to Baker's theme. Although the literature seems to be well covered, there are some obvious gaps. For example, the section on movement paths in chapter 18 does not refer to J. Paloheimo's theoretical analysis of optimal search paths, or J. N. M. Smith's refined experimental analysis of bird movement tracks. The long section on bird navigation and pigeon-homing makes no reference to the Italian work on scent maps, Keeton's work on release site bias, or Gwinner's re-appraisal of the star map work of Sauer. One general area which did not seem to be covered is the genetics of migration. The theoretical arguments assume that there is genetic variance for migration thresholds, but the work on genetic correlates of emigration in small mammals, for example, is not included.

However, these minor omissions are more than offset by the great diversity of facts and insights in the book. To mention just one example, the discussion of home range and territoriality (pp384-410) places a useful emphasis on the exploratory behaviour of non-territorial individuals. In part, the discussion centres around conditions which will favour a non-territory holder attempting to displace a resident instead of migrating to try and find an empty space elsewhere. Looking at home range defence from the resident's point of view, Baker argues that one of the most general benefits derived from exclusion of others is an increase in the accuracy with which resources in different parts of the home area can be assessed and ranked. If transient individuals are permitted to exploit these resources, then the value of exploration and ranking by the resident is reduced. As with so many of the ideas in the book, there is plenty to stimulate thought and discussion, but no hard evidence.

Baker's discussions of migration in modern industrial human societies is

certain to arouse controversy. He takes the extreme view that our present-day migration patterns are directly determined by natural selection. He also argues that natural selection is still acting in a similar fashion to that experienced by our hunter-gatherer ancestors. The argument is roughly as follows. In ancestral hunter-gatherers, newly formed pairs migrated away from their natal groups to other parts of the home range explored during adolescence. Pairs tended to settle in areas where their expected reproductive success (limited mainly by food supply) was highest. Males tended to explore larger ranges than females, partly because they could increase their reproductive success by incidental copulations on the way. Baker goes on to apply these speculative conclusions to industrial man: "I feel some justification in claiming that the migration pattern shown by modern industrial humans is that evolved during the 10-20 million years of hunting-gathering. All that remains is to consider whether the

selection that is acting at the present time on modern industrial humans is tending to perpetuate or change the pattern." His highly controversial conclusion is that our present-day migration patterns are genetically controlled, hormonally mediated ("part of the physiological syndrome that transforms an individual to physical maturity is a decreasing migration threshold") behaviours. There is likely to be much argument against this conclusion. Even if one accepts in principle that genetic selection can still influence migration behaviour in man (a view for which no evidence is given), it is not at all clear to what extent factors such as extramarital copulation and protein intake alter inclusive fitness.

Although one may not agree with all of Baker's views, the book is thought provoking, and it will be a pity if it is not widely read because of its size and price. □

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Inherited behaviour and reactions to drugs

Drugs and the Inheritance of Behavior: A Survey of Comparative Psychopharmacogenetics. By P. L. Broadhurst. Pp. 206. (Plenum, New York and London, 1978.) £12.28.

P. L. BROADHURST has written a short but surprisingly complete book in the area of the effects of inherited behaviour on reactions to drugs. The title that he has taken was not his first choice and it is somewhat misleading. As everyone familiar with the field will know, this is a relatively small and new discipline which studies the effect of genotype on an organism's reaction to drugs. Almost all of the work discussed comes from rat and mouse experiments. The title implies that the drugs are altering the inheritance of behaviour, but of course the opposite is the case, it is the influence of inheritance on the response to drugs that is being studied. Dr Broadhurst would have preferred the title *Psychopharmacogenetics* but that title was pre-empted by B. E. Eleftheriou with an edited book (Plenum, New York, 1975). Others, such as McClearn, favour the name "Behavioural Pharmacogenetics" for the area.

Whatever the name, it is a field that has been entered on the one hand by behavioural geneticists often with insufficient knowledge of pharmacology, on the other by pharmacologists with insufficient knowledge of genetics or by psychologists with insufficient knowl-

edge of both of the other two disciplines. Dr Broadhurst's book is replete with examples of the resultant chaos. He is properly critical of the investigators who have perpetrated some of the scientific monstrosities which often seem to have been undertaken with bad design, poor or non-existent controls, poorly analysed and then published repeatedly.

There are, however, numerous examples of well designed and well executed studies. Students and those

Aflatoxins

Aflatoxins: Chemical and Biological Aspects. By J. G. Heathcote and J. R. Hibbert. Pp. 212. (Elsevier, Amsterdam and New York, 1978.) Dfl. 120, \$53.50.

MANY processed food components, and the foods themselves, are potential substrates for moulding by mycotoxin-producing fungi and so it is no small wonder that aflatoxin B, being the most potent hepatocarcinogen in the rat, and related aflatoxins should be a subject of much biological and chemical research.

After opening chapters on the discovery of aflatoxins and their production under natural conditions and in the laboratory the chemistry of these substances is reviewed and their assay by physicochemical techniques and bioassay are described.

Thereafter, sufficient pathology is presented for the non-medical reader to appreciate the general nature of the effects without being blinded by technical jargon. The distinction between acute toxicity and carcinogenicity is made clear.

just entering the field would do well to read this book and take heed of the lessons that it contains. It gives examples of the use of the most popular and powerful genetic techniques including selective breeding, sex differences, strain differences, diallel crosses and recombinant inbreeding.

For the novice to the field the precautions and controls that are outlined seem excessive and unnecessary, however, the experiments usually require months if not years of study and are often exceedingly expensive. The techniques are extremely powerful but much time and money can be wasted unless careful planning is done before rather than during or after the experiment.

The book has the decided advantage of being written by one individual with a consistent style and approach. It also has the advantage over edited books of being less self-serving and providing a broad overview rather than a series of narrow reports. In his desire to be complete, Dr Broadhurst has done a remarkable job, but therein also lies one of the criticisms of the book. In his zeal he has often used information contained in abstracts. Generally this is done because the data has not appeared in an edited journal. Thus, some of the conclusions reached by Dr Broadhurst rest on some rather thin ice.

Richard A. Deltich

Richard A. Deltich is Professor and Vice-Chairman of Pharmacology at the University of Colorado School of Medicine, and Research Associate of the Institute for Behavioral Genetics, Denver, Colorado.

The chapters on biochemistry and metabolism perhaps constitute the most intellectually stimulating feature of this book and the authors, who have worked in the field, have not hesitated to make certain value judgements. They are cautious concerning the significance of the elusive derivative aflatoxin B₁-2-3 epoxide in acute toxicity and offer hemiacetal derivatives of aflatoxin as candidates for the role of carcinogen.

The book concludes with a well illustrated review of the biosynthesis of the aflatoxins and their interrelationships, followed by a chapter on control of aflatoxin contamination and current attempts at detoxification of foods.

The style of the text is clear and readable and typographical errors are rare. Those concerned with food science should read this book. I also suspect that the general reader could catch from it the fascination of aflatoxins and their mode of action.

P. G. Mantle

P. G. Mantle is Senior Tutor in Biochemistry at Imperial College, University of London, UK.

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Chemisorption of gases on metals

Chemisorption of Gases on Metals. By F. C. Tompkins. Pp. 370. (Academic: London, New York and San Francisco, 1978) £16.80.

MAJOR advances in our knowledge and understanding of the chemisorption of gases on metals have occurred during the past decade. The elemental composition, crystallography, and the electronic and vibrational properties of surface layers on defined single crystal faces are now measurable, and a wealth of detailed information is available in the research literature. But for the advanced undergraduate or beginning postgraduate student there has been a serious lack of comprehensive introductory texts in which the theoretical bases and experimental methods of the newer physical techniques are clearly explained and in which the contributions of these techniques are placed in perspective with the traditional methods of surface study.

Professor Tompkins' book is intended to fill this gap. Its plan is excellent. The early chapters deal with the terminology of adsorption, methods of preparing clean surfaces, the kinetics of adsorption and desorption, isotherms and heats of adsorption, and the thermodynamics and statistical thermodynamics of adsorption. These chapters provide a natural link with earlier texts. They are followed by outlines of the electronic theory of metals and metal surfaces. The remaining half of the book is composed of short chapters dealing with work functions, magnetic susceptibility, electrical conductivity, low energy electron diffraction, infrared spectroscopy, field emission and field ion microscopy, Auger electron spectroscopy, photoelectron spectroscopy, ion neutralisation spectroscopy, electron energy loss and appearance potential spectroscopies, and a short review of some experimental results for adsorbates on tungsten.

The book conveys quite well the interplay of theory and experiment in modern surface science, and gives due emphasis to the methods which have proven most fruitful. However, it is neither as comprehensive nor as up to date as one might have expected. Ion-scattering and secondary ion mass spectrometry are completely omitted, as is cathodoluminescence, and the only reference to high resolution electron energy loss spectroscopy is the early paper of Propst and Piper (1967). Although published towards the end of 1978, the book contains very few ref-

erences dated after 1975. Except for the treatment of ultraviolet photoelectron spectroscopy, it is not appreciably more up to date than Wedler's *Chemisorption: An Experimental Approach* (Butterworths, 1976) and it is less comprehensive in its coverage of experimental methods. More emphasis is placed by Tompkins on the theoretical background, particularly the electronic properties of metals and surfaces. However, the level of the theory is sometimes too ambitious for its proper development in an intro-

ductory book of this scope and the student will often be baffled unless he pursues the references.

Despite these shortcomings the book is a welcome addition to the literature on chemisorption. The student will find it a useful guide to an increasingly interdisciplinary field of physics and chemistry.

J. Pritchard

J. Pritchard is Professor of Physical Chemistry at Queen Mary College, University of London, UK.

Account of the bumblebee

The Life of the Bumblebee. By D. V. Alford. Pp. 80. (Davis-Poynter: London, 1978) £3.95.

THIS is a popularised and much abbreviated version of D. V. Alford's *Bumblebees* which was published by Davis-Poynter in 1975. To help the general reader Alford begins by reviewing the features of insects in general, and bumblebees in particular. He then follows the usual and successful formula of tracing the biology of the colony from its initiation in spring, its growth to maturity, and the production of males and queens. He goes on to discuss further topics (including temperature regulation, foraging, orientation, mating and hibernation) and refers to most of the more important recent findings as well as the better

known and established aspects of bumblebee biology.

The final chapter entitled "Bumblebees and Man" is most valuable in drawing the reader's attention to the importance of bumblebees as pollinators of our crops, and the need whenever possible to protect them from the hazards of insecticides, to replace sources of forage destroyed by herbicides and intensive cultivation, and to provide sites suitable for nesting and hibernation. Appendices describe the 25 British species of bumblebees. There are eight pages of good black and white photographs.

In general Alford is to be congratulated in avoiding placing undue emphasis on his own particular interests and in presenting a well balanced accurate and readable account of the bumblebee.

John B. Free

John B. Free is at Rothamsted Experimental Station, Harpenden, UK.

Biological identification methods

Biological Identification: The Principles and Practice of Identification Methods in Biology. By R. J. Pankhurst. Pp. 104. (Edward Arnold: London, 1978.) Paperback £3.20.

SYSTEMATICS as an approach to the teaching of the biologicals was becoming unpopular in the 1960s. Memory-taxing, as then taught, it carried a flavour of the elite natural history museums and culture collections: the spirit of Robbins was moving university teachers towards molecular biology and genetics which could, in some fashion, be assimilated by the many.

Meanwhile, computers were making possible numerical, multivariate and probabilistic treatments of biological classification and identification. It has taken over a decade for this to penetrate university teaching and by now there is a shortage of young systematists with an interest in the identification craft.

R. J. Pankhurst's compact book on biological identification methods begins with a splendidly lucid section on the use and construction of keys, taking British species of the genus *Epilobium* as a running example. If the rest were as easy to understand, one could begin to see a very satisfying second-year biology degree module in general systematics drawing on the second edition of C. Jeffrey's *Ecological Nomenclature* (Edward Arnold: London, 1977) for nomenclature, P. H. A. Sneath and R. R. Sokal's *Numerical Taxonomy* (Freeman: Reading, 1973) for classification, and Pankhurst for identification. Lecturers should buy their copy well ahead of the students and settle down to some supplementary reading (the short bibliography is well chosen): the section on probabilistic methods drawing on Bayes' theorem, for a start, will need cracking by a worked-out example, that given (Fig. 16) being just a section of a computer print-out, understandable only by the initiated.

Monothetic and polythetic methods, explained on page 3, would have been an academically sound way of arranging the core of the work. Instead, the

central chapters focus respectively on "conventional" and "automatic" methods: the latter "may or must involve calculations . . . so that the use of a computer is usual". Though it perhaps looks sensibly down-to-earth, this is a comparison not of identification methods but of the ways in which we find it convenient to go about them, and it leads to some confusion. Diagnostic keys are "conventional", are they not? But, wait, we can programme computers to construct keys, so that must be "automatic". Matching methods, now these are certainly "automatic": botheration, they have to go in the "conventional" section as well because we can identify by comparison without calculation. And so on, until it must

dawn on the undergraduate or advanced school student that it is identification logic to which he should address himself, not the fuss about computing and programming language (leading, incidentally, to a strange Fig 18 which actually suggests that "on-line" is a method of biological identification).

It will be observed that the reviewer has been stimulated. So will be the many others who buy this interesting, thought-provoking volume, they, too, will have to work hard.

J. E. Smith

J. E. Smith is Professor of Microbiology at the University of Surrey, Guildford, UK.

Ecological methods

Ecological Methods, with Particular Reference to the Study of Insect Populations. Second edition. By T. R. E. Southwood. Pp. 524. (Chapman and Hall: London, 1978) £10.

It is a mark of the uniqueness of this book that in the 12 years since the first edition no competitor volume has emerged that covers such a wide field. Professor Southwood is just as much at home discussing the idiosyncracies of insect trap construction as he is comparing indices of diversity.

The book is well described by its title, and like many compendia will be most useful to the expert or semi-expert. The sort of methods we choose are influenced by our current understanding of the ecological world around us. Nevertheless, in many asides the author does try to redress the balance, pointing out the relative merits of the different techniques, what they can and cannot do. It is here that the book will also be useful to the beginner.

In the past 12 years there has been a dramatic but allometric growth of ecology in two quite different areas. On the one hand the subject has im-

pinged more on public consciousness; on the other, the rapid development of computers and electronic calculators has stimulated much mathematical modelling and analysis. The result is that the scope of ecology and of this second edition is much wider than ever before.

Every chapter has been updated and new sections added, especially on dispersion, capture-recapture methods, components of predator-prey interaction, and dispersal. In particular, recent growth in the methods of construction of life-budgets, systems analysis, descriptions of diversity and species-packing models now allow Professor Southwood to crystallise out a conceptual framework for discussion which was not possible before.

Theoretical astrophysics

Theoretical Principles in Astrophysics and Relativity. Edited by N. R. Lebovitz, W. H. Reid and P. O. Vandervoort. Pp. 257. (University of Chicago Press: Chicago and London, 1978) £16.10; \$23.

Theoretical Principles in Astrophysics and Relativity offers yet another collection of papers from a conference, this one held in honour of Professor S. Chandrasekhar's 65th birthday in 1975. I found it a distinct improvement on the usual hotch-potch of disparate lectures re-hashed for the wider public with a minimum of editorial tinkering. Firstly, the book is better produced than most conference reports (which usually resemble a wedge of preprints glued together)—which probably accounts for the immense delay in publication. More importantly, however, the articles are mainly of a review rather than research variety; this is fortunate as nearly four years have

A reviewer must also criticise. The greatly expanded field of the subject (1,000 new references, with few deletions from the first edition) is covered in the space of 130 extra pages. The pace of the discussion is therefore fast and, occasionally, confusing. There are omissions (for example, the log normal distribution is mentioned twice, on pp33 and 424, without its basic model) and repetitions (for example, equations 2.26, 2.27, 2.29 and 2.30 re-appear as equations 2.37, 2.38, 2.41 and 2.42); and occasionally parameters are ill-defined (for example, I_m of equation 9.28 and S_T of 13.1) or, together with variables, given different symbols in the text and figures (for example, q and Q in Table 9.1 and equation 9.6; N_F , N_R and P_L , P_R on p382 and Fig. 10.8). Generations of students will now continue to write, incorrectly, of density dependence (Fig. 10.7, in contrast to Fig. 89 of the first edition) and of the Shannon-Weaver rather than Shannon-Wiener function (p426). There are also several small, typographical errors.

None of this, however, detracts from the significant contribution of this volume. The author and publishers are to be congratulated on producing an expanded second edition at less than the price of the final printing of the first. By far the best value on any ecologist's bookshelf.

D. J. Rogers

D. J. Rogers is Lecturer in Entomology at the University of Oxford, UK.

elapsed since the lectures were prepared.

I found the paper on stellar dynamics by Contopoulos especially lucid, and was surprised to see a very formal mathematical paper by Mullikin on the very physical subject of radiative transfer. There is a nice review by Thorne on various phenomena in astrophysics in which general relativity plays a central role, looking all the more relevant with the recent probable discovery of gravitational radiation. The article on singularities in spacetime by Penrose is masterly and thought provoking.

The range of topics covered in the conference was fairly wide, and the articles necessarily somewhat superficial. The book does have, however, a rather more permanent quality about it than comparable proceedings, and is certainly good introductory reading for a host of topics in astrophysics and cosmology. At £16.10, it is not overpriced for such a well-produced format.

P. C. W. Davies

P. C. W. Davies is Lecturer in the Department of Mathematics at King's College, University of London, UK.

● The US edition of *The Climatic Threat* by John Gribbin (for review, see *Nature*, 272, 788; 1978) has been published as *What's Wrong with Our Weather?* by Scribners: New York (\$9.95).

○ The price of *Principles of Chemical Kinetics* (for review, see *Nature*, 278, 194, 1979) was incorrectly quoted. This should be £12.65.

● The US edition of *The Visible College* by Gary Werskey (for review, see *Nature*, 276, 136; 1978) will be published in May by Holt, Rinehart and Winston.

Reports and Publications

UK & Ireland—March

Commonwealth Human Ecology Council. Organic Farming: Economic, Social and Technical Aspects. An Annotated Bibliography compiled by Brenda Hirst. Pp. 25. £1.25. Energy Use in Agriculture (Fossil Fuels). An Annotated Bibliography compiled by Brenda Hirst. Pp. 18. £1. The Human Ecology of Settlements. (Auckland Documentation, No. 15.) Edited by Dr. L. J. Hale. Pp. 165. £2.50-plus packing and postage 40p surface; £1 airmail. (London: Commonwealth Human Ecology Council, 63 Cromwell Road, SW7, 1978.) [23]

Journal of Biomedical Engineering, Vol. 1, No. 1, January 1979. Managing Editor: Christopher J. Rawlins. Assistant Editor: Mary Korndorff. Pp. 1-80. Annual subscription (4 issues) £40 (UK); Single copies £10 (UK). Edited by W. J. Perkins. (Haywards Heath, Sussex: IPC (Sales and Distribution Ltd.), Oakfield House, Perrymount Road, 1979.) [23]

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Pollution: The Neglected Dimensions. By Denis Hayes. (Worldwatch Paper No. 27.) Pp. 32. (Washington, DC: Worldwatch Institute, 1776 Massachusetts Avenue, N.E., 1979.) \$2. [123]

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nature

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QUEENSLAND INSTITUTE OF MEDICAL RESEARCH PRINCIPAL MEDICAL RESEARCH FELLOW (2 Positions)

Applications are invited for two senior positions from medically qualified investigators registrable in Queensland, Australia. Applicants should have extensive research experience in appropriate fields of medical science and will be expected to establish their own units within the Institute. Clinical association with a major teaching hospital is negotiable.

The Institute currently has commitments in infectious disease, oncology, molecular biology and genetics, with a deep interest in the application of medical science in the context of epidemiology of disease, both in Australia and in the Asian-Pacific region.

Appointment is initially for 5 years, renewable, with a salary of \$33,063 p.a.

Applicants should forward copies of their curriculum vitae, together with the names and addresses of at least 3 professional referees to Dr C. Kidson, Director, Queensland Institute of Medical Research, Brisbane, 4006, Australia, by May 30, 1979. 414(A)

BRUNEL UNIVERSITY LECTURESHIPS IN THE SCHOOL OF BIOLOGICAL SCIENCES

(1) Applications are invited for a lectureship in the Department of Applied Biology. An interest in teaching and research in the general field of Eukaryote Genetics will be a decided advantage.

(2) Applications are invited for a lectureship in the Department of Biochemistry.

The salary for both posts will be on the Lecturer scale £3,883 to £7,754 per annum plus £502 London Allowance.

Write for application form and further details for both posts to the Assistant Secretary (Establishment), Brunel University, Uxbridge, Middlesex UB8 3PH or telephone Uxbridge 37188, extension 49. Closing date May 18, 1979. 372(A)

WESTMINSTER MEDICAL SCHOOL RESEARCH ASSISTANT

Organic Chemist or Biochemist (would be suitable for recent graduate or for a person graduating this Summer).

Required to join a small team working on pigment changes in disease. Work will include the isolation and assay of pigment intermediates from the body fluids of patients undergoing treatment. Post available for one year, initially.

Salary on Whitley Council Scale for Hospital Biochemists.

Applications with curriculum vitae and the names of two referees, to Professor J. R. Hobbs, Department of Chemical Pathology, Westminster Medical School, 17 Page Street, London SW1P 2AR, by April 30, 1979. 425(A)

MEDICAL RESEARCH COUNCIL RADIOBIOLOGY UNIT, HARWELL MAMMALIAN GENETICIST OR CYTOGENETICIST

A scientist is needed to join a group studying the genetic effects of radiation and other mutagenic agents in mammals. The emphasis is on methods of exploring the sensitivity to mutagenesis of germ cells of different sexes, developmental stages and species in the intact animal. Formal genetics and cytogenetics relevant to the mutagenesis work are also included. Candidates with experience of genetics of higher organisms, or of handling rodent chromosomes, at either meiotic or mitotic stages, would be at an advantage.

Possible subjects of research include:

(1) Comparison of the sensitivity to mutagens of various mammalian species, with particular emphasis on methods of studying chromosome changes in oocytes and early embryos;

(2) Studies of the causes of chromosomal non-disjunction in mammals;

(3) Factors affecting the sensitivity of oocytes to mutagenesis.

There are excellent research facilities. The Unit also includes groups studying cell mutation, cytogenetics, pathology, and effects of radionuclides.

Applicants should hold a relevant good honours degree, with three years' research experience, and preferably a Ph.D. Salary and conditions of service according to M.R.C. non-clinical scientific scales. Hostel accommodation for single, or housing for married people, may be available.

Applications, with curriculum vitae and names of two referees, should be sent to Administrator, M.R.C. Radiobiology Unit, Harwell, Didcot, Oxon OX11 0RD, from whom further particulars may be obtained. 429(A)

MEDICAL RESEARCH COUNCIL LABORATORY ANIMALS CENTRE TECHNICIAN

There is a vacancy in the Genetics Department for a technician to assist in the institution of systems of genetic monitoring of small laboratory animals. Experience in immunological and electrophoretic techniques would be an advantage. An HNC or equivalent qualification is essential.

Incremental salary in the range £3,261 to £4,680 plus £354 London Weighting. Contributory superannuation scheme.

Applications to The Chief Technician's Office, Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM3 4EF

MRC

Medical Research Council
386(A)

APPOINTMENTS VACANT

UNIVERSITY OF NOTTINGHAM School of Agriculture DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL STUDIES DEMONSTRATORS IN ANIMAL PHYSIOLOGY

2 Demonstrator posts
are available

1: Demonstrator—Available immediately to assist in lectures, tutorials and demonstrations in animal physiology. Applicants should be trained in human or animal physiology or veterinary science and should preferably have gained a Ph.D. research facilities in animal physiology are available. The post is for two years. Salary scale £3,384 to £3,883 per annum.

2: Junior Demonstrator—To assist in tutorials, demonstrations and laboratory work. Post available from August, for up to three years. Facilities available for post-graduate research. Honours candidates graduating this year are encouraged to apply. Salary scale £3,085.

Further particulars and forms of application from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Not to be returned later than May 7, 1979. Ref No' 681.

420(A)

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF PHYSIOLOGY Applications are invited for the office of

UNIVERSITY DEMONSTRATOR IN THE DEPARTMENT OF PHYSIOLOGY

Candidates should hold a Ph.D. degree, or a Medical or Veterinary qualification, or be on the point of obtaining one. The person appointed will be expected to assist with the teaching of neurophysiology and the functioning of the nervous system, and should have research interests in this general field.

The initial appointment will be for three years from October 1, 1979. The pensionable stipend for a University Demonstrator will be on a scale of £4,133 rising by four annual increments to £5,129 (under review), with initial placing above the minimum where appropriate. The maximum tenure of a University Demonstratorship is five years. A grant is made towards removal expenses.

Candidates should send 12 copies of their application together with the names of not more than three referees to Mr G. R. Anderson, General Board Office, The Old Schools, Cambridge CB2 1TT, from whom further information can be obtained to arrive not later than May 25, 1979. 413(A)

oxford polytechnic

Faculty of Technology

Head of Department of Geology and Physical Sciences (Grade VI)

A new department is being established from September 1, 1979. The person appointed to this post, while being the administrative Head of the Department, will also be expected to offer academic leadership in at least one major area of the department's work. Applicants should have strong academic and research interests.

Salary: Head of Department (Grade VI) £9,345 to £10,305 (under review from April 1, 1979).

Applications, including a curriculum vitae and the names of three referees, should be sent by May 15, 1979, to the

Dean of Faculty of Technology,
Oxford Polytechnic,
Oxford OX3 0PB,

from whom further particulars and application forms may be obtained.

417(A)

Universiteit van Amsterdam

On the staff of the Research Group of Special Botany (vakgroep Bijzondere Plantkunde) there is a vacancy to be filled of

senior lecturer/reader (m/f) in special botany

The candidate would preferably have to be a

plant ecologist or
vegetation ecologist

(with qualifications on DSc level) who is capable of and will agree to

- taking charge of the section Botanical Ecology of the above-mentioned study group
- doing research on structure and dynamics of vegetation in mire and bog ecosystems
- taking care of the teaching plant and vegetation ecology.

Candidates for this function and those who may recommend suitable candidates are requested to apply (or to advise) within four weeks to the Nomination Committee, attention Prof. Dr. T. van der Hammen, Hugo de Vries-Laboratorium, Sarphatistraat 221, 1018 BX Amsterdam, The Netherlands.

W44(A)

ARC, COMPTON

A CELL BIOLOGIST

is required in the Department of Microbiology. The appointee will be a member of the Scrapie Project Group and required to collaborate in studies of the pathogenesis of scrapie, particularly as it may involve the lymphoreticular system. Duties will also include assistance in the development of improved cell culture systems for the scrapie agent.

Candidates should have a first or upper second class degree in an appropriate biological subject preferably including cellular immunology. At least 2 years relevant postgraduate research or other approved experience is required for appointment as H.S.O., with 4 years for S.S.O.

The successful applicant will be appointed either as a Higher Scientific Officer (Salary scale £4,101 to £5,448 p.a.) or as a Senior Scientific Officer (Salary scale £5,154 to £6,898 p.a.), depending on experience. Non-contributory pension scheme.



Application forms from the Secretary, Institute for Research on Animal Diseases, Compton, Nr Newbury, Berkshire, quoting reference 279.

397(A)

M.R.C. CLINICAL RESEARCH CENTRE (Northwick Park Hospital) Watford Road, Harrow, Middx. HA1 3UJ KERATIN PROTEIN CHEMIST

required to join Dermatological Group with special interest in Collagen Chemistry and related diseases. The appointee should develop methods of Keratin Protein separation which would allow examination of certain inherited and acquired epidermal diseases at a molecular level. This is a limited term appointment for four to five years, depending on age and experience. Salary within the range £5,367 to £7,754 plus £450 L.A. Application form and further details obtainable from Mrs J. Tucker-Bull. Please quote Ref. 110/1/4178. Closing date April 23, 1979. 376(A)

POSTDOCTORAL POSITIONS AT PENNSYLVANIA STATE

Theoretical studies of scattering of high energy ions (500-5,000 eV) from solid surfaces with the objective of understanding surface structure and bonding. A background in scattering theory, electronic processes and/or electronic structure would be desirable. The starting salary is \$11,000 per annum.

Please send a curriculum vitae and two letters of reference to Professor Barbara J. Garrison, Department of Chemistry, 152 Davey Laboratory, University Park, Pa. 16802.

Applications and nominations should be received by May 15. The Chemistry Department reserves the right to continue to process applications and nominations until the position is filled.

The Pennsylvania State University is an affirmative-action, equal-opportunity employer. W61(A)

UNIVERSITY COLLEGE CARDIFF

Applications are invited from graduates or prospective graduates for the post of

TUTORIAL FELLOW in the

DEPARTMENT OF BIOCHEMISTRY.

The appointee will have a First or Upper 2nd Class Honours Degree and will be permitted to register for a Ph.D. There will be some limited teaching duties and the appointee will join Prof. K. S. Dodgson's research group in studies on enzymes involved in the biodegradation of detergents.

The appointment will be for a period of three years. Salary range £3,384 to £3,883 per annum (under review). Duties to commence October 1, 1979.

Applications (two copies), together with the names and addresses of two referees, should be forwarded to the Vice-Principal (Administration) and Registrar, University College, P.O. Box 78, Cardiff CF1 1XL, from whom further particulars may be obtained. Closing date, May 18, 1979. Reference 1761. 418(A)

TECHNICIAN Grade 4 required in Department of Pharmacology to run teaching laboratories for Science and Pre-clinical Medical Students. Previous experience in Pharmacological/Physiological techniques essential. Five weeks annual paid holiday plus statutory and customary days providing a week at Christmas and Easter. Salary in range £3,746 to £4,232 inclusive of London Weighting. Application forms from Personnel Officer (Technical Staff FE7), University College London, Gower Street, WC1E 6BT. 416(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL (University of London) LONDON W1N 8AA SUB-DEPARTMENT OF MOLECULAR BIOPHYSICS

Applications are invited from graduate bio-scientists, preferably with research experience, for a post of

RESEARCH ASSISTANT

within the sub-department of Molecular Biophysics. The appointment, for two years, will be directed initially towards the application of modelling and optimisation techniques to radio-isotopic analytical methods, and ultimately to studies into the modelling of endocrinological control systems. Competence in FORTRAN programming, handling of differential equations, numerical methods, and particularly statistical techniques, will be advantageous. The work, which is supported by the D.H.S.S., will appeal to biological scientists with some mathematical ability. Salary, according to age and experience, but within the range £3,858 to £5,340 (including London Allowance).

Applications, including a curriculum vitae and the names and addresses of two referees, to Professor R. P. Ekins, at the above address by April 20, 1979. 394(A)

UNIVERSITY OF DURHAM DEPARTMENT OF EXTRA-MURAL STUDIES

Applications are invited for the post of
STAFF TUTOR

in Biological Sciences (Zoology) to develop further a wide range of courses for both the general public and specialist groups. Experience in teaching adults is desirable but not essential.

Salary on the scale £3,883 to £7,754 per annum (under review) plus superannuation.

Applications (three copies) naming three referees should be sent by April 21, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. 281(A)

UNIVERSITY OF LEICESTER

DEPARTMENT OF GENETICS

Applications are invited for a

POSTDOCTORAL RESEARCH ASSOCIATE

to work on an M.R.C.-supported project on the mechanism of action of penicillin and the use of this antibiotic to the processes of cell division and shape determination in *Escherichia coli*.

The position is tenable until October 1981 on the range for Research Associates, £3,883 to £6,555. Applications, including the names of two referees, should be sent to Dr Brian G. Spratt at the Department of Genetics, University of Leicester, Leicester LE1 7RH. 296(A)

BLOOD GROUP REFERENCE LABORATORY

SCIENTIFIC OFFICER

A graduate is required for research and development related to the production of anti-globulin reagents for use in blood transfusion laboratories. Some experience in immunochemistry, protein purification or radioimmunoassay desirable, but not essential.

Applications in writing with details of experience and the names of two referees to Miss K. Lomas, Blood Group Reference Laboratory, Catliff Road, off Ebury Bridge Road, London SW1W 8QJ. 354(A)

The Research Organisation of an internationally recognised European
Pharmaceutical Group located in

PARIS

requires a

**SENIOR
CARDIOVASCULAR
PHARMACOLOGIST**

to join its Department of Biology

The high success rate and rapid growth of this pharmaceutical group is related to the quality of people they employ and their continued commitment to research and development.

For this key position in the Cardiovascular Group we require a professional and highly motivated scientist who will be responsible for various projects within the group and should also be able to conduct independent research programmes. Applicants can be either male or female and should have relevant postdoctoral experience (a maximum of five years). It is essential that the candidate should have experience of the pharmaceutical industry.

A realistic salary can be expected and relocation assistance will be available with other financial benefits.

All applications will be treated in the strictest confidence and should be sent directly to:

"NATURE"
c/o Box No. W59(A)
3 DYERS BUILDINGS
HOLBORN, LONDON
EC1N 2NR

The Polytechnic of North London

Department of Chemistry

Technical staff are required for interesting and varied work in modern and well-equipped laboratories.

LABORATORY TECHNICIAN GRADE 5:

To be responsible for the organisation and daily running of undergraduate and research laboratories; and to participate in advanced work and research projects which may involve preparative chemistry and the operation of complex research equipment.

The technician should preferably hold HNC in Chemistry (or C & G Pt. II or a recognised equivalent) and have 8 years' laboratory experience. (Ref 2/79)

LABORATORY TECHNICIAN GRADE 4:

To be responsible for the organisation and daily running of an undergraduate laboratory, with duties in research laboratories involving preparative chemistry and the operation of scientific instruments.

The technician should have passed at least: GCE with two "A" levels, ONC/OND, C & G Pt. I or a recognised equivalent, and have 7 years' laboratory experience. (Ref 3/79)

LABORATORY TECHNICIAN GRADE 3:

To work in the undergraduate and research laboratories, providing technical service and assistance in operating and maintaining complex equipment.

The technician should have passed at least: GCE with two "A" levels, ONC/OND, C & G Pt. I or a recognised equivalent and have 3-5 years' relevant experience. (Ref 4/79)

Further specialised training will be given where appropriate.

Salaries Scales (including London weighting).

Grade 5 £3,675—£4,212

Grade 4 £3,441—£3,891

Grade 3 £3,162—£3,549

Further details and application forms may be obtained from:

Head of Department of Chemistry
The Polytechnic of North London
Holloway Road, London N7 8DB.

378(A)



THE WORLD HEALTH ORGANIZATION

Requires an EDITOR for its Office of Publications at Headquarters, Geneva. The incumbent will have editorial responsibility for the content, style and presentation of WHO publications, and will follow publications through all stages of preparation; on the basis of background texts or interviews; will write articles, reviews, publicity material, etc., for publication; may be required to serve as a precis, minute or report writer at meetings. Applicants should have a university degree or equivalent, and experience in the editing of publications of a technical nature, in writing articles and/or preparing of records of international conferences. Excellent knowledge of English essential and good knowledge of French.

Applications, quoting reference VNP79/10, should be addressed to:

The World Health Organization, Personnel
CH-1211 Genève 27

Not later than 30 April 1979.

Only candidates under serious consideration will be contacted.

W51(A)

UNIVERSITY OF EDINBURGH

Re-advertisement

LECTURER

IN SEED TECHNOLOGY

This post is being re-advertised as it is now possible to offer normal tenure and, therefore, the date for applications is being extended to May 9.

The Edinburgh School of Agriculture invites applications for a University Lectureship with responsibility for the M.Sc./Diploma Course in Seed Technology. This 12-month postgraduate course, which is supported by O.D.M., has emphasis on technical management for seed production, particularly overseas. Applicants should be University graduates with experience of seed production overseas.

This appointment will commence at the latest from October 1979 and will be on the Lecturer scale £3,883 to £7,754.

Further particulars can be obtained from the Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh. Please state Ref. No. 1013 with application. 428(A)

WELSH NATIONAL SCHOOL OF MEDICINE

(University of Wales)

DEPARTMENT OF MEDICAL BIOCHEMISTRY

POSTDOCTORAL

RESEARCH OFFICER

required for a D.H.S.S.-funded appointment (renewable annually for up to three years) in the above Department at Heath Park, Cardiff.

The project involves the development of chemiluminescent compounds as alternative labels to radioactive iodine in immunoassay. The aim is to establish clinically useful methods for measuring a variety of peptides and small molecules using luminescence. Candidates should have at least submitted their Ph.D. by time of appointment. Salary on the scales for University Research and Analogous Staff, commencing salary dependent upon qualifications but could be up to £4,302 per annum (under review).

Further particulars (quoting ref. No. C38/4/26) from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN to whom applications in the form of a brief curriculum vitae with the names and addresses of two referees should be submitted by April 30, 1979. 405(A)

UNIVERSITY OF BRISTOL

DEPARTMENT OF PATHOLOGY

Applications are invited from veterinary graduates for the post of

LECTURER IN

VETERINARY PATHOLOGY

The person appointed will be responsible primarily for teaching general and special pathology of domestic animals but may also contribute to courses for medical and science students. The Lecturer appointed will be expected to take part in the clinical pathology service to the Veterinary School and to undertake research, for which there are excellent facilities. Experience in teaching and diagnostic veterinary pathology is essential. Salary, which is currently under review, is on an incremental scale within the range £3,883 to £7,754 per annum.

Further particulars may be obtained from the Registrar and Secretary, University of Bristol, Senate House, Bristol BS8 1TH, to whom applications, together with names and addresses of three referees, should be sent by May 7, 1979 (please quote reference EB). 403(A)

UNIVERSITY OF GEZIRA SUDAN

Applications are invited for the following posts in the Preparatory College:—

1. PROFESSOR/ASSOCIATE PROFESSOR/LECTURER IN MATHEMATICS

2. PROFESSOR/ASSOCIATE PROFESSOR/LECTURER IN PHYSICS

3. PROFESSOR/ASSOCIATE PROFESSOR/LECTURER IN PHYSICAL CHEMISTRY

Salary scales: (excluding cost of living allowance) Professor £55,460 per annum; Associate Professor £54,030 to £54,550 per annum; Lecturer £51,500 to £53,500 per annum (£S1 equals £1.25 sterling). The British Government may supplement salaries in range £3,894 to £4,542 per annum (sterling) for married appointees or £1,830 to £2,310 per annum for single appointees (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages; free housing; annual overseas leave. Detailed applications (two copies) with curriculum vitae and naming three referees should be sent by airmail to the Vice-Chancellor, University of Gezira, P.O. Box 2667, Khartoum, Sudan by May 10, 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 395(A)

UNIVERSITY OF SYDNEY

LECTURESHIP/SENIOR

LECTURESHIP IN

VETERINARY CLINICAL PATHOLOGY

Appointee to be responsible for teaching, research and diagnostic services in the field of Veterinary Clinical Pathology, will be located in Sydney. Veterinary qualifications or equivalent required. Experience in clinical or practical pathology desirable.

The position may be filled either on a tenured basis, or in the first instance, for a probationary period of three years with the possibility of tenure being given during or shortly after that period.

Salary ranges: Senior Lectureship SA21,180 to SA24,687; Lectureship SA15,786 to SA20,737 per annum.

Applications, including curriculum vitae, list of publications and names of three referees by June 30, 1979 to the Registrar, University of Sydney, N.S.W. 2006, Australia, from whom further information available. Information also available from Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. 396(A)

ASSISTANT PROFESSOR OF PHYSIOLOGY

The Department of Biology at Clark University invites applications for a tenure track position in physiology. Candidates with postdoctoral experience in cellular, organismal and/or neurophysiology with experience in ultrastructural techniques are preferred. The candidate should be prepared to conduct an independent research programme and teach an introductory physiology course for majors and other appropriate courses at the undergraduate and graduate levels. Deadline date for applications is May 15. Applications should include a curriculum vitae with career objectives and names of at least three referees and be submitted to: Dr V. Ahmadian, Chairman, Biology Department, Clark University, Worcester, MA 01610. Clark University is an Equal Opportunity/Affirmative Action Employer. Applications from women and minorities are encouraged. W45(A)

Experienced Electron Microscopist

An experienced electron microscopist is required in the Special Instrumentation Group of Shell Biosciences Laboratory, Sittingbourne, Kent, to lead a team which provides an expert service for a number of research and development projects pertaining to the life sciences and the agricultural chemical business. The successful applicant will also be expected to conduct his own limited electron microscopy research programme. Current equipment within the electron microscopy team consists of transmission and scanning microscopes, the latter with an X-ray microprobe analysis attachment.

The person we are seeking would probably have a Ph.D. in a biological subject, followed by some years' broad experience in electron microscopy.

We offer a competitive salary and a range of excellent benefits including pension, flexible working hour system, staff restaurant and a recreational club. Please write or telephone for an application form to:

Shell Research Limited, Recruitment Division, (N1) PNEL/11, Shell Centre, London SE1 7NA. Telephone: 01-934 2948.



427(A)

FACULTY POSITION IN NATURAL PRODUCTS CHEMISTRY

DEPARTMENT OF
ENTOMOLOGICAL SCIENCES,
COLLEGE OF NATURAL
RESOURCES
UNIVERSITY OF
CALIFORNIA
Berkeley

Full-time, 11-month research (80 per cent) and teaching (20 per cent) position available in Natural Products Chemistry at the Assistant Professor level. Ph.D. in organic chemistry or other field(s) relevant to natural products required with evidence of interest in chemicals active in insects.

Responsibilities will be in basic and applied research involving bioactive chemicals from insects, plants, and other biological materials that influence insect growth, development, reproduction, and behaviour. Research will include identification and synthesis of active components, and studies on their potential use in managing insect populations. Teaching duties may include developing a course in chemical ecology, environmental chemistry, insect biochemistry, or chemical pest control.

Send full curriculum vitae, official transcripts, copies of significant publications or manuscripts, and names of at least three references by May 1, 1979, to Evert I. Schlinger, Acting Chairman, Department of Entomological Sciences, 137 Giannini Hall, University of California, Berkeley, California 94720. The University of California is an equal opportunity, affirmative action employer.

W47(A)

UNIVERSITY OF SALFORD DEPARTMENT OF BIOLOGY POSTDOCTORAL RESEARCH FELLOW

required for a study of vertebrate respiratory physiology supported by the Agricultural Research Council. Applications are welcome from zoologists and physiologists. Candidates should possess a Ph.D. or expect to gain one in the near future. Informal enquiries may also be made to Dr J. H. Brackenbury, Department of Biology. The post is tenable for up to 3 years.

Salary range: £3,883 to £4,382 per annum (under review), U.S.S. benefits.

Application forms are available from the Registrar, University of Salford, Salford M5 4WT (tel 061-736 5843, ext. 215) to whom completed applications should be returned by Friday, May 11, 1979, quoting reference B/90/N.

423(A)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF ZOOLOGY

Applications are invited for a Lectureship in the Department of Zoology. The successful applicant will assist in the teaching of ecology and will be particularly involved in research and teaching in the freshwater fisheries unit. He will take up his duties on October 1, 1979, or as soon as possible after that date.

The salary will be within the range £3,883 to £4,382 per annum on a scale rising to £7,754 per annum.

Applications, together with the names of three referees, should be received not later than May 14, 1979, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX from whom further particulars may be obtained. Quote ref. RV/555/N.

407(A)

THE PRINCE HENRY HOSPITAL LITTLE BAY

THE PRINCE OF WALES HOSPITAL RANDWICK

(Teaching Hospitals of the University of New South Wales)

THE EASTERN SUBURBS HOSPITAL BONDI JUNCTION SYDNEY, N.S.W. AUSTRALIA

Applications containing full details of qualifications and experience together with the names and addresses of three referees should be forwarded to:

The Staff Manager,
The Prince Henry, The Prince of Wales,
and The Eastern Suburbs Hospitals,
P.O. Box 233,
MATRIVILLE, N.S.W., 2036, AUSTRALIA

SENIOR STAFF SPECIALIST IN ANATOMICAL PATHOLOGY

with conjoint appointment as

CLINICAL PROFESSOR OR

CLINICAL ASSOCIATE PROFESSOR

SCHOOL OF PATHOLOGY, THE UNIVERSITY OF N.S.W.

Applications are invited from suitably qualified medical practitioners for the above position. The appointee may also be offered the title of Clinical Professor (conjoint appointment) or Clinical Associate Professor (conjoint appointment) and as such would be responsible to the University of New South Wales for teaching in the School of Pathology, with access to the facilities of that school.

The Department of Anatomical Pathology is staffed by three full-time specialists (including the Director) and seven registrars on rotation.

Salary is at a Senior Staff Specialist rate—\$37,850 per annum and appropriate administrative allowance is payable. Conditions in accordance with the Medical Officers Hospital Specialist (State) Award, including three months' paid study leave for each five years of service. Limited right of private practice in accordance with procedures laid down by the Health Commission of New South Wales is also available.

For further information contact Professor A. W. J. Lykke, telephone 662.2770 (Sydney).

Closing date May 28, 1979

W42(A)

UNIVERSITI SAINS MALAYSIA MINDEN, PENANG

APPLICATIONS ARE INVITED for the following vacancies —

- SCHOOL OF PHARMACEUTICAL SCIENCES**
Professor in the fields of PHARMACEUTICAL TECHNOLOGY or PHARMACOLOGY.
Associate Professor in the fields of PHARMACEUTICAL CHEMISTRY, CLINICAL PHARMACY or PHARMACOLOGY.
Lecturer in the fields of PHARMACEUTICAL CHEMISTRY, PHARMACOLOGY, PHARMACEUTICAL TECHNOLOGY or CLINICAL PHARMACY.
- SCHOOL OF APPLIED SCIENCES**
Associate Professor in the fields of ELECTRONIC SCIENCE AND Associate Professor in the fields of ELECTRONIC SCIENCE AND Digital System or Communications; FOOD SCIENCE AND TECHNOLOGY (experience required in Food Processing or Food Manufacture).
- SCHOOL OF BIOLOGICAL SCIENCES**
Professor in the fields of PLANT PATHOLOGY with emphasis on NEMATODES, VIROLOGY or BACTERIOLOGY, AQUATIC BIOLOGY with emphasis on MARINE or FRESHWATER BIOLOGY.
Lecturer in the fields of BOTANY with emphasis on ALGAE, BRIOFITES AND PTERIDOPHYTES, GENERAL MICROBIOLOGY with emphasis on FOOD or ENVIRONMENTAL MICROBIOLOGY.
- SCHOOL OF CHEMICAL SCIENCES**
Lecturer in the fields of PHYSICAL or INORGANIC CHEMISTRY.
- SCHOOL OF MATHEMATICAL SCIENCES**
Professor in the fields of PURE MATHEMATICS (ANALYSIS), APPLIED MATHEMATICS, STATISTICS, COMPUTER SCIENCE or OPERATION RESEARCH.
- SCHOOL OF PHYSICS**
Professor in the fields of EXPLORATION or APPLIED GEOPHYSICS Associate Professor in the field of BIOPHYSICS (RADIATION or MEDICAL BIOPHYSICS)
Lecturer in the fields of EXPLORATION GEOPHYSICS, OCEANOGRAPHY, BIOPHYSICS (RADIATION/MEDICAL BIOPHYSICS), X-RAY CRYSTALLOGRAPHY or SOLID STATE PHYSICS
- SCHOOL OF HOUSING, BUILDING AND PLANNING**
Professor in the field of ENGINEERING (STRUCTURAL & CIVIL).
Lecturer in the field of PLANNING (SOCIAL PLANNING AND DEVELOPMENT, POPULATION PLANNING, MANPOWER & EMPLOYMENT, LANDSCAPING AND ENVIRONMENTAL DESIGN).

QUALIFICATIONS/EMOLUMENTS

Applicants for the post of Professor should be relatively senior in years with a Ph.D. or a Master's degree with a wide and long teaching experience both at undergraduate and postgraduate level. Candidates should also have several outstanding publications to their credit.

Applicants for the post of Associate Professor should have a Master's degree or a Ph.D. with three to five years' experience as a Lecturer. They should be actively engaged in research and should have some research publications to their credit. Experience in supervising higher degree candidates and ability to provide academic leadership is an advantage.

Applicants for the post of Lecturer should have a Master's degree or a Ph.D. with an inclination towards undergraduate teaching and research.

Salary range per annum: Professor, from *M\$35,580 to *M\$40,380, Associate Professor, from *M\$30,300 to *M\$34,380; and Lecturer, from *M\$15,420 to *M\$29,100. Passage assistance, medical benefits and gratuity provided.

Apply to REGISTRAR, UNIVERSITI SAINS MALAYSIA, MINDEN, PENANG, MALAYSIA. Enclose personal vitae and references. The closing date for receipt of applications May 12, 1979.

*Check with your local bank for up-to-date exchange rate. W49(A)

UNIVERSITY OF ST ANDREWS DEPARTMENT OF ASTRONOMY

Applications are invited for a lectureship in the Department of Astronomy tenable from October 1, 1979. Candidates should be experienced observational astronomers with research interests in binary systems and galactic structure. Teaching experience essential.

Salary at appropriate point on scale £3,975 to £8,250 (under review), starting salary probably not above £5,830, plus F.S.S.U./U.S.S.

Applications (two copies preferably in typescript) with the names of three referees should be lodged by April 27, 1979 with the Establishment Officer, The University, College Gate, St Andrews, Fife, from whom further particulars may be obtained. 398(A)

MUSEUM CHEMIST—The Metropolitan Museum of Art, New York City, seeks a young Ph.D. chemist with several years' postdoctoral experience to work in collaboration with the staff of the Paintings Conservation Department. The applicant should be conversant with the following techniques: X-ray diffraction; gas chromatography; mass spectrophotometry; electrophoresis; radio-amino assay and wet chemical analysis. Responsibilities will include the identification and testing of materials, particularly organic, that have been used in the fabrication and treatment of paintings of all periods as well as long term research projects. Some knowledge and interest in paintings as works of art is essential. The candidate must be flexible and practical and willing to work outside one area of expertise. Forward application to: Mr. John M. Brealey, Conservator, Metropolitan Museum of Art, Fifth Avenue and 82nd Street, New York, New York 10028. W54(A)

FACULTY POSITION IN INSECT PATHOLOGY DEPARTMENT OF ENTOMOLOGICAL SCIENCES, COLLEGE OF NATURAL RESOURCES UNIVERSITY OF CALIFORNIA Berkeley

Full-time, 11-month research (75 per cent) and teaching (25 per cent) position available in insect pathology at the Assistant Professor level available July 1, 1979. Expertise mandatory in one of the following areas: bacteriology, comparative virology, mycology, or protozoology. Ph.D. and background in pathology required and post-doctoral experience desirable.

Responsibilities will be in basic and applied research and teaching in insect pathology and pest management. Experience in pathogen-insect relationships, pathogenicity, and the manipulation of pathogens for insect control will be weighting factors when judging applicants.

Send full curriculum vitae, official transcripts, copies of significant publications or manuscripts, and names of three references by May 1, 1979, to Evert I. Schlinger, Acting Chairman, Department of Entomological Sciences, 137 Giannini Hall, University of California, Berkeley, California 94720. The University of California is an equal opportunity, affirmative action employer. W46(A)

UNIVERSITY OF NAIROBI KENYA

Applications are invited for the post of

ASSOCIATE PROFESSOR IN DEPARTMENT OF BIOCHEMISTRY

Applicants must have a Ph.D. or equivalent in Biochemistry and have research and teaching experience at University level. The appointee will be required to teach biochemistry to the undergraduate students in the faculties of Medicine, Science, Veterinary Medicine and Agriculture. He/she will also be required to supervise M.Sc. and Ph.D. students and at the same time pursue their own research projects in biochemistry. Salary scale KES 864 to KES 4,488 per annum (KEI equals £1.30 sterling). The British Government may supplement salary by £5,784 per annum (sterling) for married appointee or £3,876 per annum (sterling) for single appointee (currently under review and normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages, superannuation scheme, medical aid scheme; subsidised housing, various allowances. Detailed applications (two copies) with curriculum vitae and naming three referees should be sent by air mail to Registrar, University of Nairobi, P.O. Box 30197, Nairobi, Kenya by May 29, 1979. Applicants resident in the UK should send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 415(A)

HARVARD MEDICAL SCHOOL NEUROBIOLOGIST

The Department of Neurobiology is seeking a new faculty member at the assistant-professor level to begin 1979 or 1980. Applications from women and minority people are encouraged. Please send curriculum vitae and a brief outline of research plans, and have three letters of reference sent to

Search Committee
Department of Neurobiology
Harvard Medical School
25 Shattuck Street
Boston MA 02115

W52(A)

THE HANNAH RESEARCH INSTITUTE FOR STUDIES RELATING TO THE PRODUCTION AND UTILISATION OF MILK METABOLIC PHYSIOLOGIST

A vacancy exists for a Higher Scientific Officer in the Physiology Department to study metabolism in goats and cows in relation to the control of milk secretion and to nutritional and environmental factors.

Applicants should possess a First or Upper Second Class Honours Degree in a relevant discipline with, preferably, a Ph.D.

Salary will be in the range £4,101 to £5,448. At least two years' relevant postgraduate experience is required for appointment to HSO. Non-contributory pension scheme in operation.

Further particulars may be obtained from

The Secretary
The Hannah Research Institute
AYR KA6 5HL

to whom applications, which should include a curriculum vitae, list of publications, and the names and addresses of three referees, should be sent by: April 20, 1979. 387(A)

MCGILL UNIVERSITY DEPARTMENT OF BIOLOGY

PCSTDOCTORAL FELLOW

Required to work in a project on the interaction of the killer toxin protein with cell surface receptors in yeast. Experience in protein chemistry and/or yeast genetics an advantage. The appointment is for one year initially and may be renewed for a further two years. Initial salary \$13,000 per annum. Applications and two letters of recommendation should be sent to Dr H. Bussey, Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, Quebec, Canada, H3A 1B1. W50(A)

THE POLYTECHNIC OF NORTH LONDON DEPARTMENT OF FOOD SCIENCES Applications are invited for the following post: **TEMPORARY LECTURER II IN NUTRITIONAL BIOCHEMISTRY** for an initial period of one year from 1st September 1979 with a possible extension for a further year. The Lecturer will be required to teach and supervise projects at both degree and diploma level. The main biochemical teaching is required for the 18 month Diploma in Dietetics course but the Lecturer will play an important part in integrating the teaching of biochemistry with the nutrition taught at degree level. Applicants should hold a degree in which biochemistry in relation to man was a major area of study, and must be able to relate their teaching to the field of human nutrition. Experience in teaching would be an advantage. Salary Scale: £4,575 to £7,032 (including London Allowance). Application form and further particulars from the Establishment Officer, The Polytechnic of North London, Holloway Road, N7 8DB. Tel. 01-607 2789, Ext. 2018. Closing date—two weeks from the appearance of this advertisement. 406(A)

TWO postdoctoral positions are available immediately to study the structural organization of the nitrogen fixing gene cluster of *Klebsiella*. One additional position will be available starting July 1, to clone plant DNA in E. coli and in yeast. Requirement: Ph.D. in biochemistry or in molecular biology. Candidates with research experience in phage, viral, or plasmid systems preferred. Send curriculum vitae and two references to: Dr A. A. Szalay Boyce Thompson Institute at Cornell University, Tower Road, Ithaca N.Y. 14853. W56(A)

THE UNIVERSITY
OF LEEDS
DEPARTMENT OF
PHYSIOLOGY

Applications are invited for a temporary post of RESEARCH FELLOW in the above Department. The successful candidate will work with Professor B. R. Jewell in a small team with interests in various aspects of cardiac muscle physiology. Candidates should possess a Ph.D. degree in Physiology and Biochemistry. The appointment will be made for a fixed period of three years.

Salary at an appropriate point on the LA scale for Research and Analogous Staff (£3,883 to £6,555) (under review), according to age, qualifications and experience.

Informal enquiries about the post may be made to Professor Jewell (telephone (0532) 31751, ext 7267).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 104/7/D. Closing date for applications May 25, 1979.

401(A)

UNIVERSITY OF
EDINBURGH
FACULTY OF MEDICINE
LECTURESHIP IN
PHYSIOLOGY

Applicants must have a good Honours degree in Physiology and at least three years' research experience. Salary on the scale £3,883 to £7,754 per annum (under review).

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL, with whom applications (6 copies) including a full curriculum vitae and the names and addresses of three referees should be lodged not later than May 18, 1979.

Please quote Reference 1035.

399(A)

UNIVERSITY OF READING
DEPARTMENT OF PHYSIOLOGY
AND BIOCHEMISTRY

Applications are invited for a Lectureship, for a fixed term of one year beginning on October 1, 1979. The Department teaches Physiology, Biochemistry and Nutrition in the Faculty of Science and in the Faculty of Agriculture and Food. Applicants should hold a good honours degree in an appropriate subject and have experience of research and preferably of teaching. The person appointed would be expected to join in the research activities of one of the established groups in the Department.

The appointment will be in the Lecturers' salary scale within the range £3,883 to £4,882 (under review).

Further information may be obtained from the Registrar, (Room 214, Whiteknights House), The University, Whiteknights, Reading RG6 2AH, to whom applications should be received not later than May 26, 1979.

406(A)

UNIVERSITY OF BATH
Applications are invited for a
LECTURER
IN BIOCHEMISTRY

The starting salary will be set in the lower part of the salary range £3,883 to £7,754 per annum.

Further particulars and application forms obtainable from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting reference number 79/70/N.

Closing date for applications May 14, 1979.

379(A)

Medical Development Project Co-ordinators

May & Baker is an international Company, manufacturing and marketing a wide range of research based pharmaceuticals.

In connection with the internal expansion of our pharmaceutical development organisation, we have created vacancies for Medical Development Project Co-ordinators. These are key posts carrying responsibility for the planning and progress of the development of new medical specialty products. The work involves close liaison with senior staff in all areas of R & D.

Candidates should possess a good honours degree in a biological science or chemistry and an ability to communicate clearly in discussions and in written reports. Preference will be given to those applicants with experience in the pharmaceutical industry, particularly in the field of medical product development. Familiarity with modern project planning techniques and a working knowledge of the French language would be an advantage.

Benefits of employment include competitive salaries, flexible working hours, subsidised meals and a contributory pension scheme with life assurance. Assistance with relocation expenses will be given where appropriate.

Please telephone 01-592 3060 extension 2506 for an application form or write with full details of career to date to



M&B May & Baker

Mrs. P. Clark, Personnel Officer,
May & Baker Ltd, Dagenham, Essex RA10 7XS
quoting reference number 120 N 1.

430(A)

IMPERIAL COLLEGE OF
SCIENCE AND
TECHNOLOGY
CENTRE FOR
ENVIRONMENTAL TECHNOLOGY
Mathematical Models of the
Population Biology of Human
Infectious Diseases in
Urban Environments

Applications are invited for a Postdoctoral Research Post in the Centre for Environmental Technology as part of a contract from the Department of the Environment.

The work involves dynamic modelling of the population biology of infectious diseases (measles, mumps, whooping cough and sexually transmitted diseases) with particular reference to human density and other environmental parameters in urban settings.

Candidates should preferably possess a Ph.D. in applied mathematics of mathematical ecology with ideally experience of epidemiological work.

The appointment will be for a period of two years in the first instance. Salary will be on scale 1A, £3,883 to £6,555 per annum (under review), plus London Allowance.

Applications including curriculum vitae and names of two referees should be sent to Dr R. M. Anderson, Centre for Environmental Technology, Imperial College of Science and Technology, London SW7 2AZ by June 1, 1979.

392(A)

A faculty position is available for an individual with a Ph.D. in Physiology and demonstrable training with scientific accomplishments in the area of smooth muscle electrophysiology, mechanics and/or biochemistry. Only those who meet these qualifications should send a curriculum vitae and selected reprints to: Dr Stanley G. Schultz, Professor and Chairman, Department of Physiology, University of Texas Medical School at Houston, P.O. Box 20708, Houston, Texas 77025 (An Equal Employment Opportunity, Affirmative Action Employer) W57(A).

SEVERAL junior and senior faculty positions are available for individuals with Ph.D.'s in Physiology and demonstrable scientific accomplishments in the areas of ion transport and electrophysiology of epithelial tissues; a familiarity with computer sciences is also desirable. Only those who meet these qualifications should apply. A curriculum vitae and selected reprints should be forwarded to: Dr Stanley G. Schultz, Professor and Chairman, Department of Physiology, University of Texas Medical School at Houston, P.O. Box 20708, Houston, Texas 77025, (An Equal Employment Opportunity, Affirmative Action Employer) W58(A).

M.R.C. CLINICAL RESEARCH CENTRE
(Northwick Park Hospital)

Watford Road, Harrow, Middlesex HA1 3UJ

KERATIN PROTEIN CHEMIST

required to join Dermatological Group with special interest in collagen chemistry and related diseases.

The appointee should develop methods of keratin protein separation which would allow of certain inherited and acquired epidermal diseases at a molecular level.

This is a limited term appointment for four to five years depending on age and experience.

Salary within the range £5,367 to £7,754 plus £450 London Allowance.

Application form and further details obtainable from Mrs J. Tucker-Bull. Please quote reference 110/1/4178. Closing date April 23, 1979.

431(A)

The Research Organisation of an Internationally recognised European Pharmaceutical Group located in Paris requires a

TECHNICAL RESEARCH OFFICER

The high success rate and rapid growth of this pharmaceutical group is related to the quality of people they employ and their continued commitment to research and development

To strengthen the research effort. The research organisation requires a professional and highly motivated scientist who will be responsible for:

- Advising on the implementation of the GLP standards within research and within a proposed new research centre.
- Assisting with the planning and organisation of research projects.
- Organising the collation and storage of research data.

Applicants can be either male or female and should be a graduate in the biological sciences. It is essential that the candidate should have experience of the pharmaceutical industry.

A realistic salary can be expected and relocation assistance will be available together with other financial benefits.

All applications will be treated in the strictest confidence and should be sent to 'Nature', c/o Box No. W53(A), 3 Dyers Building, Holborn, London EC1N 2NR. W53(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF PHYSIOLOGY

Applications are invited for a temporary post of LECTURER in the above Department, tenable from October 1, 1979 for a fixed period of three years. The Department teaches Science, Medical and Dental students, and has research interests in the major fields of physiology.

Salary on the scale for lecturers £3,883 to £7,754 (under review), according to age, qualifications and experience.

Informal enquiries to Professor B. R. Jewell, Department of Physiology (telephone 0532) 3175, ext. 7267.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 104/6/D. Closing date for applications May 7, 1979.

402(A)

UNIVERSITY COLLEGE LONDON DEPARTMENT OF CHEMISTRY POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a position as postdoctoral research assistant to work with Dr B. P. Roberts on ESR, and chemical studies of nitrogen-, oxygen-, and sulphur-centered free radicals. An appreciable amount of synthetic organic chemistry will be involved. Appointment for two years, starting from October 1979 (earlier starting dates are possible). Starting salary in range £3,909 to £4,408 per annum, plus £502 London Allowance, depending on age and experience. Applications, including curriculum vitae and names and addresses of two referees to Dr B. P. Roberts, Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ. 375(A)

IMPERIAL COLLEGE DEPARTMENT OF CIVIL ENGINEERING LECTURER IN SOIL MECHANICS

Applications are invited for the post of Lecturer in soil mechanics. The soil mechanics section is linked, under the general heading of geo-technical engineering, with groups specialising in engineering rock mechanics, engineering geology and engineering seismology.

The section runs a large M.Sc. course, as well as being involved in undergraduate teaching and a wide variety of experimental and theoretical research. It also forms part of the S.R.C.-sponsored London Centre for marine structures and materials.

The Lecturer will be involved in both undergraduate and postgraduate teaching, and will be expected to play an active part in the conduct and supervision of research.

Applicants with varying practical experience and a variety of specialisms at research level will be considered, although a knowledge of numerical methods and/or dynamic problems would be of particular interest. Subject to suitable postgraduate experience, applicants need not have a first degree in civil engineering.

Salary is the scale £3,883 to £7,754 per annum plus London Allowance.

Applications and enquiries should be made to Professor B. G. Noel, Head of Civil Engineering Department, Imperial College, South Kensington, London SW7 2AZ. 391(A)

PLANT TISSUE CULTURE DEPARTMENT OF MICROBIOLOGY AND CELL SCIENCE UNIVERSITY OF FLORIDA

Applications are invited for a tenure track faculty position currently open at the ASSISTANT PROFESSOR rank for individuals with a Ph.D. and demonstrated expertise in plant tissue culture. Individuals working in the areas of biochemical genetics and molecular biology are particularly solicited.

Responsibilities will include development of a vigorous research program utilising plant tissue culture techniques to explore molecular and subcellular phenomena involved in plant development. After the program becomes established, part of the responsibility will be to provide advice and consultation to other scientists in the general area of plant tissue culture.

Applicants should submit curriculum vitae, a statement describing current research activities and detailing future plans, and at least three letters of recommendation to:

Dr Paul H. Smith, Chairman
Department of Microbiology
and Cell Science
1059 McCarthy Hall
University of Florida
Gainesville, Florida 32611

The University of Florida is an Equal Opportunity and Affirmative Action Employer. W41(A)

CAMBRIDGE UNIVERSITY DEPARTMENT OF GENETICS Applications are invited for an ASSISTANT TECHNICAL OFFICER

to be responsible for the organising of the maintenance of Drosophila.

The scale of stipends, for a person not normally resident in College, is at present £3,634 per annum, at age 23, rising by annual increments to £5,367.

Applications, with the names of not more than three referees, should be sent to the Secretary of the Appointment Committee of the Faculty of Biology "A", Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX, from whom further particulars may be obtained. Applications should reach him by not later than May 12, 1979. 385(A)

MONASH UNIVERSITY Melbourne, Australia DEPARTMENT OF CHEMISTRY JUNIOR RESEARCH FELLOW (THEORETICAL CHEMISTRY)

To undertake theoretical investigations into the Auger electron spectra of small molecules with Dr F. P. Larkins for a period of two years subject to a satisfactory review one year from the date of appointment. The successful candidate should have a Ph.D. degree or equivalent qualifications with a strong background in theoretical chemistry or physics. A sound knowledge of molecular orbital theory would be advantageous. A salary in the range \$A13,083 to \$A14,606 per annum is offered with some provision for travel costs. Applications including three referees and curriculum vitae to the Academic Registrar, Monash University, Wellington Road, Clayton, Victoria, Australia, 3168. Further details available from Dr Larkins. Applicants should quote reference No. 41061. Closing date April 30, 1979. 389(A)

UNIVERSITY OF EDINBURGH DEPARTMENT OF ZOOLOGY

Applications are invited for the post of Lecturer in the Department of Zoology. Salary scale £3,883 to £7,754 per annum (under review), with placement according to qualification and age, and with superannuation benefit.

The successful candidate will be expected to take up duty on October 1, 1979.

Further particulars may be obtained from the Secretary to the University of Edinburgh, Old College South Bridge, Edinburgh, with whom applications (six copies), giving the names of two referees, should be lodged not later than May 5, 1979. Please quote Reference 1032. 400(A)

PROFESSORSHIP IN MOLECULAR GENETICS

Seeking individual for Professorship in Molecular Genetics. Individual must be established investigator for research and teaching in molecular genetics.

Position in Cancer Center with broad laboratory research programmes including cellular genetics and development, tumour immunology, virus oncology, and chemical carcinogenesis.

Salary negotiable dependent on level of experience and academic rank of applicant. Send curriculum vitae and inquiries to: Chairman, Molecular Genetics Professor Search Committee Cancer Center/Institute of Cancer Research, Columbia University, 701 West 168th Street, New York, New York 10032.

An Equal Opportunity Employer. W60(A)

TECHNICIAN/RESEARCH OFFICER

required for Tumour Virology Laboratory, for work involving tissue culture (preparation of primary cultures and handling of normal and virus-infected cell lines), virus assays, cytological techniques (cell-fusion, karyology) and nucleic acid biochemistry (DNA extraction, enzyme assays, hybridisation). The technician will share routine laboratory duties but would also work on research projects without day-to-day supervision.

Degree/HNC required plus at least two years relevant laboratory experience. Salary range £3,582 to £4,902 plus L.W. Appointment available May 1979. For application form write or telephone Miss S. M. Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2 on 01-242 0200 ext. 305. 412(A)

Avon Area Health Authority (T. —Southmead Health District SOUTHMEAD HOSPITAL BRISTOL RESEARCH ASSISTANT

Research Assistant required for a 3 year supervised project to study alterations in cell membrane glycoproteins, surface charges and enzyme cytochemistry in human leukaemia. Some tissue culture work will also be carried out.

Applicants should possess an honour degree in Biochemistry or related subject. The post is available from June 1, 1979. Salary range: £3,486 to £4,275.

Applications to and further details from Dr I. D. Fraser, Department of Haematology, Southmead Hospital Bristol BS10 5NB Tel: Bristol (0272) 505050 by April 30, 1979. 404(A)

EDITORIAL SERVICES CONTROLLER

We need an experienced, literate and adaptable Editorial Controller to work in our Higher and Further Education division, managing a wide variety of typescripts in subjects ranging from undergraduate science to semi-technical hobbies. The job involves working closely with the Further Education, Published, copy-editing and proof-reading, as well as dealing with authors, freelance staff, and our own production staff. Accuracy, and the ability to rewrite where necessary are important. Training will be provided for a person with the required academic and work record.

We are based at Basingstoke, close to the M3. The right person will be offered a competitive salary, and will be entitled to 20 days' holiday per year. We also have a subsidised bar, canteen, social club, pension scheme, and free transport to and from the town centre.

Send your application in handwriting, along with a detailed curriculum vitae, to: **Sheilagh Browne, Personnel Manager, The Macmillan Press Ltd., 4 Little Essex Street, London WC2R 3LF.** 333(A)

**M MACMILLAN
PRESS**

UNIVERSITY OF YORK CANCER RESEARCH UNIT RESEARCH ASSISTANT

Applications are invited for the above post in the Cancer Research Unit, which is shortly moving into new laboratories. The post is suitable for someone who has, or expects to have, an honours degree in Biochemistry or Biology, preferably with some microbiological experience.

The appointment will be made as soon as possible and is for a two-year period. The research work will involve the study of microbiological methods of detecting reactive metabolites of chemical carcinogens.

Further information concerning the work may be obtained from Dr R. C. Garner (Tel. York (0904) 31805).

Salary will be on the Range 1B, starting at £3,384, currently under review.

Four copies of applications, naming two referees, should be sent by May 4, 1979, to the Registrar, University of York, Heslington, York YO1 5DD. Please quote reference number 11/7078. 424(A)

THE UNIVERSITY OF MANCHESTER RESEARCH ASSOCIATE IN PATHOLOGY

Applications invited for this post, tenable for three years from a date to be arranged. The project is to investigate the nature and significance of intraduct proliferative breast lesions and their relationship to carcinoma in humans. A variety of techniques will be employed including electron microscopy, microdensitometry and immunofluorescence. Initial salary range per annum: £3,883 to £4,382. Superannuation. Applications (curriculum vitae and three referees) should be sent to the Registrar, The University, Manchester M13 9PL by April 30. Quote Ref: 75/79. Further information from Dr M. Harris, Department of Pathology, University Hospital of South Manchester, Manchester M20 8LR (Tel: 061-445 8111, Ext. 2760). 409(A)

THE POLYTECHNIC OF NORTH LONDON SCIENCE AND TECHNOLOGY FACULTY RESEARCH ASSISTANT

required to (a) assist in the development and teaching of a new course "Science and the Making of the Modern World" taken by all students on the B.Sc. Science degree; the course surveys developments in science since the 17th century and outlines some of the ways in which science has shaped the modern world; (b) conduct research—for a higher degree, if appropriate—on some topic in the history and/or social aspects of science which is relevant to the above course. The post is tenable for one year in the first instance, renewable for up to three years. Salary Scale: £3,087 to £3,261 (including London Allowance). A good first degree in Natural Science or covering the historical, philosophical or social aspects of science is required; some teaching experience an advantage. Further particulars are available from Dr J. Marks, Physics Department, The Polytechnic of North London, Holloway Road, N7 8DB. 377(A)

UNIVERSITY OF OTAGO Dunedin, New Zealand LECTURER IN ZOOLOGY AND MARINE SCIENCE

Applications are invited for the position of Lecturer in Zoology and Marine Science. This position is a joint appointment between the Department of Zoology and the Portobello Marine Laboratory, and the person appointed will be expected to contribute to the teaching programme of the Department of Zoology and to devote approximately half his time to research and other duties at the Marine Laboratory. Some preference may be shown towards candidates whose research interests are in marine field of studies which utilise the resources of the sea-going research vessel of the Marine Laboratory.

Salary Scale—NZ\$11,894 to NZ\$14,615 per annum. Further information is available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University. Applications close on June 15, 1979. 388(A)

Microbiologist

G. D. Searle is a leading Pharmaceutical company with its main U.K. site and Research laboratories at High Wycombe in Buckinghamshire.

Within the laboratories we are involved in a growing number of research and development programmes in various fields of medical science.

The position of Microbiologist would ideally suit an experienced man or woman who would join the Research and Development Division to take on responsibility for several branches of microbiological work, primarily the monitoring of sterile production and testing facilities, but also providing microbiological advice for programmes on fermentation development and microbiological research. The person appointed will report to the Biological Quality Control Manager.

Candidates should have several years' experience in this field, probably with a PhD and possibly with specific experience of microbiological aspects of tissue culture based vaccine production. All applicants should have at least a working knowledge of GMP requirements and should enjoy a real challenge – with the potential satisfaction of making significant advances in the world of medicine.

In addition to a competitive salary, conditions of employment include 4 weeks' holiday per year, a contributory pension fund, subsidised private health insurance, sports and social club and staff cafeteria.

Applications are requested, quoting reference M/R16, either in writing, giving career details, or by telephoning for an application form to: G. Jenkins, Personnel Manager, Research & Development, G. D. Searle & Co. Ltd., Lane End Road, High Wycombe, Bucks. (Tel. High Wycombe 21124 ext. 3374).



426(A)

SEARLE

THE DISTILLERS COMPANY LIMITED Yeast and Food Division MICROBIAL BIOCHEMIST

The Yeast and Food Division of The Distillers Company Limited require a graduate to work in areas of Microbial Biochemistry in their research and development laboratories, Glenochil Technical Centre, Menstrie, Clackmannanshire.

Applicants, who should be under 28 years old, must have a degree in microbiology, biochemistry or closely related subjects and preferably have postgraduate experience.

Further details together with an application form can be obtained by writing to: The Yeast Research Manager, The Distillers Company Limited, Yeast and Food Division, Glenochil Technical Centre, MENSTRIE, Clackmannanshire FK11 7ES. 305(A)

FELLOWSHIPS

OXFORD UNIVERSITY
and ICI JOINT RESEARCH
SCHEMEResearch
Fellowship

Applications are invited for a Joint Research Fellowship, to work on the THEORETICAL INVESTIGATION OF SOLVENT EFFECTS, to be held in the Physical Chemistry Laboratory, Oxford University.

The proposal is to study the effects of water on solutes by statistical mechanical methods allied with quantum mechanical calculations. The work will be carried out in Oxford and in collaboration with Dr K J Heritage, ICI Plant Protection Division, Jealott's Hill Research Station, Bracknell, Berkshire.

The Fellowship will be tenable for two years at a starting salary of up to £4,382 (under review) with membership of USS.

Applications with curriculum vitae and the names of two referees should be sent to Dr W G Richards, Physical Chemistry Laboratory, South Parks Road, Oxford, OX1 3QZ. 382(E)



Plant Protection
Division

POSTDOCTORAL
FELLOWSHIPS:
MEDICAL

OR

Ph D. GRADUATES

The School of Pathology of the South African Institute for Medical Research and the University of the Witwatersrand offers one-year fellowships (renewable) for scientists interested in working in any of the following fields:—

- Histopathology
- Haematology and/or Immunohaematology
- Medical Biochemistry
- Microbiology
- Tropical Pathology (including parasitology)
- Vaccine Production
- Mycology
- Cytology
- Human Genetics (including cytogenetics)
- Immunology
- Epidemiology
- Electron Microscopy

Clinical material and laboratory facilities are available for research in these fields. The fellowships will provide opportunity for challenging research in diseases of importance for developing countries.

For further details contact —

Head, Personnel Services, The South African Institute for Medical Research, P O Box 1038, Johannesburg 2000, South Africa. Tel 724-1781. W48(E)

POSTDOCTORAL
FELLOWSHIP

A postdoctoral fellowship is open for work on the structure and dynamics of fluids with the neutron scattering group at the University of Guelph. Present projects include the study of three body forces and correlations in atomic gases and molecular orientation effects in molecular gases. Duties include participation in neutron scattering experiments (for extended periods) at A.E.C.L., Chalk River and (for short periods) at I.L.L., Grenoble, France. This position will be open until filled. Initial appointment for one year with renewal for a second year by mutual agreement. Salary approximately \$12,000 per year. Candidates with Ph.D. in any area of condensed matter or nuclear physics will be considered, and should send C.V. and names of two referees to: Dr P A. Egelstaff, Department of Physics, University of Guelph, Guelph, Ontario, Canada N1G 2W1. W43(E)

UNIVERSITY OF BRISTOL
SCHOOL OF CHEMISTRY
POSTDOCTORAL
FELLOWSHIP IN
LASER SPECTROSCOPY

Applications are invited for a Postdoctoral Research Fellowship for two years from October 1, 1979, to work on the infrared spectroscopy of unstable molecules and free radicals. The project will make use of modulated CO₂ lasers in conjunction with Stark and Zeeman tuned absorption cells, and will be oriented to problems of astrophysical importance.

The post is S.R.C. funded, and the initial salary will be in the range £3,883 to £4,382 per annum (scale under review).

Applicants should send a curriculum vitae and the names of two referees to: Dr Geoffrey Duxbury, School of Chemistry, The University, Bristol BS8 1TS, U.K. 419(E)

BRANDEIS UNIVERSITY
Rosenstiel Center

A postdoctoral fellowship is available for the study of structure and assembly of tobacco mosaic virus by X-ray diffraction from oriented gels. Extension and refinement of present 4 Å structure, study of other helical TMV protein aggregates and of related viruses. Project is related to larger program of Dr D L. D Casper on virus structure. Computing ability is desirable and preferably crystallography, protein chemistry or biological structure experience. Available immediately, for up to two years. \$12,500–\$15,000.

Contact Dr G Stubbs, Rosenstiel Center, Brandeis University, Waltham, MA. 02154, USA (617)-647-2467. W55(E)

ASSISTANTSHIPS

UNIVERSITY OF
ST. ANDREWS
DEPARTMENT OF
APPLIED MATHEMATICS
POSTDOCTORAL
RESEARCH
ASSISTANTSHIP

Applications are invited for a position as Research Assistant, from October 1979, to work with Dr B Roberts on the theoretical nature of solar intense magnetic fields. The programme will involve the development of theories for spicules (transient flows in a magnetic tube) and for the propagation of waves in intense tubes. Links with the observations may be maintained by visiting Kitt Peak and Sacramento Peak Observatories. Ideally, applicants should have a Ph.D. in Applied Mathematics or Theoretical Physics.

The appointment is supported by S.R.C. and is initially for one year with good prospects for an extension to two or three years. Starting salary will be in the range £3,975 to £4,505 (under review).

Applications should be lodged by May 4, 1979, with Dr B. Roberts, Department of Applied Mathematics, University of St. Andrews, Fife, from whom further particulars may be obtained (Tel: St Andrews 74317). 422(P)

UNIVERSITY OF DURHAM
DEPARTMENT OF PHYSICS

Applications are invited from an electronics engineer or physicist with an electronics background for a senior research assistantship, tenable for two years, from as soon as possible. The work will involve the development of silicon diode arrays for low light level imaging in astronomy and their associated data acquisition systems. Experience with minicomputer systems would be an advantage.

Initial salary in the range £3,883 to £5,129 (under review) on range 1A plus superannuation.

Applications (three copies) naming three referees should be sent by May 11, 1979 to the registrar and secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. 410(A)

UNIVERSITY OF DURHAM
DEPARTMENT OF PHYSICS
POSTDOCTORAL
SENIOR
RESEARCH ASSISTANTSHIP

tenable for three years from 1st October, 1979. The successful applicant will participate in a new research project devoted to the theoretical study of low energy molecular collisions.

Initial salary in the range £3,883 to 4,917 (plus superannuation).

Applications (three copies) naming three referees should be sent by April 30, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. 280(P)

UNIVERSITY OF DURHAM
DEPARTMENT OF
CHEMISTRY

Applications are invited from chemists, materials or polymer scientists, or solid state physicists for an S.R.C. funded postdoctoral Senior Research Assistantship tenable for two years from October 1, 1979 (or earlier by arrangement) for an interdisciplinary investigation of the formation and properties of thin films and of structure and reactivity of polymeric systems, and composites in general, primarily by E.S.C.A. The appointee will assist in setting up a custom designed spectrometer dedicated to the research.

Salary £3,883 to £4,133 on range 1A plus superannuation.

Applications (three copies) naming three referees should be sent by June 1, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. 411(P)

STUDENTSIPS

UNIVERSITY OF
EDINBURGH
RESEARCH STUDENTSHIP

Applications are invited for a Medical Research Council Studentship to investigate various biochemical and immunological methods for detecting carriers and pre-clinical cases of muscular dystrophy.

Applicants should have, or be likely to graduate with, at least an Upper Second-Class Honours Degree in a relevant field and will be expected to register for a higher degree.

Please send C.V. and the names of two referees to:

Professor A. E. H. Emery,
University Department of Human Genetics,
Western General Hospital,
Edinburgh EH4 2XU. 421(F)

UNIVERSITY OF LONDON
REACTOR CENTRE
(Ascot, Berkshire)

POSTGRADUATE RESEARCH

The Science Research Council is preparing this year to offer suitable candidates a limited number of Research Studentships, tenable at the University of London Reactor Centre. The Reactor Centre, operated for the University by Imperial College, provides facilities and supervision for students to engage in research leading to the degrees of M.Phil. and Ph.D. of the University of London.

Current fields of research include low energy nuclear physics, neutron activation analysis, radiochemistry and reactor physics.

Applicants holding, or expecting to obtain in 1979, a good honours degree in a physical science, can obtain further information and an application form by writing to: The Registrar, Imperial College of Science and Technology, London SW7 2AZ. 374(A)

THE UNIVERSITY
OF LEEDS
DEPARTMENT OF
PLANT SCIENCES
M.A.F.F.
POSTGRADUATE
AGRICULTURAL
STUDENTSHIP

Applications are invited for a postgraduate studentship for research on techniques for the inoculation of field crops with vesicular-arbuscular mycorrhizal fungi.

A first or upper-second class Honours degree is essential.

Application (before end of May) and enquiries to: Dr F. E. Sanders, Department of Plant Sciences, Agricultural Sciences Building, University of Leeds, Leeds LS2 9JT. 384(F)

STUDENTSHIPS—continued

THE OPEN
UNIVERSITYBRAIN
RESEARCH GROUPResearch
Studentships

Ph.D. studentships are available in the Open University Brain Research Group, (Head, Professor Steven Rose) for graduates with good biochemistry/cell biology degrees to work on projects in the area of developmental neurobiology and biochemical and cellular correlates of experience and learning.

Further particulars and application forms are available from the Higher Degrees Office, The Open University, P.O. Box 49, Milton Keynes, MK7 6AD. Please quote reference (RR/9). The closing date for receipt of applications is 26th April. 373(F)

UNIVERSITY OF
NOTTINGHAM
FACULTY OF
AGRICULTURAL SCIENCE
POSTGRADUATE
STUDENTSIPS

Applications are invited for Ph.D. studentships in the Department of Physiology and Environmental Studies for research in the following fields:—

(i) Interactions between endocrine status and sexual behaviour in the ram.

(ii) Endocrine control of mammary gland metabolism (including cardiovascular parameters) in lactating ruminants.

Applicants should possess, or expect to obtain this summer, a first class or upper second class Honours degree in an appropriate subject.

Further details may be obtained from the Secretary, University of Nottingham, Faculty of Agricultural Science, Sutton Bonington, Loughborough, Leics. LE12 5RD, to whom completed applications including a full curriculum vitae and names and addresses of two referees should be sent by April 30, 1979. 381(F)

UNIVERSITY OF
LEICESTER
DEPARTMENT OF BOTANY
N.E.R.C.
RESEARCH STUDENTSHIP

Applications are invited from graduates, or those expecting to graduate this summer, for a N.E.R.C. Research Studentship. The Studentship is available for one only of the following research projects:

(1) Effect of real and simulated vegetation canopies on the maturation and subsequent germinability of weed seeds.

(Supervisor: Professor H. Smith)

(2) Available genetic resources of turf grasses—C.A.S.E. studentship with the Sports Turf Research Institute.

(Supervisor: Dr C. A. Stace)

The first project will be of interest to a plant physiologist/ecologist whereas the second project would be more suited to a plant taxonomist. All promising candidates will be invited to the Department for informal discussions.

Requests for further information and applications should be addressed to Professor H. Smith, Department of Botany, University of Leicester, Leicester LE1 7RH. 313(F)

UNIVERSITY OF
WARWICKC.A.S.E. STUDENTSHIP
KINETICS OF DIAZONIUM
SALT REACTIONS BY FAST
REACTION METHODS

Applications are invited for a three-year S.R.C. (C.A.S.E.) Research Studentship in the Department of Chemistry and Molecular Sciences, starting October 1, 1979, and supervised by Dr T. J. Kemp in collaboration with Dr J. Atherton (ICI Organics). The programme will involve a kinetic study of the decomposition and coupling reactions of ArN_2^+ ions using stopped-flow spectrophotometry combined with a rapid-scanning spectrometer which provides complete u.v.-visible spectra in milliseconds. Applicants should hold or expect to hold at least an upper second class honours degree in chemistry. Further details may be obtained from Dr Kemp, Department of Chemistry and Molecular Sciences. Applications with the names of two referees should be sent as soon as possible to the Academic Registrar, University of Warwick, Coventry CV4 7AL. Please quote Ref. No.: PG/41/79. 383(F)

UNIVERSITY OF LONDON
REACTOR CENTRE
S.R.C. C.A.S.E. STUDENTSHIP

Applications are invited for a Research Studentship tenable for three years at the University of London Reactor Centre.

The project, which will be carried out in collaboration with the Division of Radiation Science and Acoustics of the National Physical Laboratory, will involve careful measurements of neutron spectrum shapes at U.L.R.C. and at N.P.L. The effect of changes in epithermal neutron spectra on the measurement of resonance integrals will be investigated.

Applicants should hold, or expect to obtain in 1979, a good honours degree in Physics, and should have been resident in the United Kingdom for at least three years.

Application forms and further particulars are available from Dr T. D. MacMahon, University of London Reactor Centre, Silwood Park, Sunninghill, Ascot, Berkshire SL5 7PY. 393(F)

GRANTS

THE ROYAL SOCIETY
SCIENTIFIC PUBLICATIONS
GRANT

Applications for grants from the first allotment of the Scientific Publications Grant for 1979 should be made not later than May 22, 1979 on forms obtainable from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG. The grant is for the assistance of scientific publications in the United Kingdom, particularly, but not exclusively, those of scientific societies and institutions.

Applications are receivable only from societies and institutions in the United Kingdom and individuals of British nationality. 380(N)

COURSES

IMPERIAL COLLEGE
M.Sc. IN ATMOSPHERIC
PHYSICS AND DYNAMICS

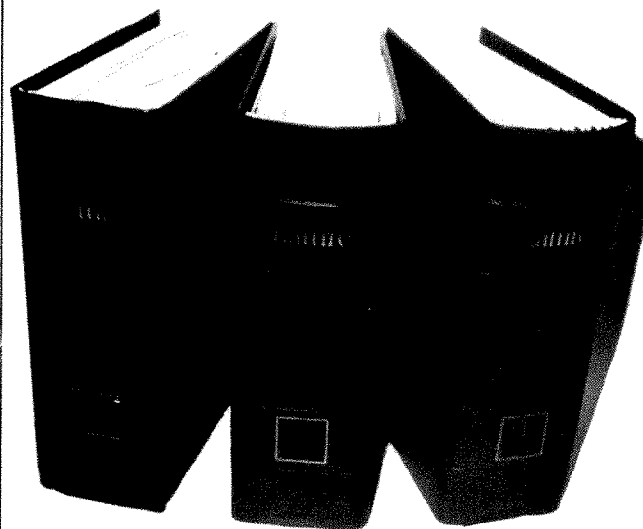
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Further enquiries to: Dr J. S. A. Green, Atmospheric Physics Group, Imperial College, London SW7 2BZ. 390(D)

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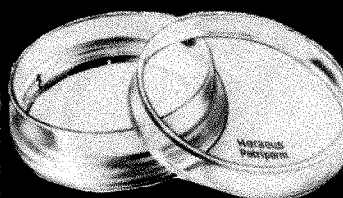
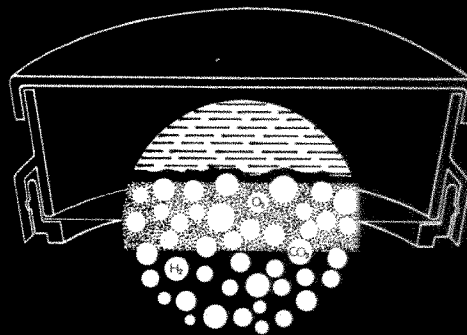
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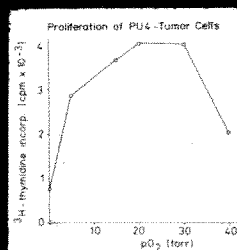


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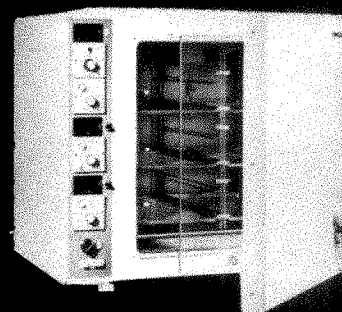
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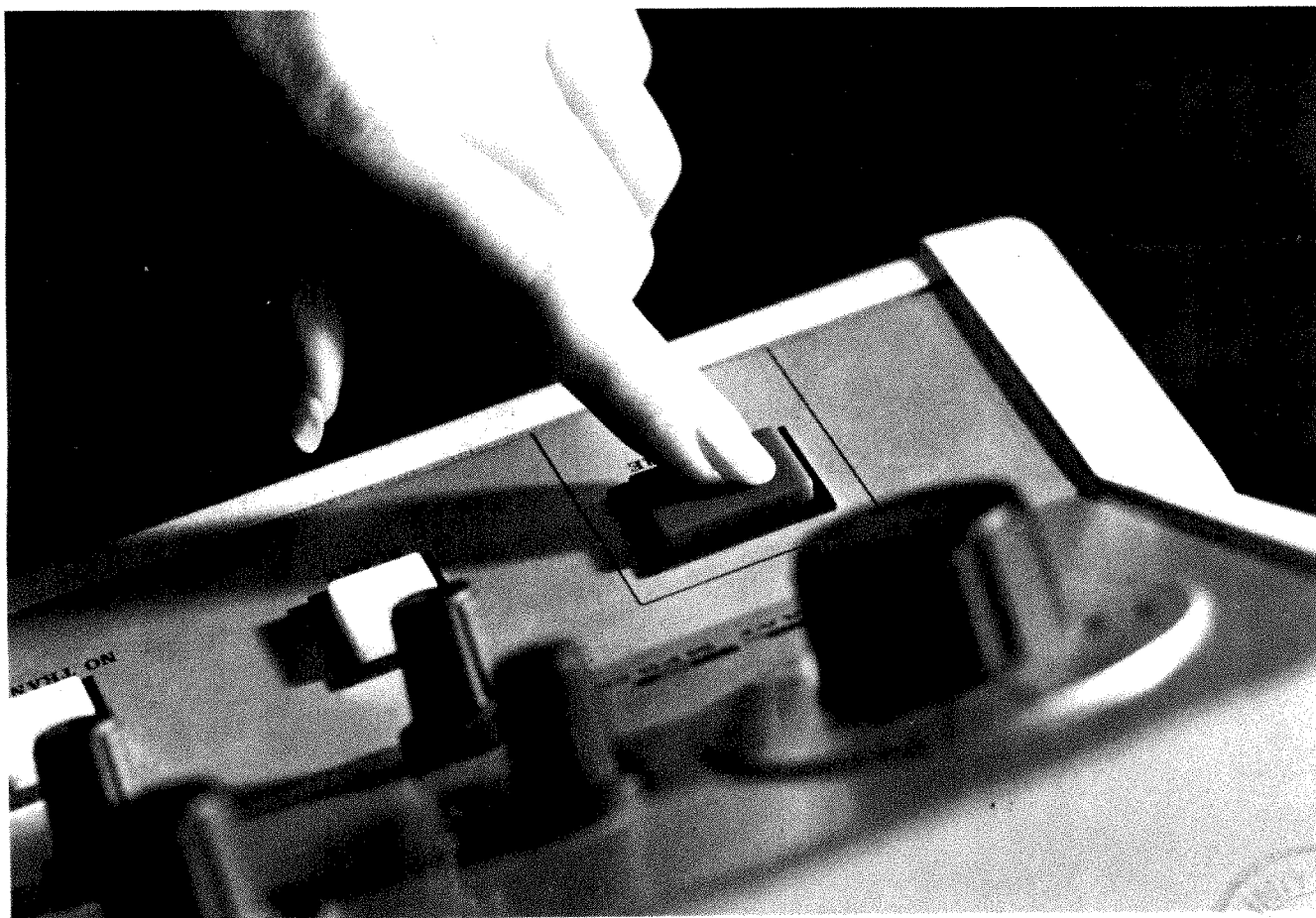


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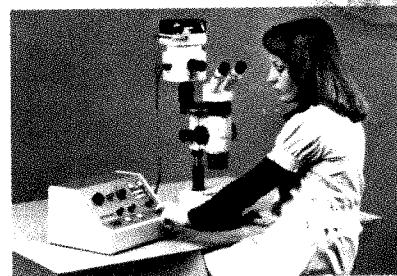
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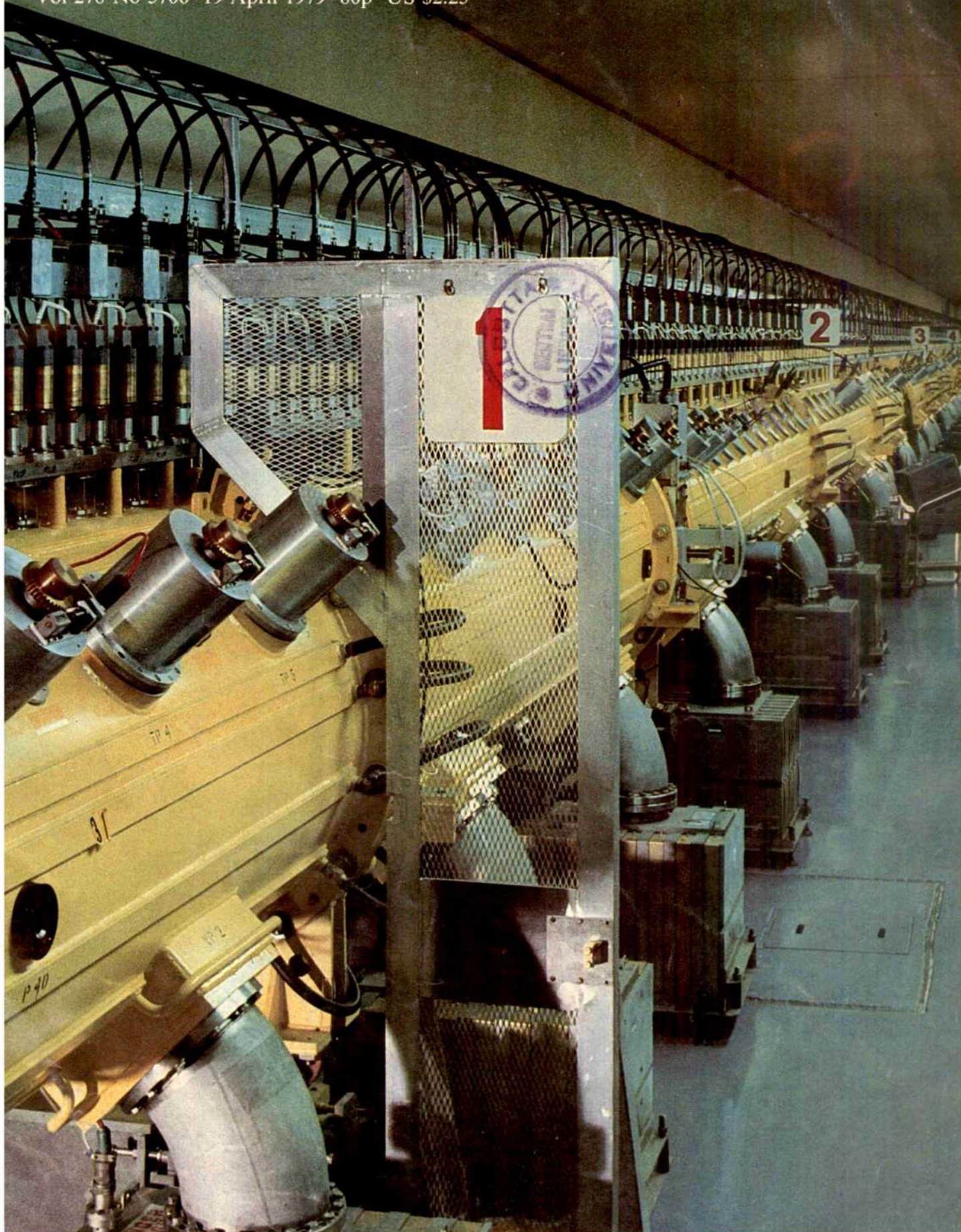
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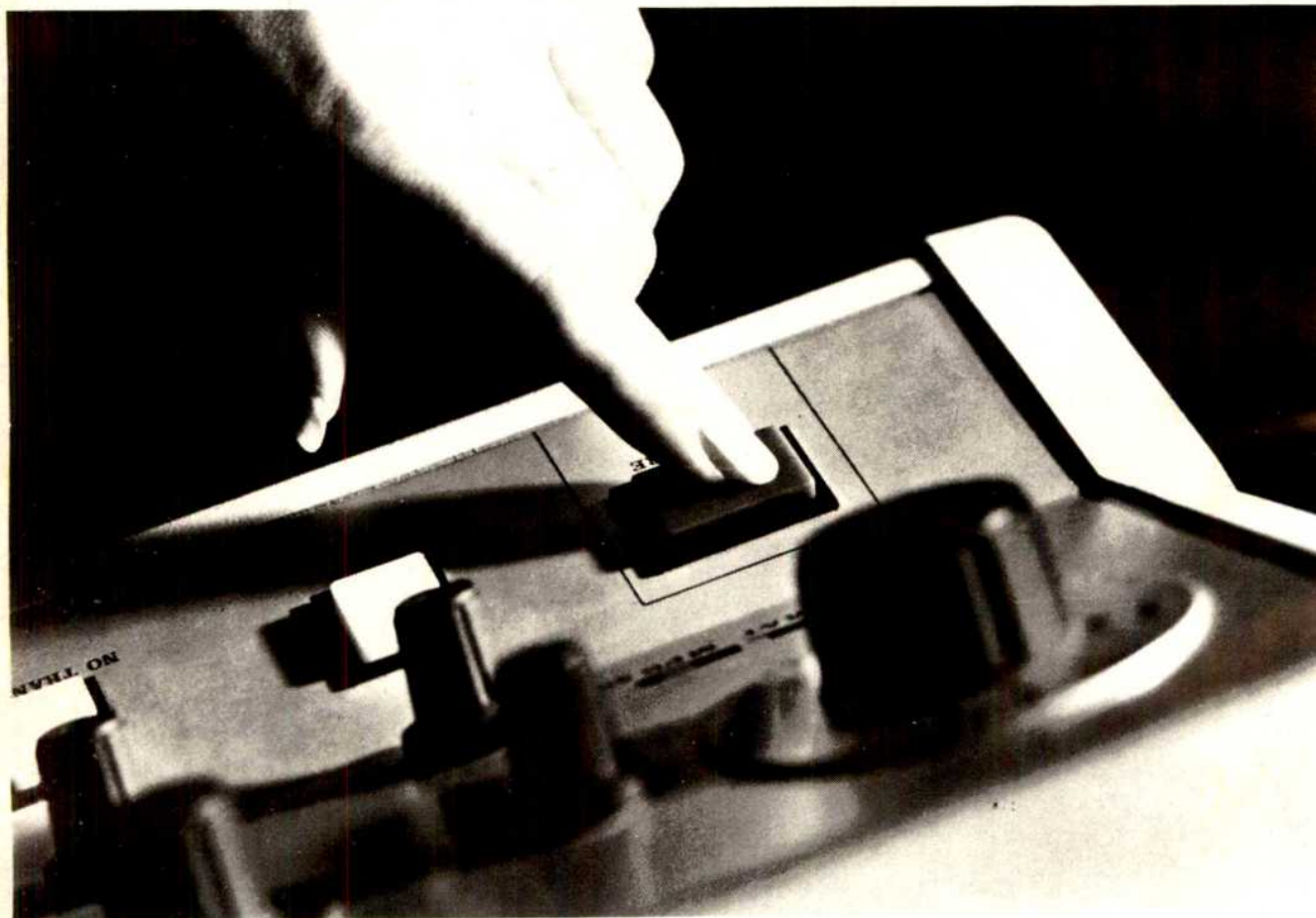
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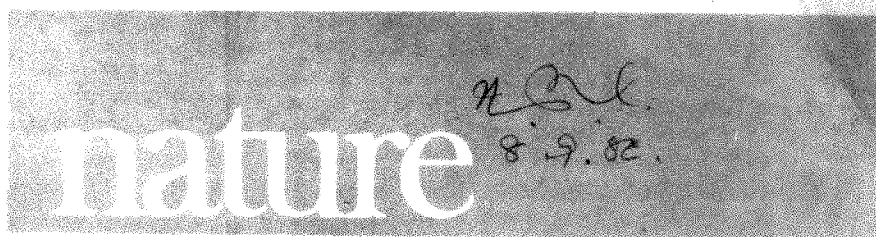
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Brookhaven's 200 MeV linear accelerator. Pierre Grand discusses on page 693 the use of such accelerators in the nuclear fuel cycle. They could be used in place of fast breeders to convert uranium-238 to plutonium.

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From 1 May 1979 the recently opened New York office of *Nature* will take over from the Washington office the responsibility for handling manuscripts. Manuscripts which would in the past have been submitted to Washington should now be sent to New York.

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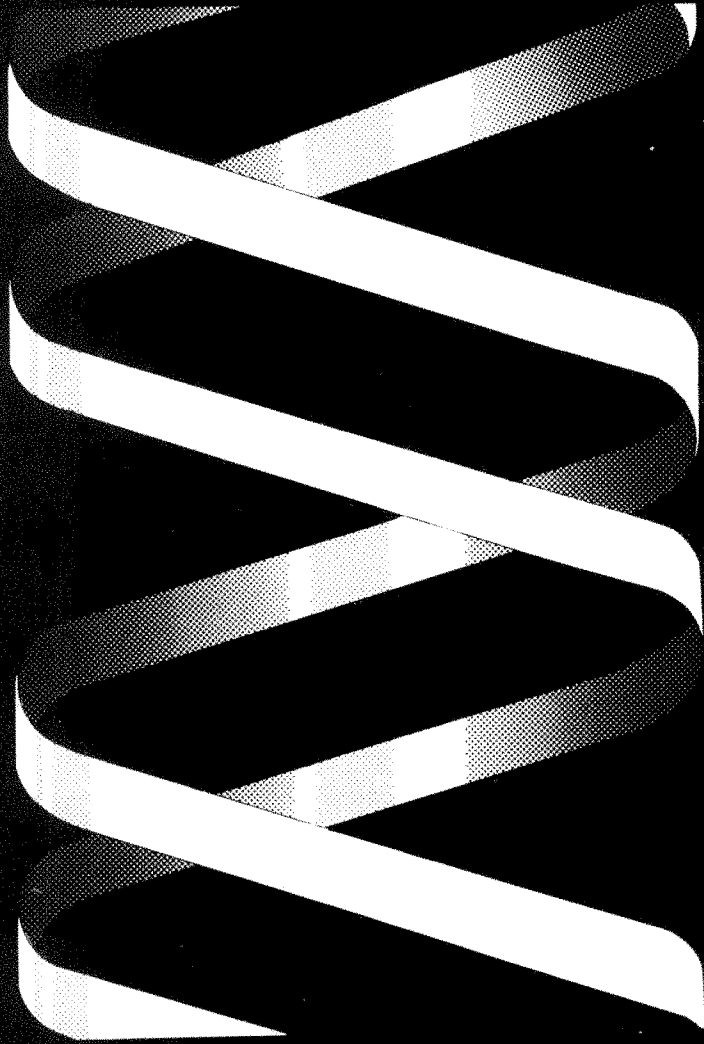
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nature

19 April 1979

The voice of the people—which one?

ONE of the unmistakable trends of the 1970s has been for the question of risk—its measurement, perception and acceptance—to move progressively away from being a purely technological problem and towards the centre of the political stage. Scientists and technologists may have been able to come up with numerical values and probabilities and indeed as a result of their work immense strides have been made in raising safety standards. But a never-ending succession of accidents, major and minor, continues to remind us that the risks of a technological world, be they from unsafe drugs, faulty nuclear reactors, wrecked supertankers or whatever, come largely from human and institutional frailty and not from imperfect materials or inappropriate differential equations. Small wonder then that risk is now a political issue.

Many scientists and technologists are bitter about the way apparently technical issues have been taken over by social scientists, bureaucrats and politicians. It would be wrong totally to dismiss this bitterness as professional narrow-minded jealousy without giving some thought to whether an embittered profession will attract the ablest young people to it. Nevertheless, the problematical nature of the interaction of humans with technology, as controller as well as potential sufferer inevitably means that wider circles of opinion are now being sounded out, and that even wider circles may have to be consulted in the future. But what exactly does public participation in the handling of technological risk questions mean? This was one of the important issues raised at a recent seminar in Berlin organised by the European Economic Community and the European Committee on Research and Development (CERD).

If decisions are not simply to be handed down from remote technocratic elites, responsible only to themselves, it is all but inevitable that there will be dissension, some of it vigorous. And as Dorothy Nelkin of Cornell put it, "resolving conflicts is much like seeking 100% risk-free technology—hardly a feasible goal". What has to be done is that all sides must see that their case is at least taken into account by whoever makes the decision, and they must be satisfied that the choice of decision-maker and the form of inquiry does not prejudice the case. Apparently harmless criteria—yet it was striking how many unsatisfactory cases could be cited where the decision was a foregone conclusion or where objectors found themselves seriously hindered.

That said, however, a striking theme to emerge from the seminar was that objectors to technological developments on grounds of unreasonable risk should be smoked out on what they really stand for. This, it was suggested, could be done in two ways. First, they should be asked whether they were aware of the consequences of saying no to particular projects, and had thought them through as carefully as they had thought through their objections. Second, many objectors to technological projects do so not because they disapprove of one particular proposal at one particular place, but because such projects go against their total sense of values. In the words of Jerry Ravetz of Leeds "we must recognise that we can no longer follow the politics of 'economism', where quantitative differences can be negotiated between opposing sides; rather we must accept some features of the politics of 'ethnicity', where deep differences of values must be confronted and then used for the mutual education of both sides".

It is clear that there is absolutely no agreement about just how widely views on risk should be canvassed. When three out of four drivers, given a chance, will spurn the use of a seat-belt for no apparent reason whatever, it is obvious that thinking by the man-in-the-street about risk-taking is immensely complex, not to say irrational. So how do we represent—indeed who represents—this man-in-the-street, and is this element of irrationality to be despised or to be allowed for? The problem is that we may be in danger of replacing a technological elite by yet another elite which thinks it knows what the public wants or ought to want. The long-term answer, and this should be regarded as a long-term problem not just here for a few years, must surely lie in much more effective education at all levels in thinking about technology and risk. Few technologists are taught to think about social matters. Few social scientists are taught to think about technology in depth. It is here that progress must be made.

The European Economic Community could feel pleased with this seminar. It is a subject peculiarly appropriate to the community. Individual nations cannot at present muster the resources to take an omnidirectional view of the subject. Yet each is having to cope with a set of broadly similar problems in its own distinctive way. What more valuable than to let these varying patterns rub off on each other under EEC auspices. □

Anti-war movements—heading for a 60s revival?

David Dickson reports from California on how critics of US military policy are beginning to tap the grass-roots energy of the anti-nuclear movement

It might have become a formula for instant nostalgia. But when the film version of the 1960s anti-war musical *Hair* opened in California a few weeks ago, it was reviewed—and widely praised—at face value, with few claiming that its sentiments belonged to the previous decade. And taken with the success of two other anti-war films in last week's Oscar awards, the popularity of *Hair* suggests that these sentiments remain close to the surface.

In Washington, concern about the security of Middle East oil supplies is already generating the type of discussion about 'protecting natural interests' that preceded the military intervention in Vietnam. And a question now being debated on many campuses throughout the US is whether opposition to a growing military budget and to an increasingly hawkish administration is likely to coalesce into something comparable to the anti-war movements of the 1960s.

To judge from the situation in California, such a coalescence may not be too far away. At present, various groups are concentrating on a number of different objectives, some only distantly related to one another. But growing concern about US military policy in general—together with unease about nuclear energy in all its forms after the Harrisburg accident—are beginning to draw these groups together.

Some of the more recent events include:

- At the University of California's Berkeley campus, a senate policy committee has recommended that the university sever all links with its Lawrence Livermore and Los Alamos laboratories, claiming that the large proportion of the activities of the two laboratories devoted to weapons research make the involvement of the university—currently responsible for the management of each—"totally inappropriate".

- A number of Californian and anti-nuclear groups are expected to take part in demonstrations outside the university demanding that the laboratory's skills and resources be turned "totally towards peaceful research" as a way of putting the cap on the arms race.

- At Stanford University, a group of students is mounting a campaign to oppose legislation being discussed in Washington for revising the draft. One of the first speakers to address a

university meeting on the issue was David Harris, student president in 1966-67 who spent 20 months in prison for draft evasion.

- A number of requests has been received by the American Association for the Advancement of Science to place public discussion of the US military budget and its implications on the agenda of next year's annual meeting in San Francisco.

- A Berkeley group has published, through the Washington-based Institute for Policy Studies, a pamphlet on "resurgent militarism", claiming that "all struggles for social progress . . . must confront the new militarism", and that otherwise "the likelihood of nuclear war will surely increase".

Such concerns are by no means confined to the West Coast. At a national level, church and religious leaders have, in particular, put military spending at the top of their agenda of items of social concern, both on a national and an international basis.

Similarly anti-nuclear groups, once concerned primarily with the safety parameters of energy policies, are making the links to military policy. "If you've done your homework you know you cannot separate the two issues" says Marta Daniels of the Trident Conversion Campaign, the sponsors of a mass demonstration in Groton, Connecticut two weekends ago at which 229 persons were arrested for trying to block shipyard entrances to guests arriving for the launch of a new nuclear submarine.

But it seems to be on the West Coast, with its high concentration of both defence-related industries and veterans of the anti-Vietnam campaigns, where the linkages are being most tightly drawn.

One focus in particular has been the relationship between the University



Flashback to the 1960s: anti-war protest fills the street.

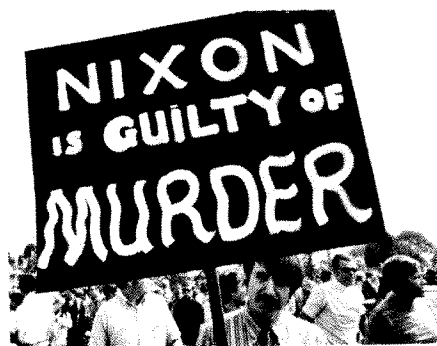
of California and the two weapons laboratories which it manages for the Department of Energy. This link has long been a source of contention among university faculty and students and moves to sever it in recent years have been given a boost by a broader anti-militarism campaign.

Supporters of the current arrangement argue that being run by the university means the laboratories can both maintain the high quality of their scientific research, and remain free of industry or Washington-dominated influences to express their views. "The university connection is the best way to serve the people" says Ed Hammel of the Los Alamos laboratory.

Critics point out that every nuclear weapon in the US armoury was conceived, developed and tested by the two laboratories. "The laboratories are not academic institutions, they epitomise 'science with a purpose'", says one long-time opponent, Professor Charles Schwarz of Berkeley's Department of Physics.

Ironically, those on both sides of the debate agree that, despite wide disparities in the technology, concern over the health hazards of nuclear power have linked the energy and weapons issues together in the public's mind.

Dr David S. Saxon, President of the University of California, sees public attitudes on both as a source of concern—but a situation for which scientists must share part of the blame. "The present unreasoning fear within this country and abroad of anything associated with the word nuclear is a reflection of an almost inexcusable failure within the scientific and pro-



Now nuclear power is seen as the guilty party.

fessional community involved in nuclear energy", he said recently.

"There has been a consistent unwillingness to react in responsible ways to the questions that members of the public ask. In too many instances the official attitude has been overbearing, arrogant and intolerant."

Dr Schwarz, on the other hand, feels that the type of concerns raised by the Harrisburg incident may—legitimately—spill over to what he considers a far greater health issue, namely the spread of nuclear weapons. "We have to show that the same institutions that run the nuclear power programme also run the nuclear weapons programme—and that we have been told the same lies. I am not against nuclear power in itself, but I am opposed to it because, as with nuclear weapons, I don't trust the people in control of it at the present time".

The university has itself been reconsidering its relationship to the two laboratories. A committee established in 1977 under the chairmanship of Dr William Gerberding, Chancellor of the University of Illinois, issued a report last year, suggesting that the university should continue to manage the laboratories, but through the operation of a powerful board appointed by the university, which would play a much closer role in overseeing the operation of the two laboratories than under present arrangements.

In an attempt to counteract charges that even under a board such as that proposed by the Gerberding committee, there would be little public discussion of the laboratories' activities, one university vice-president, Dr William Fretter, has proposed as an alternative arrangement the establishment of an advisory council to the university president, containing scientists, faculty members and students.

"I did not find the Gerberding proposals realistic, since the programmes of the laboratories—especially the weapons laboratories—are determined in Washington by the federal government, and it seemed unrealistic that these could be influenced substantially by a university committee", Dr Fretter said last week.

He argues that although the committee which he has proposed would only be an advisory one, it should

operate in public "since both the university community and the general community need more information on what goes on in the laboratories". Critics such as Dr Schwarz, however, maintain that since the committee would not deal with the laboratories' classified activities, and since it would have no executive responsibility—which would remain in the hands of President Saxon—it would lack any real teeth, and hence be little improvement on the current situation.

Debate within the university on the future relationship to the laboratories has been put temporarily on ice following the announcement in December that the Department of Energy was itself planning a review of the current arrangements. The department's report is due to be presented to Energy Secretary James Schlesinger next month, and is awaited with interest (particularly since, although being prepared by a subcommittee, the report is officially to be submitted by DOE's Energy Research Advisory Board—and this contains a number of individuals from the public interest community as specialists in alternative energy sources).

But if there is a pause in the official debate, grass-roots opposition is continuing to grow. The future of the weapons laboratories was among the issues raised at a mass rally held in San Francisco two weeks ago to protest at the opening of the Diablo Canyon nuclear power plant in the state, a meeting held under the shadow of the Harrisburg incident. And organisers of next month's demonstration at Livermore predict many thousands will turn up for a mass march, rally and 'peace conversion fair', with groups fasting at the laboratory gates, and others walking to the laboratory from all over California.

To judge from reactions elsewhere such activities are unlikely to be warmly welcomed by the university authorities. Last week a film crew was thrown out of Harvard University while trying to make a film about the 1960s protest movement. The film's director, Mr Jim Zinneman, admitted that a three-story mural showing some American soldiers trampling on Vietnamese "upset some faculty members and alumni". Nostalgia is not what is used to be. □

ISABELLE team denies setback

RUMOURS that the US ISABELLE project is having difficulties producing superconducting magnets for its 400 GeV proton-proton storage rings were denied last week by Dr Jim Sanford, the project head. In an interview with *Nature*, he said there were no problems with quadrupole magnet production, although they had run into difficulties with the dipole magnets.

Overall, progress was "90% satisfactory". "We are having problems in meeting the ultimate field of 5 teslas. We have reached 5 teslas in a model but not in the production prototypes. They are operating now at around 4½ teslas," he said.

The dipole magnets were first designed to operate at 4 teslas. The team made 13 model magnets and most of these exceeded this field. Recently the design was modified to operate at 5 teslas, and one model reached 5.1 teslas. However, when this was put into production, the magnets only achieved a field of 4-4½ teslas, Dr Sanford said. "We are not entirely sure what is causing this problem. Maybe it is the materials in the superconducting braid. We wanted to have a bigger margin than 5.1 teslas and so we added more niobium-titanium in the production, and in the process we may have lost some of the stability of the materials."

Production of the superconducting magnets is the major technological challenge of the ISABELLE project. The project has already had one setback—the US Department of Energy will only fund ISABELLE over seven years and not the five years the scientists had hoped for. However, Dr Sanford is confident the magnet problem will be solved by the summer so the contract for production can be issued by the end of the year. This will keep ISABELLE on schedule, and may even provide an argument for advancing to a 5-year target, said Dr Sanford.

In Europe, ISABELLE's competition in the race to produce the heavy intermediate vector boson, the CERN proton-antiproton experiment, is expected to produce hard evidence by 1982, said a CERN spokesman. □

UK and Sweden attack lead poisoning studies

A NEW US report on lead pollution published in *The New England Journal of Medicine* by a team of researchers from Harvard Medical School and the Children's Hospital in Boston has not been openly welcomed in Britain.

The study indicates that currently accepted lead levels may be set far too high to be safe for children in urban

areas. The team, headed by Dr Herbert Needleman, examined lead levels in children's milk teeth, which is considered to be a better indicator of long term exposure than blood lead levels.

Starting with a sample of 3,329 children, two samples matched for social and environmental variables including social class, mother's age and

parental IQ were constructed. The 58 high lead children (20ppm in dentine) and the 100 low lead children (less than 10ppm) were given standard psychological and mental tests. (The mean blood lead levels in the two groups were 355 µg/l and 233 µg/l, both of which are well within the normal range for children in UK urban areas.)

The results showed that the high lead children performed less well on all the tests including reaction time, word processing and standard IQ tests.

Professor Pat Lawther of St Bartholomew's Medical School in London and chairman of a Department of Health and Social Security working party on lead pollution in the UK calls the Needleman study "the result of very careful work" but says that the statistics "have to be gone into in great detail" before he could evaluate it. "There are 39 variables subject to regression analysis, and besides, behavioural studies are very difficult" said Professor Lawther.

According to Anthony Tucker, science correspondent of *The Guardian* newspaper, the DHSS working party has been primarily concerned with criticising earlier studies that showed lead induced behavioural changes in children in the UK. "But since no studies of this standard or scale have been carried out in Britain or elsewhere it is difficult to see how its findings can be refuted," writes Tucker.

Meanwhile one of Britain's specialists

on lead whose work is supported by Needleman's study, Professor Derek Bryce-Smith of the University of Reading, was subjected to some extremely unprofessional behaviour when he visited Stockholm recently to take part in a panel debate on lead and children. His visit followed a minor furor caused by the publication (in the journal *Ambio*) of an article which he co-authored, alleging that body lead levels considered normal in children actually appear to be pathogenic.

The article had prompted denials from two Swedish researchers, one of whom—Professor Åke Swensson—agreed to take part in the debate. In deference to Bryce-Smith, the Royal Academy of Sciences, which arranged the debate, announced that it should be held in English. This was duly printed on the programme and all the Swedes complied—except one. Docent Carl-Johan Göthe, ignoring the chairman's admonition and in spite of the fact that there was no official interpretation provided, announced that he would speak Swedish. He then proceeded to attack Professor Bryce-Smith, coming to the

very edge of personal insult. A little later he answered a question in English.

But Bryce-Smith himself was more disturbed by the behaviour of Professor Magnus Piscator, the other critic of the *Ambio* article. Piscator published his critique in a Swedish-language medical journal, accusing Bryce-Smith—among other things—of distorting information in some of his reference material. He made no attempt to contact Bryce-Smith to make his charges in English, and even refused to participate in the panel debate, saying it was stacked with people sharing Bryce-Smith's views—which was not the case.

"The allegation of distortion is untrue and libellous as far as we are concerned", says Bryce-Smith. "Piscator's article was extremely discourteous and unprofessional. His behaviour is very irresponsible and not in the best traditions of science."

Professor Bryce-Smith was not impressed, either, with the level of the debate. "These people do not seem to be aware of recent literature on the subject", he said, citing the Needleman paper. □

Soviet science boss refused Academy membership

SERGEI P. Trapeznikov, the Soviet top Party boss for science, has been refused full membership of the Soviet Academy of Sciences. So far as is known, this is the first time that the academy has ever refused an application from a member of the Central Committee of the Communist Party of the Soviet Union.

Trapeznikov is chairman of the Science Commission of the Supreme Soviet, and as such, formally has both the State Committee for Science and Technology and the Academy of Sciences under his jurisdiction. His appointment, however, was made on political grounds, not scientific. He has the reputation of a hardliner, and during the 1960s vigorously opposed the economic reforms proposed by Professor E. G. Liberman, which were aimed at loosening the Party's hold over the economy.

Since his appointment as Chairman of the Science Commission in 1968, Trapeznikov has tried several times to get elected to the academy. In 1976, he finally achieved the rank of "Corresponding Member" (i.e. Associate Member). Election as Corresponding Member, however, does not require a vote from the full General Assembly of the Academy, but only confirmation by the general assembly of a decision already made by the relevant section.

Applications for full membership, however, must be discussed by the general assembly, and are then decided

by secret ballot—the academy is the last remaining body in the Soviet Union to exercise this method of voting. It is due to this right of secret ballot that Academician Andrei D. Sakharov remains a member of the academy, in spite of several attempts to have him ejected.

Sakharov, indeed, took part in the debate on Trapeznikov's candidacy, stating that the latter's works on the collectivisation of agriculture were not of sufficient scientific value to qualify him for admission. Trapeznikov was rejected by a majority of 212 to 137.

Regarding these figures, it is interesting that Mark Popovskii, a journalist who emigrated from the Soviet Union in 1977 claimed in his book *Controlled Science*, that secret ballot notwithstanding, there are certain members of the academy who would never dare vote against the election of the chairman of the Science Commission. Discussing just such an election as an example of the degree of "humility" the academy shows towards the Party, Popovskii states that the Chairman of the Science Commission (i.e. Trapeznikov) has within his competence the appointment of the directors of scientific institutes, "one of the most longed-for jobs in the country". Secret ballot notwithstanding, Popovskii suggests that in such an election, although the "rank and file" academicians can "play their game of democracy", those who hold the post of director of an

institute simply would not dare.

Popovskii also relates a number of other cases when persons of political status but insufficient scientific quality have been turned down by the academy. These include the present Minister of Education, V. P. Elyutin, and the Minister of Health B. V. Petrovskii (The latter has in fact good medical qualifications, but medical personnel, however distinguished are not admitted to the Academy of Sciences—they must apply to the less prestigious Academy of Medical Sciences).

Some years back, however, the Academy of Sciences seems to have been more complaisant—during his term as Minister of Geology, Aleksandr V. Fedorenko managed to become a Corresponding Member (1953) and then a full Member (1966).

Why though should Trapeznikov and other politicians whose work verges on science be so keen for election to the academy? Is it simply a desire for increased status? Possibly. However, membership of the academy brings more than status; it includes a generous salary, and a number of valuable fringe benefits in housing and the like. Moreover, these are held, effectively, for life. Expulsion from the academy is virtually impossible, although it is reported that during the last few months the academy's statutes have been amended to ensure that anyone deprived of his Soviet citizenship would be automatically expelled.

Vera Rich

Czechoslovakia strengthens science and technology

LAST year, Czechoslovakia embarked on what was described as a "complex experiment in effectiveness and quality control"—which to Western observers seemed virtually a reform of the economy—save that "economic reform", to the Czechs, is an expression tainted by memories of the Dubcek regime. Last month, the meeting of the Czechoslovak Federal Assembly carried the tendency further, by virtually rejecting the figures for the next Five Year Plan—an event unprecedented in the history of the Czechoslovak Socialist Republic.

One change associated with the "complex experiment" was the re-organisation of the pay structure for scientific and tutorial staff at universities and other higher educational institutions. Addressing the Federal Assembly last month, Prime Minister Lubomir Strougal observed: "The salary conditions of scientific and tutorial staff of the higher educational institutes [polytechnics and universities] were regulated with effect from 1 October, 1978. We believe this, however, to be only one partial step designed to raise the interest of specialists from scientific and research institutes [which do no teaching] and from practical places of work in taking up jobs in higher educational institutes."

Although details of the new "con-

ditions" are not forthcoming, the trend accords with the general tenor of Strougal's speech and the other discussions in the General Assembly.

Czechoslovakia is the most reluctant member of the Comecon bloc when it comes to the purchase of foreign technology or know-how; the economic proposals outlined last month envisaged a cut-back in imports of all kinds and a greater dependence on home resources.

Officially, this change was linked to the word: energy situation. Since September, 1976, when the Soviet Union suddenly increased oil prices to Comecon, Czechoslovakia has had a growing energy problem, which has been only partially met by the staggering of working hours. Strougal's speech contained six major proposals in the energy sector: expansion of capital expenditure on home fuel and energy production, a crash programme for the development of the North Bohemian lignite reserves, intensified research into the "complex of problems" ranging from investment to social welfare) associated with plan fulfilment in the deep mines of the Ostrava-Karvina area, an extension of nuclear generating capacity to 3,500 MW by 1978 and 8,000-10,000 MW by 1990, further participation in transit programmes, allowing "various forms

of energy" (eg natural gas) from the Soviet Union to cross Czechoslovak territory and the implementation of fuel-saving programmes, aimed at saving some 20m tonnes of fuel per year.

Regarding this energy programme, Strougal noted that the total savings envisaged would not be possible immediately, but that substantial progress "must be registered in the remaining years of the current Five Year Plan, and especially in the next Five Year Plan". In his main speech the Prime Minister did not, of course, make any reference to the accidents which the Charter 77 movement allege took place at the Jaslovské Bohunice nuclear power station in January 1976 and February 1977.

It was left to a relatively minor member of the Assembly, Deputy Dana Kancirova, to raise this inauspicious subject. Even she approached it in a roundabout manner, asking for an explanation concerning the current Austrian press campaign about the safety of nuclear power stations. Mr Strougal answered, somewhat airily, that "our present and planned nuclear power stations are of the Voronezh type, different from the one at Zwentendorf". The alleged fatal accidents at Jaslovské Bohunice, he added, were simply "a fabrication of bourgeois propaganda". **Vera Rich**

Islamic scientists prepare for UNCSTD

Ziauddin Sardar discusses the Muslim countries' views on development and the role of Islamic science

THE Islamic Secretariat is to submit a position paper, representing the views of its 42 member countries to the United Nations Conference on Science and Technology for Development in Vienna next August. The paper is the final outcome of a conference held in Jeddah on 17-21 March and has at least two novel features: it is the first effort by the Islamic bloc to represent an Islamic viewpoint on science and technology at the level of the United Nations and it forces the Muslim countries to recognise that there can be no effective development without the evolution of indigenous science and technology.

Implicit in the first point is the assumption that there is an Islamic alternative to the conventional practice of science and technology. That the Jeddah conference could not go beyond vague statements of this alternative is not surprising: the Muslim civilisation has been dormant for over three hundred years and current ideas on Islamic

science and Muslim technology emerged only at the turn of the decade. Clearly there is a great deal of work and much analysis to be done before anything concrete emerges. The Islamic Secretariat paper provides recognition to these ideas and should give them an international hearing.

The second feature is important because, when the paper is endorsed by the Tenth Islamic Conference of Foreign Ministers in May, the Muslim countries will become actively committed to the establishment of ministries and/or other high-powered bodies for science and technology. This may not appear to be much of an achievement from the perspective of the industrialised countries, but only half a dozen of the 45-odd Muslim countries currently have ministries and other national bodies devoted entirely to science and technology.

Billed as "The First Muslim Science Conference", the Jeddah meeting was convened by the Ninth Islamic Confer-

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



الإسلامية العالمية للعلماء والتقنيين

Emblem of the Islamic secretariat

ence of Foreign Ministers, held in April 1978. The science conference itself was a shambles. Of the thirty-odd delegates who attended only seven could be classified as scientists; the rest were all career diplomats. Although it was never openly admitted, the dominant presence of the civil servants was clearly embarrassing to the scientists who were eager to produce a powerful position paper.

What is surprising is that the scientists managed to carry the conference and produce a coherent and

reasonably well argued paper. They see UNCSTD as the first step towards the establishment of a "new world order in science and technology". Dr A Abou El-Azm, who is tipped to be the first director of IFSTD, argued for a strong action-orientated paper that does not undermine the position of the Group of 77, and Dr Ali Mohammed Kamel, a consultant to the Arab League Educational, Cultural and Scientific Organisation (ALESCO) repeatedly asked for the paper to be connected with the call for a New International Economic Order.

Dr Waqar Husaini of the Civil Engineering Department, King Abdul Aziz University, was more concerned with emphasising the issues of ethics and morality of science. "Islamic development", he argued, "is concerned with individual and self-purification and with growth that does not transcend the ethical boundaries of Islam. Science and technology do not have an independent existence under Islam. To the contrary, they are subject to the value principles of Islam"—a point that was also echoed by other delegates.

Dr Yalgin Tuncer of Middle East Technical University was not very optimistic about the outcome of UNCSTD and argued that the Islamic position paper should be addressed not so much to UNCSTD but more to Muslim countries. "We should have a crystallised model of Islamic science and technology and its role in development; and we should offer this model to Muslim countries for study and application", he said. It was finally agreed that the paper will address the UNCSTD meeting, the Muslim countries and IFSTD.

The paper airs many concerns common to the developing countries; the lack of awareness in the Muslim world of the key role of science and technology in development; the quality and quantity of available manpower; the brain drain; the exploitative practices of western governments and multinational corporations in the field of technology transfer; lack of information on the choice of available technology; and the adverse repercussion of the politics of aid.

Unfortunately, examination of these issues is devoid of self-analysis. Placing the shortcomings squarely at the door of the West has become a common practice with the developing countries. The Islamic Secretariat's paper could have provided a lead by closely examining the attitudes and outlooks that prevent science and technology from taking indigenous roots in the Muslim world.

Also absent from the paper is an awareness of the ideological stance of science. The Islamic Secretariat's paper asserts that "there are no ideological obstacles to a vigorous introduction of science and technology in Muslim coun-

tries". This is not altogether true. Traditional Muslim intellectuals do not see science as an entirely value-free, apolitical activity; and their experiences have confirmed their views. The Islamic Secretariat's paper largely overlooks this viewpoint and asserts that problems only exist in the realm of transfer and adoption of technology.

However, a slight awareness that the situation may be more complex appears in the statement that "it is not always possible to separate science and technology from the philosophical background and social forms attached to them in their country of origin". Hence, it is argued, it is necessary to develop an Islamic philosophy of science. The paper states: "Science is a common human heritage and almost all nations have contributed to it at one time or another. Muslims, in particular,



have made some of the most decisive contributions. . . . Hence it is an absolute fallacy to see science and technology as the sole product of Western culture". But there is an inductive leap that leads the paper to conclude that it is therefore possible to fit conventional practices of science and technology into Islamic social and ideological systems. The first step towards this goal is said to be the popularisation of education, training and the professional practice of science and technology. Here there is an acute shortage of good technical translators and qualified scientists who can teach in indigenous languages. Those who can teach do so only in a foreign language, for they themselves were either trained abroad, or by foreigners locally. Scientists and technologists trained abroad, particularly, present a tremendous resistance against popularisation.

The paper rightly blames the frustration of local scientists on the *en masse* transfer and purchase of western technology. This is why the Muslim countries "have not developed locally effective institutional mechanisms and expertise to ensure performance of various functions for assessment and

selection of appropriate technologies and for successful implementation and adoption of these technologies in the productive sectors". But in turn, this technological weakness is the main reason for the failure of technology transfer and for the technological dependence of the Muslim world. The prescription for shaking off this dependence asks Muslim governments to individually and collectively: formulate their policies for choice, transfer, and development of technology; build up an infrastructure for creating and using technology; and develop competent manpower.

Although it is not specifically stated, the paper assumes that at present Muslim governments do not have science and technology policies worthy of the name. A comprehensive science and technology policy is seen as a "fundamental pre-requisite for self-reliance and self-development". Further, this policy "should be integrated with the Islamic socio-economic objectives and policy and be consistent with its development priorities . . ."

The best way to turn the brain drain into the brain gain, says the paper, is to introduce and strengthen the internal "pull-factors". Such "pull-factors" could include "an Islamic socio-political environment, facilities for intellectual growth and professional satisfaction, logistic support at home and place of work that enables the scientists and technologists to be efficient and productive, proper salaries and privileges, merit-based promotion and recognition through a system of rewards, in-service training, continuing professional advancement, visits to advanced countries etc."

This oft-preached solution has one unusual recommendation: the developed countries should be persuaded to adopt legal measures against immigration and employment of skilled Muslims from developing countries without their governments' consent.

Science and technology resources in the Muslim world are scarce. The paper's plea to the Muslim countries to share and pool their resources, therefore, makes a great deal of sense. Many Muslim countries face similar development problems. Obstacles to development in these areas could be eliminated by effective cooperation and joint action programmes. IFSTD, the paper states, will be a catalyst in this process of cooperation and coordination.

Despite its many shortcomings the importance of the Islamic Secretariat's paper for the Muslim world cannot be underestimated. Even if only half of its recommendations are actually followed up by the Muslim countries, it could change the status of science and technology in the Muslim world. And that would be no small achievement. □

Gargamelle may be scrapped: CERN's heavy liquid bubble chamber, Gargamelle, which was the first to detect the weak neutral current and has been in operation since 1970, may be at the end of its life. Last year a leak was detected in the chamber that proved to be easily identifiable and repairable. But the chamber was sent to the factory for further inspection, to check the welded joints, and micro-cracks were discovered in the welds. The cracks are currently under investigation and a decision will be taken on the chamber's future at a meeting on 19 April. Engineers have given negative advice saying that repairs will be very costly, of the same order of magnitude as the cost of building a new chamber. Another consideration is whether the chamber would be in violation of Swiss safety standards. Gargamelle is used as a neutrino detector and its loss would leave only the BEBC heavy liquid chamber for neutrino physics, a considerable cut in detecting power. The loss would come at a particularly inopportune moment, as CERN is to reach 450 GeV this summer, doubling the neutrino flux to the two chambers.

Soviets respond to Harrisburg: Soviet nuclear planners have taken the opportunity of the Harrisburg disaster to defend their own commitment to fast breeders. Within the last few days, Moscow radio has put out two major interviews which, while not mentioning Harrisburg explicitly, are a clear comment on the situation.

In the first, an English-language transmission to North America, Dr Andronik Petrosyants, the Chairman of the USSR State Committee for the Utilization of Atomic Energy, said that although to date the Soviet energy programme had been based mainly on 'conventional reactors' (with experimental fast-breeders at Obninsk, Dmitrovgrad and Shevchenko), long-term plans envisage a change-over to fast-breeders, a field in which Soviet scientists "cooperate with their counterparts in the US". Petrosyants added "Only such reactors will enable the industry to develop sufficiently fast and on a large enough scale". Later he explained that the Soviet applications of fast-breeders, which "are limited by scientifically motivated standards, present no radiation hazards".

In a programme for the home audience, Anatolii Aleksandrov, President of the Academy of Sciences of the USSR described Soviet plans for constructing nuclear power stations in urban residential areas, which should, he claimed, halve the cost of district heating. Plans are also under way, he said, for using power stations as a source of heat to cut the cost of "relatively expensive technological processes such as those involving powder metallurgy".

Orlov told to stop issuing research notes: Yuri Orlov, the Soviet physicist at present serving a 7-year labour-camp sentence for his activities in defence of human rights has been forbidden to include in his fortnightly letters home any mathematical formulae or other material which the camp guards cannot understand. Ironically, his wife received this information about 10 days before *Nature* published extracts from his letters, in which he described his attempts to keep up some form of theoretical research.

Banning of the intellectual activity of dissidents is nothing new in Russia. In 1847, the Ukrainian poet, Shevchenko, was sentenced to 25 years in an army penal battalion, "with the express prohibition of writing or painting", added to his sentence in the Tsar's own handwriting. It is not known at what level the ban on Orlov's intellectual activity originated. Mrs Orlova thinks that it probably originated with the camp guards themselves. However, a friend of the Orlovs told *Nature*, no guard would take any initiative

he did not think would be approved higher up. This friend also pointed out that in Orlov's last "scientific" letter from the camp—a paper on wave probability—he indicated by a periphrasis that he hoped it would be published abroad.

Portugal doubtful about Soviet Atlantis find: Recent claims by Soviet oceanographers that they have discovered traces of a city on the floor of the Atlantic have been treated somewhat sceptically by their Portuguese opposite numbers. After discussing the "finds" with the Soviet team, Dr Marcello Vasconcelos, head of the Portuguese National Institute for Fishing Research, admitted only that "It is undeniable that they have found something". He and his colleagues at the Institute could not give a professional opinion on the reported find, he explained.

Dr Vasconcelos said that the Soviet scientists appeared to be "highly optimistic" about their photographs which, they claim, show ruins of a city some "320-480 km" off the coast of Portugal, in the direction of its "mid-Atlantic island possession, Madeira". Dr Aleksei Aksenov, the head of the Soviet team, claims to have taken eight photographs, showing "vestiges of walls and stairways". Further details, including the depth of the alleged finds, will, according to Dr Aksenov, be announced after further study of the photographs in Moscow. Soviet opinion, he explained, was that the discovered remains could well be the "lost continent of Atlantis".

Soviet oceanographers would appear to be having great success recently in explaining Atlantic "mythology". Last year, another oceanographic expedition reported huge "vortices" in the Bermuda triangle region.

UK proposes major microprocessor research and training programme: The Science Research Council in the UK has adopted the recommendations of a Council panel which call for a major programme of additional academic research to "help the UK get maximum benefit" from microprocessors and programmable electronic technology. The panel makes specific recommendations in the areas of education and training, new computer applications and new computer technology. In education, the panel proposes a centrally coordinated programme of six five-year projects to expand existing commitments such as the National Development Programme in Computer Assisted Instruction. The panel envisages 1,000-2,000 "work stations" that will use the new technology for education and it calls for the appointment of a Programme Director "at an early stage". In addition the panel proposes 50 research studentships per year in computers and microelectronics, conversion courses for "arts graduates (or unemployed teachers)" and additional fundings to attract PhD and MSc students.

In new computer applications the panel emphasises the importance of industrial robotics both for UK application and for export. The panel asks the SRC to appoint a Programme Director to carry out an "in depth study" of the UK situation over the next 12 to 18 months and it recommends the creation of an SRC professorship in this field "in a major location." The Rutherford Laboratory is proposed as a centre for research into increased uses of microprocessors in measurement and instrumentation as well as for research into the next generation of silicon chip design and test automation.

The SRC has appointed an Implementation Group chaired by Professor J. Brown of Imperial College to draw up detailed costs and to liaise with other concerned governmental bodies.

Steady progress on the 'long march' to modernisation

Jen Tsi Yang, professor of chemistry at the University of California, San Francisco, on the revitalisation of science in China

THE People's Republic of China is embarking on a grand programme of Four Modernisations: in agriculture, industry, national defence, and science and technology. An aphorism by the late Premier Chou-En-lai, "Dare to think, dare to say, dare to do! Work hard, work with energy, work with ingenuity!" can serve as a battle cry for what is being called a New Long March (*Xin Chang Zheng*) or even a New Liberation (*Xin Jie Fang*). After a turbulent decade from 1966 to 1976, an openness (within limits, of course) and a new mood of enthusiasm are evident. During a recent visit to China my hosts talked to me uninhibitedly about many subjects, both scientific and more wide-ranging. Amongst them were some horrific stories—for which the now toppled villains were blamed in nearly all my conversations—which are better left for future historians to sift through. My observations on science in China are not intended to be comprehensive, but will indicate the progress being achieved.

Education is the key to the modernisation programme. China is racing to train a vast phalanx of scientists and technologists. The country lost a generation of educated manpower during the Cultural Revolution, starting in 1966. Schools were closed for several years, and the students of universities, colleges and secondary schools went on a rampage while the teachers were subjected to repeated confrontations and inquisitions. Yet these students received promotion every year and "graduated" automatically. Millions of them were later sent into the countryside and still live there. The sudden change in political climate in October 1976 has produced a renaissance fervour for learning. The few cities I visited, including Shanghai, Nanking and Peking, reflect this new atmosphere now sweeping the country. Books on science, technology and primers of foreign languages, especially English and Japanese, are in great demand, and a new English-Chinese dictionary has already sold out. The libraries are once again crowded.

Nationwide college entrance examinations were held in December 1977 and July 1978. The applicants were appraised morally and physically as well as intellectually. No longer, I was assured, are the children and grandchildren of workers, peasants and soldiers automatically favoured for admission. To avoid corruption in the form of backdoor dealings, each appli-

cant was allowed to check his or her score, calculated to the first decimal, against the announced pass mark, which was adjusted geographically to give a balanced distribution of successful applicants. Having cleared this first hurdle, each applicant had a physical checkup and an evaluation of his or her moral character. Both former 'graduates' from the bad years and those fresh from high school competed for this educational opportunity. Many did well in the exams by dint of cramming, either alone, or with the aid of tutors.

The present education system comprises periods of five, three and two years at elementary, junior high and high schools respectively, and four years in college or university (five years in medical college). Currently, 88 institutions are designated as *chong dian* (literally, main-point) or key universities. Initially, about 200,000 freshmen were to be accepted in 1978, but this number has been enlarged, perhaps even doubled, and many universities have set up branches to accommodate the increased enrolment. In addition, the graduate training programme has been restored as a three to four year course, although details are still to be worked out. This has led to the danger that the teaching staff could be overburdened by the combination of increased teaching loads with new activity in research. More serious is the risk that tensions may build up between the educational "haves" and "have-nots", especially those millions sent to the countryside, who do not get as far as the entrance exams.

In the 1950s China adopted the USSR educational model, which is now being modified. At present a comprehensive university is essentially a large college covering both letters and science, while schools of engineering, agriculture and medicine are separate colleges or universities (depending on size). Under the old system, incoming freshmen were required to choose a specialised subject (*zhuan ye*), and the students were denied a spectrum of courses beyond their specialities. China's new plan is to teach basic courses for 2½ to 3 years and the students will have freedom to choose one *zhuan ye* in their final year.

Three major speeches delivered at the National Science Conference in March 1978 have now been published in the "Documents of the National Science Conference" by the People's

Publishing House (*Renmin Chubanshe*). They contain the blueprint for the Four Modernisations. The State Science and Technology Commission, headed by Vice Premier Fang Yi, now administers all the research programmes in the Academia Sinica, universities and colleges under the Ministry of Education, and various industrial ministries.

The Academia Sinica (or the Chinese Academy of Sciences) is traditionally the cornerstone of basic research in the natural sciences. The academy has many branches all over the country and it also administers four universities. (In addition, there are municipal and provincial academies of sciences.) At present the academy has more than 100 research institutes in some 20 cities, Peking having 40 and Shanghai 15. There are about 30,000 researchers and 20,000 administrative and non-technical staff. Research institutes also train their own graduate students. It seems to me that these institutes could benefit from bringing in researchers trained elsewhere, and from having their scientists participate in university and college graduate training. Such cooperation between the institutes and schools is crucial to the crash programme of modernisation. While a certain degree of competition between institutions is an incentive for excellence, Chinese researchers face a real problem in "mountain-topism" (the establishment of rival empires); because of the shortage of scientists, both the universities and research institutes want to keep their best trained graduates. I believe that this is a waste of manpower, and furthermore, the in-breeding does not expose the graduates to a suitably broad spectrum of knowledge. Ideally, the universities should train the graduates and the institutes train the post-doctoral staff (although no degrees are offered in China).

Gone are the anti-intellectual Workers' Propaganda Teams which once controlled the institutions, and established scholars have again been called upon to administer institutions, albeit under party leadership. While the loss of a generation of educated young is most tragic, one should also spare a thought for the frustrated middle-aged researchers who have wasted the most creative decade of their lives. At present China's institutions have many senior faculty and researchers, but not enough well-trained junior investigators under the age of 35. This gap will close when a new crop of graduates joins the rank of their elders, for junior faculty and staff are again being professionally evaluated for pro-



Education is the key to modernisation

motion, after a lapse of more than ten years. They will succeed the ageing generation of Western-trained scholars and will have to shoulder the main burden in the immediate future. I can attest to the fact that the new political climate has brought about a euphoria amongst Chinese educators and researchers, both young and old.

Under the incentive to catch up rapidly, "glamorous" research projects are naturally the most attractive, and I think China must be very careful that fundamental research does not suffer as a result. Genetic engineering is a case in point, for despite considerable enthusiasm for such topical research, the universities and research institutes are really so poorly equipped at present that I think it unwise to seek glory and neglect the basic facilities.

Visiting scientists are welcomed as foreign guests (*waibin*) and given VIP treatment. Such visits expose Chinese researchers to the current state of knowledge and level of thinking in their fields. China-born scientists now living abroad, often called the people's relatives, are especially sought after, for their ability to teach with no language barrier and to advise with the benefit of a common cultural background. Many of us, with close kinfolk in China, have made repeated visits in recent years, for while we are loyal to our adopted countries, we are proud of our heritage and most of us are willing to contribute regardless of ideological differences. Normalisation of Sino-American relations is bound to multiply such scientific exchanges. Not many such visitors can extend their stay for too long without affecting their own research but sabbatical leaves and collaborative research projects may provide longer-term scientific contact. China is now sending abroad hundreds of graduate students for training, experienced researchers to expand their knowledge and numerous scholars to attend international meetings. The impact of these returned scientists and technologists is bound to be immense and not confined to science.

Chinese scientific journals are being revitalised. The bimonthly *Scientia Sinica*, a comprehensive journal for the natural sciences, with some articles on agriculture, medicine and technology, resumed publication in 1973 and is scheduled to be published monthly in 1979. Most, but not all, papers in the Chinese and foreign (predominantly English) editions are identical. Currently, the editor-in-chief is a vice president of the Academia Sinica; he is assisted by an associate editor and an editorial committee of 106 members as well as an editorial staff. Other Chinese journals such as *Acta Biochimica et Biophysica Sinica* have both Chinese and English abstracts. In a departure from the formats of early years, political slogans and quotations are generally absent from scientific texts, and collective (anonymous) authorship by a laboratory may also be declining. *Kexue Tongbao* (*Science Bulletin*), which was also re-launched in 1973, will concentrate on short communications in sciences in 1979. This monthly publication will soon become semi-monthly, and a foreign edition is being planned. Recently, *Nature* has accepted the first paper from the People's Republic of China (Ho, Y.S. & Tsou, C. L. *Nature* **227**, 245-246 (1979)). This could presage a trend for Chinese scientists to summarise their best work in foreign journals. English has now become the language of science, and Chinese students once again study English from the third grade through the college freshman year.

The term 'science management' is frequently heard in China these days. The best use of available manpower and resources, and the financing of the astronomical cost involved, are central to the success of modernisation. In October 1978 Vice Premier Fang Yi toured Western Europe, and Yu Wen, secretary general of the Academia Sinica, headed a delegation to study US science policy-making, peer review, and research and development management. Similar delegations of science managers are also being sent abroad.

But mobilisation of bureaucrats in any country is not easy. In Peking a street sign for pedestrians reads: "one, slow; two, look; three, cross the road," and this rubric has been applied to those cadres who, uncertain in which direction to proceed, hesitate and prefer to cleave to established routines. The New Liberation should be a liberation of thought, for only then can "a hundred flowers blossom!" The unparalleled vicissitudes of the past decade have left many people with a sense of *xin you yu f* (the heart still palpitates).

It is often said that Chinese scientists work at a slow pace. This I believe is no reflection on their energy and competence; they have simply been left with so appalling a legacy of problems that they need time to re-adjust. Shortage of technical assistance and lack of modern facilities must be remedied. One mundane fact is that Chinese intellectuals must spend much time on domestic tasks. Young people could easily spend one third of their working hours on non-scientific chores. It might take a woman one hour or so for the daily grocery-shopping (the majority do not have refrigerators). They wash clothes by hand. Couples with babies too young to go to the nurseries must cope with the baby-sitting problem. In addition, both husband and wife work; this further reduces their working hours on science. I believe that it would be detrimental to the national interest to treat the intellectuals as a superior class, which may become alienated from the masses, but workers would become more efficient if they were relieved of some of the more acute problems of daily life.

China can accomplish almost anything, but not all at once. There is always a danger of trying to do too much too fast when political and economic conditions may not yet be ripe to absorb such drastic changes. After nearly 30 years the people are demanding a modern nation with a higher standard of living, and over 80% of China's population live in rural areas (compared to under 4% in the US), where the struggle for the necessities of life is more arduous than in the cities. The scientists and high officials all want to speed up the modernisation programme, but the bureaucrats may be too slow to make the necessary response. China-watchers estimate that the programme will require several hundred billion dollars over the next eight years. With Chinese oil still in the ground, and little to export for raw materials, China's economic problems can be tremendous. But the Chinese people are pragmatic and determined, and given unity, stability and unanimity of grass-roots support, China should succeed in her long march towards modernisation. □

correspondence

Association of Geoscientists for International Development

SIR,—Your timely editorial "After geodynamics, what?" (15 February, page 503) was read with interest.

From the tone of the editorial, however, it appears that you have not yet become familiar with the Association of Geoscientists for International Development (AGID). Originally conceived at a symposium on the role of the geosciences in international development at the 1972 International Geological Congress in Montreal, AGID has grown to a membership of 1,100 geoscientists in 95 countries in its roughly five years of active existence. In this time it has held several symposia and workshops aimed at fostering technology on problems in developing countries, and, more particularly, at increasing the capabilities of developing country geoscience communities. Such topics as geoscience education in developing countries (Australia, 1976), geochemical exploration in the tropics (Zambia, 1977), and mineral exploration in the tropical rain forest (Venezuela, 1977) have been treated in this manner.

Among AGID's several publications are a quarterly newsletter, a directory of university geoscience departments in developing countries, a bibliography of groundwater reports pertaining to LDC's, and a critique of geoscience-related development aid.

Though it has kept a low profile in many 'developed' nations, AGID is well known in its area of emphasis from the abovementioned workshops and publications, for its surplus literature exchange (enabling organisations with insufficient foreign exchange for purchasing geological publications to obtain such materials that were donated as surplus by others), its appropriate technology referral and other services, and by the activities of its members.

AGID has sometimes been considered mainly as a means of technology transfer. But technology transfer is not AGID's only purpose, nor, certainly, is this an activity unique to AGID. Perhaps its unique challenge is to overcome the lack of emphasis on, and knowledge of, the peculiar geological environments found in developing countries which by themselves currently have limited resources for attacking these problems individually. Basic problems such as the effects of tropical weathering on geochemical dispersion, and of seasonally fluctuating water tables and surface wetness on geophysical exploration in tropical environments have received almost no attention; and engineers are often still caught designing structures around the assumption that a granite will hold a steep slope indefinitely (when the feldspars quickly weather to clay in the humid tropical environment, producing rapid slope failure). For the above reasons the need for an international institute, somewhat similar to the International Centre for Theoretical

Physics at Trieste but located in a tropical environment, is even more critical to the geosciences than to theoretical physics. Although AGID's workshops have attacked some of these problems, the need for a continuous institution directed at improving geoscience capabilities worldwide has been a goal of the Association since its inception. It has just completed an overall plan for such an institute, based on the experiences gained from its workshops and of its members.

The Association has received generous support from the Canadian International Development Agency, the (Canadian) International Development Research Centre, and the governments of Venezuela and Nigeria. Modest contributions have come from other sources, such as the Commonwealth Foundation. Nevertheless, AGID's accomplishments have been achieved on a shoestring budget.

From this you can see that the "secretariat" that you are seeking already exists. It has an advantage over attempts to organise such an effort from other existing institutions in that it is already in existence solely to develop the geoscience community where it most needs development. With its broad international base and its headquarters in Caracas it is less likely to become misdirected in its efforts than might a newly created secretariat in Europe or North America.

AGID heartily welcomes partners in its activities. People interested in its goals, as well as financial support are solicited. It is also interested in assisting in ongoing or planned projects, should such assistance be desired, and consistent with its goals. Correspondence can be addressed to Dr A. Bellizzia, Secretary, Association of Geoscientists for International Development, Ministerio de Minas and Hidrocarburos, Torre Norte, Piso 19, Centro Simon Bolivar, Caracas, Venezuela.

Yours faithfully,

DAVID HASTINGS
Department of Geology and
Geological Engineering,
Michigan Technological University,
Michigan, USA

Nigeria needs scientific leaders

SIR,—I was interested in Lynette Hamblin's report of Professor Ekong's speech at a recent UNESCO symposium (18 January, page 168), and particularly that she considers lack of scientific leadership to be the principal reason for the low scientific output.

At the time when I left the University of Ibadan, I felt that the leadership of that university, in organic chemistry at least, was in good hands with Professor Ekong; but within a very short while he himself had left to take up university administration.

Professor Ekong is not the only leading chemist in Nigeria to have left university teaching and research for administration, in some cases at least quite unwillingly. In Nigeria there are a number of able administrators of one kind and another, whereas there are very few capable scientific leaders, and I question whether the move of scientists into administration is really beneficial, whatever may be the case in more developed countries.

Yours faithfully,

D. A. H. TAYLOR
Department of Chemistry,
University of Natal.

Soviet reactor safety

SIR,—In your report on Soviet energy (25 January, page 258) Vera Rich reports on the "accident" in BN350, the Russian fast reactor on the Caspian sea. By the use of her words "it is presented as" she seeks to imply that the "accident" was not in the steam generators and that some accident occurred in the reactor core itself. In fact, the full details of this "accident" have been known for many years in the West and have been fully reported in international conferences, for example at the 1978 Bologna Conference (D. S. Yurchenko, *et al.*, *Operating Experience with the BN 350 Fast Reactor (1972-77)*; IAEA Symposium on the Design, Construction and Operating Experience with LMFBRs, Bologna, 10-14 April 1978), and many designers have visited BN350 subsequent to the "accident".

The presentation adopted in the article in *Nature* seems likely to mislead and to cast unwarranted doubt both on the integrity of the Soviet physicists concerned, and on the safety of the fast reactor as a power generator.

Yours faithfully,

R. D. SMITH
UKAEA, Risley, Cheshire, UK

Intuitive energy figures

SIR,—P. T. Landsberg (5 April, page 502) expresses the hope that his energy figures carry an intuitive meaning. As a basically illiterate mathematician, may I say that they do. They convey the impression that Mr Landsberg wishes to encourage the belief that natural sources of energy are entirely sufficient for our needs; and that he considers it prudent to publish figures setting out the possible power yield from deserts, without demonstrating the energy investment required to achieve it.

I will gladly sacrifice the assistance of 60W bulbs per capita—even for 10⁶ joules—in the service of comprehensive energy budgets on all energy projections—illustrative or real, alternative or conventional.

Yours faithfully,

DAVID GREEN
Pembrokeshire, Wales

news and views

Nonradial and nonlinear stellar pulsation

from Douglas Gough*

IN the past two decades the theory of the pulsation of the Classical Cepheid and RR Lyrae stars has reached a high degree of sophistication. This kind of pulsation is the simplest mode of oscillation a star can undergo: it is periodic and the motion is purely in the radial direction. Although there remain some niggling discrepancies between theory and observation the agreement is sufficient to convince most astrophysicists that our ideas are basically correct. Thus we seem to have a firm foundation from which to extend our studies to stars whose oscillations are nonradial and non-periodic. In recent years considerable attention has been devoted to such stars, and last month observers and theorists who work in this field attended a workshop* at which β Cephei stars, δ Scuti stars, ZZ Ceti stars and the Sun, were discussed.

Walter Fitch (Steward Observatory) reviewed observations of variable stars of δ Scuti and related types. These stars pulsate at low amplitude, and many exhibit two characteristic frequencies which bear a ratio close to that of two small integers. Consequently much of the subsequent discussion concerned direct resonances between two modes of oscillation. Arthur Cox (Los Alamos Scientific Laboratories) reported his failure to reproduce this behaviour theoretically from initial value integrations, even when the resonance conditions had been carefully engineered, and Robert Stellingwerf (Rutgers University), who had analysed the stability of pairs of singly periodic limit cycles of potential double mode Cepheid models, never found both cycles to be simultaneously unstable to linear perturbations. The double mode behaviour remains unexplained. During discussions of parametric resonances, it was pointed out that many of these stars seem to exhibit characteristics of strange attractors. Some δ Scuti stars appear to pulsate in many modes simultaneously;

Wojciech Dziembowski (Copernicus Astronomical Centre) estimated that nonlinearities developed by any one mode alone were too small to limit its amplitude to a value as low as those observed, and concluded that mode interactions must be responsible. Definitive calculations have not yet been performed, but techniques developed for studying oceanic gravity wave interactions and plasma turbulence are available for tackling problems of this kind. We can therefore anticipate considerable advances in this study in the near future.

A class of stars for which there is no convincing explanation is characterised by β Cephei. Myron Smith (University of Texas at Austin) reviewed the observations. The stars are of spectral type B, and lie in a strip in the Hertzsprung-Russell diagram a little above the Main Sequence. Mike Jerzkiewicz (Wrocław University Observatory) and C. Sterken (Free University of Brussels) reported new observations and pointed out that almost all stars in the β Cephei strip show signs of variability. They appear to pulsate in both radial and nonradial modes. As with the δ Scuti stars simple period ratios are found, which suggest again that resonance mechanisms are operating. Frequency splitting, presumably by rotation, has been measured, which offers the exciting prospect of trying to infer the angular velocity within these stars. Unfortunately we are not yet in a position to do this because the modes of oscillation have not been unambiguously identified; different workers expressed contradictory opinions about the modes they thought were responsible for the variability.

An intriguing property of some β Cephei stars is that they switch from one mode of oscillation to another on a timescale of no longer than about 10 periods. Smith argued that this is evidence that the oscillations are confined to a rather thin outer layer of the star, because otherwise it would be difficult to envisage how dissipative processes could effect the enormous energy transfer that would be involved. Others contradicted that claim, citing simple nonlinear oscillators that can alter their character on a dynamical

timescale. It is unlikely that it will be established in the near future whether such oscillators actually represent the behaviour of β Cephei stars in any way, because the basic physics of the variability is not yet understood.

How are the oscillations driven? Morris Aizenman (National Science Foundation) reviewed the many ingenious ideas that have been proposed in the past, all of which have failed. A recent suggestion by Stellingwerf that the mechanism is no more than the Eddington valve that drives classical Cepheids and RR Lyrae stars was debated. He proposed that interpolation in opacity tables on too coarse a grid had led to an underestimate of the efficacy of the valve, but Arthur Cox, one of the chief suppliers of opacity data to the astrophysical community, claimed that though finer resolution decreased the stability of the theoretical models it was never sufficient to render them unstable. The suggestion was made that if all the mechanisms reviewed by Aizenman were operating in unison the sum of their contributions might be sufficient to maintain the pulsations against dissipation. This idea, which is reminiscent of an early suggestion for solving the solar neutrino problem, was unenthusiastically received.

Solar oscillations

A significant fraction of the workshop was devoted to discussing oscillations of the Sun. First, Henry Hill (University of Arizona) reviewed the observations spanning the period range 3^{min}–160^{min} and discussed the evidence already in the literature for the oscillations in the data being of solar origin. There followed a sequence of presentations of new observations and discussions of the longer period oscillations. Discussion concentrated mainly on two issues: the form of the oscillations in the upper atmosphere and the evidence for phase coherence.

By comparing different diameter measurements made at the Santa Catalina Laboratory for Experimental Relativity by Astrometry (SCLERA), Hill argued that the amplitudes of oscillation rise more steeply with height above the photosphere than can be

*The workshop 'Nonradial and nonlinear stellar pulsation' was organised by Professor Henry Hill and held at the University of Arizona on 12–16 March 1979. The proceedings of the workshop will be edited by Professor Hill and published in about 18 months' time by Springer Verlag in the series 'Lecture Notes in Physics'.

comfortably accommodated within the framework of linear theory. At first sight this should be no cause for alarm, because the amplitudes thus inferred in the low chromosphere are so great that nonlinearities must surely be important. Nonetheless several participants were uneasy with the result, because in the photospheric regions, where presumably linear theory is valid, the eigenfunctions have the appearance of waves penetrating an evanescent region from above yet having frequencies characteristic of the resonating cavity beneath. Tuck Stebbins (Sacramento Peak Observatory) presented measurements of relative oscillation amplitudes at different positions in the wings of a spectrum line which support Hill's conclusion, but Timothy Brown (High Altitude Observatory) found contradictory evidence from shape changes of the limb darkening function. The issue is currently unresolved.

For many people, the most convincing evidence that the oscillation data arise from genuine dynamical solar vibrations is their phase coherence. Peter Worden (Sacramento Peak Observatory) criticised early analyses of the SCLERA data, but Thomas Caudell (University of Arizona) presented new measurements of solar equatorial diameter variations which, it was finally agreed, convincingly maintained phase over an interval of 22 days. This did not terminate the discussion, however, because as Maurice Gabriel (Université de Liège) pointed out, unless there were a considerable difference in the amplitudes of eastward and westward propagating waves one would not expect phase coherence over a period longer than half the mean rotation period of the Sun. Polar diameter measurements, which have not yet been analysed, are not subject to this criticism.

One of the most exciting aspects of solar oscillations is their potential for providing diagnostics of the solar interior. To realise this potential it is essential to examine the theory quite meticulously to ensure that the physics is well understood, and that measurable properties of the oscillations of theoretical models can be computed accurately. In this respect greatest attention has been paid to the 5-min oscillations, because it is of these that the most refined measurements have been made. Investigations by groups at the University of California at Los Angeles and the Observatoire de Nice have revealed that of the uncertainties beneath the photosphere only the value of the adiabat deep in the convection zone substantially influences the oscillation frequencies; in addition, the Nice group have found that the frequencies of all but the chromospheric

modes are insensitive to variations in the structure adopted for the solar atmosphere, within the framework of the usual linearised theory. The results of both groups seem to imply that the adiabat must be that of a convection zone whose depth is at least 20% of the solar radius. This conclusion is similar to the findings of Beckers and Gilman, who reported last September at the EPS Workshop on Solar Rotation in Catania (*Publication No. 162 of the Astrophysical Observatory of Catania* (Eds Belvedere & Paternò) 1978) that they could not explain the observed absence of a polar vortex unless the depth of the convection zone were at least of the order of 40% of the Sun's radius.

The case for so deep a convection zone is not completely closed. Participants of the workshop were reminded of the EPS conference on solar physics (*Pleins feux sur la physique solaire* (Ed Rösch) CNRS, Paris, 1978) held in Toulouse last year, where Dziembowski and Pamjatnykh pointed out that the observations by Hill and Caudell (*Mon. Not. R. astron. Soc.* **186**, 327; 1979), apparently of solar g modes of degree about 30, are difficult to explain in terms of the so-called standard solar model (see *Nature*, **274**, 739; 1978). At the Arizona workshop Jørgen Christensen-Dalsgaard (Université de Liège) presented computations of g modes in a solar model with low interior heavy element abundance Z, such models have shallow convection zones through which modes of the kind reported by Hill and Caudell can penetrate with ease. Solar models with low Z also predict low neutrino fluxes in agreement with Davis's measurements, but they do pose many problems, such as how to explain the frequencies of the 5-min oscillations. Ross Rosenwald (University of Arizona) pointed out that if the claim of the SCLERA group concerning the nonlinear behaviour of the oscillations high in the solar atmosphere were correct, the linear calculations performed to date may not be relevant, and this problem may disappear. The claim has not yet been substantiated.

Another new observation which may shed light on this issue was reported by George Isaak (University of Birmingham). His group has measured variations in the Doppler shift of a sodium line in light integrated from the entire solar disk, and have measured discrete frequencies of what appear to be acoustic oscillations of low degree with periods of about 5 min. Unlike the most common 5-min modes, these penetrate beneath the convection zone,

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and provide an integral measure of the structure deep in the solar interior. A preliminary theoretical analysis favoured a model with a somewhat lower value of Z than that of the standard model, and a shallower convection zone. This model lies between the two extremes discussed above, and so serves to remind us how uncertain we are of the Sun's internal structure.

Degenerate variables

The final day of the meeting was devoted to degenerate variables. Edward Robinson (University of Texas at Austin) gave a stimulating review of the observations, many of which have been obtained only in the past few years. Most of these variables appear to be DA white dwarfs lying in a narrow range of spectral type with $B-V \approx 0.2$, though John McGraw (University of Arizona) in particular has observed variable white dwarfs of different colours. Of the DA dwarfs, about 25% are observed to be variable, and aside from their variability show no other distinguishing feature. Luminosity amplitudes range from a few tenths of a magnitude down to the limit of detectability.

The low amplitude variables have stable periods; one such star, R548 is even more stable than the Crab pulsar. The larger the amplitude the longer the period, and the less stable the power spectrum of the pulsations. It is not unusual for the stars with larger amplitudes also to switch entirely from one mode to another.

Oscillation periods range between about 100 s and 1,000 s, which are much too long to be p modes. Explanations in terms of g modes have been attempted, but it is sometimes difficult to fit the periods satisfactorily. Moreover, with the longer period variables it is difficult to explain why neighbouring g modes in the densely spaced spectrum are not observed, a problem faced also by those who argue that the 2^h 40^{min} oscillation of the Sun is a g mode. Carl Hansen (Joint Institute for Laboratory Astrophysics) suggested that perhaps the oscillations were toroidal elastic modes of a rather mushy material, but reliable estimates of the elastic properties of these degenerate stars are not available to test this hypothesis. In any case it was difficult to explain why only oscillations of the periods observed should be driven, and the others damped. Arthur Cox pointed out that (with the eye of faith) one could imagine the region in the HR diagram where pulsating white dwarfs have been found to be an extension of the classical Cepheid instability strip. He found models that were unstable to radial pulsations, but the periods of such modes are only about 1 s. □

More reliable counts of chromosomal aberrations

from John R. K. Savage

A NOVEL application of a staining method widely used to identify newly-synthesised DNA to the problem of obtaining reliable dose-response curves for the effects of radiation damage in cells is reported in this issue of *Nature* (page 756). The number of chromosomal aberrations induced per cell is the usual measure of radiation exposure. But the precise correlation of dose with the number of chromosomal aberrations it produces is complicated by (amongst other factors) the difficulty of differentiating between cells which have divided a second time after treatment and before analysis. The inclusion of these second division cells in the total cell count inevitably leads to an underestimate of the number of chromosome aberrations produced by any given dose, as the probability of any cell's surviving a second division decreases the larger the number of aberrations it carries. Thus there is selection for less-damaged cells which dilute the scoring sample and reduce the observed yield of aberrations per cell. In a desynchronising cell population like the *in vitro* blood lymphocyte system, the contamination by second and subsequent division cells will increase progressively with time from initiation and a concomitant fall in the yield of asymmetrical aberrations will be observed.

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In the past, many workers, aware of this problem, have sampled as early as possible after stimulation, on the principle that the earliest samples are purer first divisions. In practice, a compromise is made to ensure that a sufficient number of mitoses for scoring is present, and for human lymphocytes a time ~48 h after exposure is usually chosen. Even at this time, second division contamination may not always be negligible and careful workers attempt a further purification of this sample by omitting from their score cells with obviously modified aberrations or those lacking a full complement of acentric fragments.

Now, as Scott and Lyons elegantly demonstrate (page 756) application of the FPG 'harlequin' technique goes a long way towards solving this problem. The technique provides differential staining of the chromatids in second and subsequent division cells based on the semi-conservative segregation of bromodeoxyuridine incorporated in the DNA during synthesis phase. Consequently, by confining scoring to cells with chromosomes not showing differential staining, only first post-treatment division cells will be included in the sample, irrespective of sampling time. As Scott *et al.* show, the asymmetrical chromosome-type aberration yield induced in these cells by 200 rad X-rays is constant, independent of sampling time over the range of 40-72 h. This supplies some evidence for the homogeneous radiosensitivity of

the initial population, and provided the constant yield holds for other doses (which seems to be the case from our own preliminary work), the form of dose-response curves obtained with this method should be much more consistent than hitherto. No enhancement of aberration yield was recorded at the concentration of bromodeoxyuridine used or from its method of application. There was, however, a concentration-dependent perturbation of cell progress, but this has little relevance if the primary population is really homogeneous.

The technique is essentially very simple to apply, and there is no reason why it should not be routinely used in all future dose-response curve work. When one considers the tremendous mathematical effort which goes into the fitting and parameter derivation from such curves and their interpretation, it is surely worth-while to ensure that they have a reliable shape before starting.

The advantages of the method are not confined to dose-response curves; one can see many applications in the field of chromosomal aberration studies. For example, in the study of chromosomal mosaics (individuals whose tissues contain mixtures of two or more karyotypically different cell types) the observed relative frequencies of the cell types are in many cases influenced by selection at cell division. Confining scoring to first division cells may help towards reducing the between-sample variability frequently found.

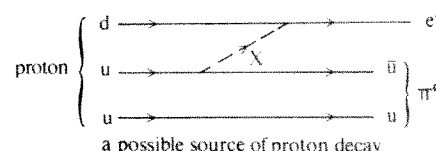
Are protons unstable?

from F. E. Close

THE experimental lower limit on the proton lifetime is well over a trillion times the age of the Universe and this has led people to believe that the proton is indeed absolutely stable. Recent theoretical attempts to construct theories that unify the strong force with the weak-electromagnetic force (see *Nature* 277, 349; 1979), however, have the consequence that the proton is not absolutely stable. A lifetime as 'short' as 10^{31} years could be possible (about an order of magnitude greater than the present lower limit).

Quantum electrodynamics (QED) and quantum chromodynamics (QCD) (the theory of the strong interaction) are very similar field theories and appear in many ways to be profoundly and intimately related. Recently devel-

oped ideas on grand unification of the natural forces do just this and postulate that they are related in a way that is made precise by group theory. Mathematically, QED is a U(1) field theory and QCD is an SU(3) ('non Abelian') field theory, the U(1) essentially displaying the fact that there is but one kind of electrical charge (which can be positive or negative like the set of real numbers) and the SU(3) displaying the existence of three kinds of colour charge (often called red, yellow and blue by analogy with the primary colours). In a nutshell, QCD is like QED with 3×3 matrices in place of real numbers in the 'photon' (coloured gluon) couplings. (Photons are the quanta of the electromagnetic force; coloured gluons those of the strong interaction.) To unify these, find a group G which contains $SU(3) \times U(1)$



a possible source of proton decay

and postulate that there exists a corresponding field theory. The SU(3) QCD and U(1) QED will then be two particular subclasses of this grand field theory. Unification of QCD with the electromagnetic and weak interaction can be achieved if G is the group SU(5) or SO(10). A consequence of this unification is that at energies typical of the world about us the SU(3) colour force is stronger than the U(1) electromagnetic. This is in accord with observed atomic structure: the protons and neutrons (quark systems) are bound in the nucleus by the strong force while the electron (lepton) orbits the nucleus at large distances held by the electromagnetic force.

An outcome of such a unification is that in addition to the photon and the

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coloured gluons of QED and QCD, there will exist a new ultraweak force whose quanta carry and couple both to electrical and to colour charge. These quanta have been called 'leptoquarks' because they have the remarkable property of being able to cause quarks to change into leptons, and hence to make the proton decay.

Leptons and quarks are very similar, the only essential difference being that quarks have colour whereas leptons do not. Imagine the effect of a leptoquark quantum being absorbed by a lepton (the electron for example). The leptoquark couples to electrical charge and so can fuse with the electrically charged electron. But the leptoquark also carries colour and so 'paints' the electron transforming it into a quark. The leptoquark quanta carry electrical charge which are fractions of an electron charge so the quark produced from the lepton will have fractional charge, as it must.

Just as electrons could be turned into quarks so could quarks decay into leptons, and this is the source of the predicted instability of the proton. The specific nature of the predicted decay products tends to be rather dependent on the particular model under consideration. In the SU(5) scheme a final state of an antilepton (positron, e^+) and neutral pion (π^0) is predicted and seems to be the one most widely favoured by theorists. The way that this happens is illustrated in the figure and is due to the predicted leptoquark quantum, X. This has electrical charge $4/3$ and can transform a u(up) quark (charge $2/3$) into an antiup ($\bar{u}$) of charge $-2/3$ on being emitted. Absorbed by a d(down) quark of charge $-1/3$ it produces an antilepton (e^+) of charge $+1$. Colour and electrical charge are conserved throughout and the proton (two up quarks and a down) decays into $u\bar{u}$ (π^0) and e^+ at a rate of 1 in about 10^{31} to 10^{33} years.

Experiments that may be sensitive to such decays are now being planned and one is already underway in Montana, USA. If one hopes to detect at least 1 such decay per year then over 10^{21} protons are needed for study, hence over 3,000 tons of material are called for. The cheapest is water and typical experiments plan to have a large swimming pool of about 20 m in each direction, built deep underground to reduce cosmic ray backgrounds. Deep mines are one possibility and in Europe the Mont Blanc tunnel (on average 3,000 metres below 'ground') has been suggested. Detectors of Cerenkov radiation are needed; the signal being some 500 MeV of radiation detected in one direction in coincidence with 500 MeV in the opposite direction. One set will be from the antilepton in the proton decay, the other will be from

the π^0 (this having decayed into two photons which in turn produce electron-antielectron pairs, these latter giving rise to the Cerenkov light).

There are many technical experimental problems to be overcome but the idea seems feasible and the stakes are high. Typically two years preparation and three years experimentation may be involved. However, the experiment currently in progress in the USA may be sensitive to about 10^{31} years' lifetime and could produce results in a year or so. If the lowest theoretical estimates are correct then we may soon have evidence for proton decay. □

Adsorbates on transition metals

from Marvin L. Cohen

THE recent renaissance in surface science has been motivated partly by a desire to explain and exploit the catalytic properties of d-electrons near transition metal surfaces. However, many technologically relevant problems connected with catalysis were put aside to await reproducible experiments and extensions of theoretical techniques which could deal with the properties of clean surfaces and surfaces with adsorbates. Experimental developments over the past 5 years in high vacuum science, photoelectron and electron loss spectroscopy, and low energy electron diffraction have provided accurate data. On the theoretical side, very recent progress has produced calculated spectra suitable for comparison with experiment, but much of the theoretical emphasis has been on semiconductors (Appelbaum & Hamann *Rev. Mod. Phys.* **48**, 3; 1976; Schlüter, Chelikovsky, Louie & Cohen *Phys. Rev. B* **12**, 4200; 1975) where the electronic states of interest have not been d-states.

The semiconductor research has provided convenient tests of the theory. One major point has become clear. A surface is not just the undeformed edge of a perfect crystal. Rearrangements take place, and the electronic charge distorts in response to the surface perturbation. The readjustment of the electronic charge density causes a change in the electrostatic potential felt by the electrons near the surface. This change has to be included in the theoretical calculations. This is done through a self-consistent or feed-back technique. The electronic charge density is calculated for an ideal surface, and the change in the potential arising from the surface redistribution of charge is fed back through the potential to produce a new charge density. This

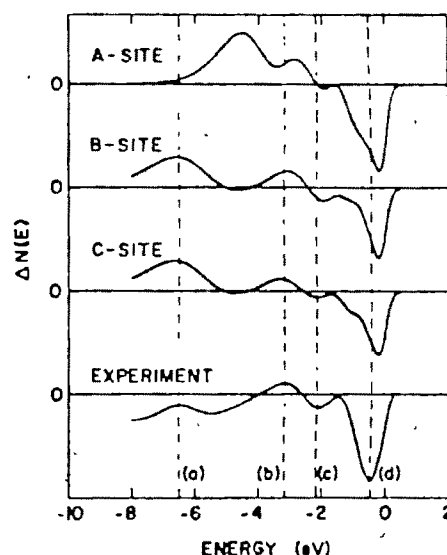


Fig. 1 Comparison of the calculated H-induced photoemission difference spectra with the experimental difference curve from Demuth *op. cit.*

is repeated until the input and output charge densities agree. When the calculation is self-consistent, the detailed surface properties can be examined. Many surface properties are determined by electronic surface states which are found localised near the surface. The surface states are affected by adsorbates, defects, steps and the detailed crystal structure of the surface. Self-consistent calculations for semiconductors have yielded a significant amount of information about these cases.

For transition metals, difficulties in handling the localised d-electrons have resulted in slower progress. However, S. G. Louie (*Phys. Rev. Lett.* **42**, 476; 1979) has recently completed the first fully self-consistent calculation for the electronic structure of an adsorbate on a transition metal. His work focused on the interaction of hydrogen with the Pd (111) surface. This calculation should serve as a model for future adsorbate studies on d-band materials. It also provides useful information for hydrogen-storage technology.

Louie's calculation is based on a self-consistent pseudopotential approach. The pseudopotential is an effective potential which determines the electron-ion interaction in the crystal. The self-consistency allows for charge readjustment at the surface and for charge flow between the adsorbate and the transition metal. This approach had already been used successfully for three studies of clean transition metal surfaces, Nb (001) (Louie *et al. Phys. Rev. B* **15**, 5627; 1977), Pd (111) (Louie *Phys. Rev. Lett.* **40**, 1525; 1978) and Mo (001) (Kerker, Ho & Cohen *Phys. Rev. B* **18**, 5473; 1978). The new extension which adds the hydrogen adsorbate uses the clean surface results

FLOWERS provide nectar for bees, and in return a bee acts as a kind of flying penis, carrying pollen from one plant to fertilise the ovaries of another. Plants and their pollinators are a classic example of mutualism: they have coevolved through evolutionary time in a reciprocal beneficial relationship. However this superficially harmonious view may conceal the true nature of the interaction. It is perhaps more accurate to think of bees having evolved towards efficient exploitation of plants as a food source, while plants have evolved adaptations to minimise the price they pay for getting bees to transfer pollen. G. H. Pyke (*Oecologia* 36, 281; 1978) has used this line of argument to develop a new insight into the structure of certain inflorescences and the foraging behaviour of bees. He studied three plant genera *Delphinium*, *Aconitum*, and *Epilobium*, all of which are cross-fertilised by bumblebees (*Bombus* spp.), and have their flowers arranged in a vertical spike.

Bumblebees feeding on these plants follow a characteristic movement pattern. They almost invariably start at the bottom of an inflorescence, move up the spike, and leave to fly to the next plant before reaching the top. Within the inflorescence, a bee typically moves vertically upwards from one flower to the nearest one above. The flowers of all three plant genera are arranged in a spiral around the vertical spike, so that by moving vertically upwards, a bee misses out some of the flowers. The bees' movement rules are related to nectar availability. Flowers at the bottom of an inflorescence are older, bigger and contain more nectar than those at the top, hence by starting at the bottom the bee visits the most profitable

Coevolution of bees and flowers

from John R. Krebs

flowers first. As it moves up the inflorescence the bee experiences gradually diminishing returns from the successively smaller flowers, until there comes a point at which more nectar per second could be obtained by travelling to the bottom of the next inflorescence. Pyke's measurements suggested that bees roughly follow this optimal departure rule, and this accounts for the fact that a bee usually leaves before reaching the top of an inflorescence. At first sight one might think that it would pay the bee to visit every single flower on its way up the inflorescence, rather than missing nectar-rich flowers near the bottom. However, the spiral arrangement of flowers reduces the benefit of this movement rule. The extra energetic costs to the bee involved in manoeuvring sideways to follow a spiral path more than offset the benefit of visiting all the flowers. The rule of moving straight upwards gives a higher net energy gain.

Bumblebees seem to have evolved a set of rules for efficient exploitation of nectar, but the plants are equally under an evolutionary pressure to exploit bees, and Pyke suggests that the flower arrangement can be interpreted in this way. The larger, older, nectar-rich flowers at the bottom of an inflorescence are female, while the younger flowers at the top are male (the plants are protandrous hermaphrodites, meaning that each flower turns from male to female as it gets

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older). This arrangement of flowers ensures that a visiting bee arrives at the female flowers near the base having just come from the male flowers near the top of the previous plant. In this way the chance of outcrossing is increased, and it is known that in *Delphinium* at least outcrossing results in a higher percentage seed set (Price & Waser *Nature* 277, 294; 1979). The spiral arrangement of flowers around the stem is also important to the plant. A bee does not usually carry enough pollen to fertilise all the female flowers on a plant, so if it visited every flower it would extract some nectar without providing pollen in return. The plants' evolutionary answer seems to be to arrange its inflorescence in such a way that an efficient bee actually does better by missing some of the female flowers.

Given the design of plants, bumblebees seem to approximate an optimal set of movement rules for maximising net rate of harvesting nectar. At the same time the plants have evolved a design which makes highly efficient (perhaps optimal, but not enough is known about benefits to plants) use of bees as pollinators. It might appear that the coevolutionary struggle has reached a stable point where bees and plants are doing as well as they can, but there is another aspect to the problem. Different species of plants (as well as different individuals of the same species) compete to attract pollinators, and a complete account of the coevolution of bees and plants must be able to explain why different plants living in the same area are designed to offer nectar in different ways. The answer to this sort of question may emerge from approaches such as Pyke's.

for comparison to determine the changes caused by the adsorbate.

A convenient way to explore the effects of the adsorbate is to calculate the surface density of states with and without the adsorbate and to examine the difference in these two functions $\Delta N(E)$. Since the density-of-states function determines the number of electronic states in each energy interval, surface or adsorbate states show up as peaks in this function in the energy interval corresponding to the binding energy of the state. The difference function $\Delta N(E)$ demonstrates how the states near the surface are affected by the adsorbate.

The site where the hydrogen atom is adsorbed on the Pd (111) surface isn't known, but three different sites are likely. Louie's calculation models all three sites (A, B and C) and computes the $\Delta N(E)$ function for each case

(Fig. 1). This function can be compared with a difference photoemission spectrum (Demuth *Surface Sci.* 65, 369; 1977) to determine which geometry fits the measured results.

One prominent feature common to all the $\Delta N(E)$ curves is the dip near the metal Fermi energy (0 eV). This dip reflects the adsorbate correlated movement of states away from this region to lower energy. The peaks at lower energies arise from the new hydrogen induced states formed from hydrogen 1s and Pd d-states. Comparison of the $\Delta N(E)$ spectra rules out site A and favours B and C which differ little in their environment. Louie concludes that the hydrogen atoms prefer a threefold hollow over the top site. This geometry yields an occupied H-Pd bonding surface band centred at 6.5 eV below the Fermi energy (Fig. 1) in good agreement with experiment

and an unoccupied antibonding H-Pd surface band at high energies (not shown in Fig. 1). Other surface structure is found and analysed.

The features of the H-Pd bonding state can be studied using charge density contour maps. These illustrate the details of the mixing between the hydrogen and Pd orbitals. Louie has also calculated the total electronic charge distribution and finds that some charge spills out into the vacuum above the surface. The values for the number of valence electrons per surface unit cell are 0.2 in the vacuum above the surface and 9.7, 10.1, 10.0, 10.0 for the first, second, third and fourth layers into the bulk (10 is the bulk value). The calculated work function is in excellent agreement with experiment.

Although Louie's calculation only considers a specific adsorbate on a specific face of one transition metal,

his success in getting information about the adsorbate site and bonding configurations provides strong motivation for future studies. The next level of complexity would be studies of molecular adsorbates (for example CO) on different surfaces of a variety of transition metals. Calculations of this kind should be possible soon, and theoretical results on systems like these should provide useful data for understanding surfaces and for developing new catalysts □

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Surface structure by SEXAFS

from D. T. Clark

THE wide variety of techniques which have been developed to take particular advantage of the special characteristics of synchrotron radiation have previously been notified in these columns and (*News and Views* 276, 672; 1978) span fields as diverse as the band structure of metals and the study of time-dependent conformational changes for proteins. The benefits of having a dedicated synchrotron source as opposed to operating in parasitic mode to basic high energy physics experiments has been appreciated for some time in the UK and in consequence a national facility is currently nearing completion at SRC's Daresbury Laboratory. It is already clear from the published work, particularly of a number of research groups in the USA, that synchrotron sources will have a vital role in surface science in particular. The benefits of a highly collimated, continuous spectrum which includes wavelengths not available from other sources is self evident in the investigations of structure and bonding of the surfaces of solids since differential changes in photoionisation cross sections and different sampling depths (consequent upon the strong dependence of electron mean free paths on kinetic energy) are thereby routinely available.

In addition, however, there are three properties of synchrotron radiation sources which enhance their potential for research in surface science. First since the electron beam is extremely stable in energy, position and intensity the light-emitting areas are endowed with the same characteristics. Second the substantial degree of polarisation of the photon source after monochromatisation provides a means of studying

asymmetry parameters and channelling phenomena and finally since the synchrotron source typically operates in the UHV regime there is direct compatibility with the requirements for carrying out experiments on uncontaminated surfaces.

The experiments which have so far been carried out in the area of surface science have largely centred on the investigation of the valence bands of metals and semiconductors and this has been a fruitful area of research particularly in the US in the research groups of Eastman, Spicer and Shirley.

In an important paper recently appearing in *Phys. Rev. Lett.* (42, 104; 1979) Bianconi and Bachrach have shown for the first time that measurements of surface extended X-ray absorption fine structure (SEXAFS) provides a powerful alternative to LEED in the investigation of surface geometries of single crystals. This obviously extends the scope of synchrotron radiation in the investigation of surfaces and it is worthwhile briefly considering the background to this work.

The basis of EXAFS, in particular as it relates to the investigation of metalloprotein systems, has recently been considered in these columns (*News & Views* 277, 89; 1979) and one need only note that previous theoretical proposals relating to the use of SEXAFS have now been implemented in the paper described here. The important question of the structure determination of surfaces have until now been addressed by one technique only, namely low energy electron diffraction (LEED). The technique is limited to single crystal analysis and the investigation of chemisorbed atoms or molecules with long range order and has relatively poor resolution. Despite the difficulties in theoretically modelling the experimental data the technique has provided important data which illustrate for example the reconstruction (change in interatomic distances) for most semiconductor surfaces.

LEED experiments on Al (100) and Al (111) surfaces have previously been reported (Martin & Samorjai *Phys. Rev.* B7, 3607; 1973; Jepson, Marcus & Jona *Phys. Rev.* B6, 3684; 1972; 5, 3933; 1972) and have been interpreted in terms of no reconstruction for the former and a 5–10% increase in interatomic spacing for the latter face. Since LEED typically provides a resolution of ~ 0.1 Å there is clearly some ambiguity in interpreting small changes in spacings. By contrast Bianconi and Bachrach demonstrate that the substantially higher sensitivity (with respect to spacing) of the EXAFS measurements allows an unambiguous assignment of both the sign and magnitude of the restructuring of the Al

(111) surface compared with the bulk.

The experiments described by Bianconi and Bachrach involved measuring the quantum yield of secondary electrons originating from inelastically scattered LVV Auger electrons generated in the decay of the absorption produced core holes. Using this partial yield technique measurements may be made of the $L_{2,3}$ surface soft X-ray absorption spectrum of a surface layer of aluminium single crystals; the thickness being determined by the effective escape depth of the secondary or Auger electrons with the selected kinetic energy.

The experiments were carried out on the grazing incidence monochromator fitted to the 4° beam line at the Stanford Synchrotron Radiation Laboratory. The photoelectron energy was selected with a cylindrical mirror analyser (detection cone at $\theta=42^\circ$) using a 3 eV window. The linearly p polarised light was incident near grazing ($\theta \leq 10^\circ$) and this strongly emphasises the interatomic distance normal to the surface. The Al single crystals were cleaned by ion sputtering and annealing at 4×10^{-10} Torr and in separate experiments the residual oxygen contamination was shown to be < 0.001 monolayer.

Surface soft X-ray absorption spectra of the Al (100) and Al (111) faces were measured collecting the inelastically scattered Auger electrons at 45 eV and 4 eV. At 45 eV final state energy an average of ~ 2.9 Å thick layer contributes to the spectrum which corresponds effectively to the surface layer since the interatomic spacing is 2.86 Å for the bulk metal. For a 4 eV final state energy the escape depth is closer to 20 Å so that *a priori* one might expect a comparison with data for the bulk. Structural information is extracted from this data by fitting the first large EXAFS modulation. The almost exact correspondence of the data for bulk aluminium and for the Al (100) surface shows conclusively that within the limits of the experiment there is no restructuring for this surface. By contrast the fact that the EXAFS structure shifts towards higher energy on going from bulk to surface shows directly that for the Al (111) face there is a decrease in interatomic spacing. By using the bulk aluminium EXAFS as reference this contraction may be quantified as $\Delta r = 0.15 \pm 0.05$ Å.

These experiments confirm the great potential of SEXAFS in the investigation of the surface structure of single crystals and it will be of interest to have comparisons with LEED data on other materials for which surface restructuring has been inferred. It should be noted that SEXAFS can also in principle be applied to the study of amorphous surfaces and we may antici-

pate that the technique will be extensively applied to such materials over the next few years. $\square$

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Nuclear giant dipole resonance

from P. E. Hodgson

LONG ago it was found that the cross sections of many reactions of gamma rays with nuclei, in particular those in which a nucleon is ejected, show broad resonances at quite high energies, usually around 20 MeV for light nuclei and falling to about 10 MeV for heavy nuclei. It was soon suggested that these resonances are due to a special type of collective excitation in which the neutrons vibrate against the protons. The resonance was found to be E1 (electric dipole) and a macroscopic calculation based on the liquid drop model gave approximately the correct variation of resonance energy with atomic weight.

Subsequently, detailed microscopic models have been developed that interpret the resonance as due to the coherent superposition of all possible particle-hole excitations from one major shell to the next. This was able to account for the width and the energy of the giant dipole resonances in several light nuclei.

The macroscopic models in terms of neutron and proton fluids were also developed, and these have the advantage that they give results for a whole range of nuclei for which the microscopic calculations would be very complicated. The most detailed of the macroscopic models are first that of Goldhaber and Teller (*Phys. Rev.* 74, 1046; 1948), which assumes that the oscillations of the neutron and proton fluids each take place within a fixed boundary, and this predicts that the excitation energy varies as $33 A^{-1/6}$. A second model, due to Steinwedel and Jensen (*Z. Naturforsch.* 5A, 413; 1950) assumes that the neutron-proton

oscillation takes place within one fixed boundary, and this gives $80 A^{-1/3}$ for the energy dependence. More recently, Myers and Swiatecki (*Phys. Rev.* C15, 2032; 1977) have developed a more detailed theory based on their droplet model of the nucleus, and this gives an energy variation of $A^{-1/2}$, which is nearer to the experimental results than the other models. The models also predict distinctly different transition charge densities.

To test these models, Pitthan and colleagues at the Naval Postgraduate School, Monterey, California have recently made some careful measurements of the inelastic scattering of electrons by ^{140}Ce (*Phys. Rev. Lett.* 41, 1276, 1978). This nucleus was chosen because of all the nuclei investigated it shows the most favourable separation between the various possible resonances. The spectrum of inelastically scattered 91.2 MeV electrons that they obtained is shown in Fig. 1, with the background already subtracted. A number of peaks are visible, corresponding to the electric resonances of various multipolarities. The spectrum is analysed into the contributions from the different multipolarities, and the one of interest for distinguishing between the different models is the dipole resonance at about 15.3 MeV. Similar measurements were made at a number of incident electron energies from 65 to 150 MeV.

The results for the dipole resonances at different electron energies were compared with the models of the excitation process by plotting the ratio of the experimental cross section to the Mott cross section at a particular angle as a function of the momentum transfer, and this is compared with predictions of the three theories in Fig. 2. It is clear that the Myers-Swiatecki model agrees very well with the data, whereas the Steinwedel-Jensen model predicts cross sections that are too high.

The Goldhaber-Teller model predicts cross sections that are too low, but it is just possible that the shortfall could be attributed to the presence of an undetected monopole (breathing mode) resonance. This state, in which the nucleus oscillates radially, has been predicted theoretically, and some evidence for its presence has been found in previous experimental work (see for example *News & Views* 271, 212; 1978). To study this possibility, the difference between the predictions of the Goldhaber-Teller model and the experimental results was compared with distorted wave calculations of the contribution to be expected from a monopole excitation. The overall dependence

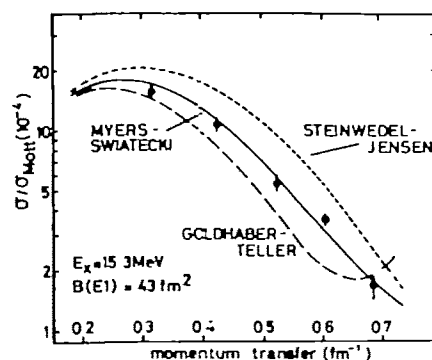


Fig. 2 Comparison between the experimental results of the electric dipole resonance at several values of the momentum transfer and the predictions of three theories of the excitation process.

on momentum transfer is found to be the same, and if it is indeed monopole it exhausts about 45% of the energy-weighted sum rule. This interpretation is however doubtful partly because the variation with A is already in disagreement with the Goldhaber-Teller model, and partly because if the monopole interpretation is correct we would expect to see all the energy-weighted sum. Thus at present the evidence favours the Myers-Swiatecki model for the giant dipole resonance. In addition, there is some discrepancy between the evidence in favour of the monopole state obtained from measurements of inelastic alpha-particle scattering and its absence in the more thoroughly understood inelastic electron scattering. Further measurements of both electron and hadron inelastic scattering are needed to resolve this discrepancy. $\square$

Physics in archaeology

from Robert Hedges

TRADITIONALLY, Archaeometry Symposia focus on the development and application of physical methods to archaeology, and there were no papers on environmental or biological issues, although two studies on the survival and extraction of organic residues were presented. Broad categories, often leading to parallel sessions, of dating, prospection, artefact analysis with the aims both of reconstructing ancient technologies, and determining the original point of manufacture, and mining and extractive metallurgy continued to provide the bulk of the research reports.

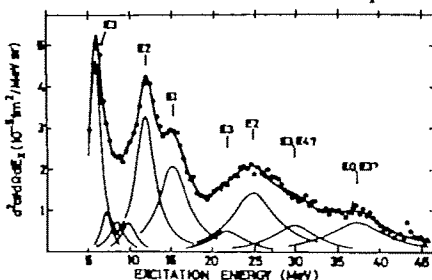


Fig. 1 Spectrum of 91.2 MeV electrons inelastically scattered from ^{140}Ce at 90° , showing the peaks due to the electric resonances of the multipolarities indicated. This spectrum is analysed into its components.

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*The 19th International Symposium on Archaeometry and Archaeological Prospection was held on 28-31 March in London.

Dating was emphasised by the presentation of three longer reviews, outlining recent progress. D. Walton (University of Oxford and McMaster University) described the measurements made of the strength of the Earth's magnetic field from small samples taken from dated Greek pottery, using a SQUID magnetometer. The resulting curve shows rapid fluctuations in the field strength which, apart from their general interest, he pointed out might eventually be useful for precise dating. This account was nicely contrasted by one from J. Pilcher (University of Belfast) who reviewed the latest story on radiocarbon calibration. Here the rapid fluctuations ('wiggles') in the calibration curve have been a source of contention for several years, but it seems that, with the development of more accurate measurements, an honourable compromise is being reached. The new possibilities opened to radiocarbon dating through the development of detection by accelerators were also reviewed. Several papers were devoted to the dating of calcitic cave deposits from the disequilibrium found in the daughter products of uranium incorporated into the calcite. This approach, which bridges the gap between postassium/argon and radiocarbon dating, is clearly becoming more useful. Technical papers on thermoluminescence dating of pottery, flint and calcite showed that the time when it can provide a problem-free dating service is still some way off. The same also applies to dating by amino acid racemisation.

Research into prospection methods has diminished somewhat over the years, most papers being application reports or describing advances in data-processing techniques. The analysis of artefacts remains a very strong centre of interest, however. The sourcing of such raw materials as jade, obsidian, sanukite, marble, as well as pottery and glass, from measurements of characteristic trace element concentrations (usually by neutron activation) and also of stable isotope ratios were described. The relationship of metal composition to the parent ore also was the subject of discussion, since the smelting process, and especially the addition of fluxes, can complicate this. Fortunately the excavation of mining sites, such as the Bronze Age site of Timna (in the Negev), in which ore, slag, smelted metal and artefacts are all available for study, can add much information to a perennially-disputed area. Although no radically new method or material for determining provenance has been introduced, the quality of work now able to exploit the general principles

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Unified theories?

THE analogy to Maxwell's unification of electricity and magnetism and the so-called 'unified' theory of weak and electromagnetic interactions expressed in F. Close's report in *Nature News and Views* 277, 349; 1979) "an intellectual achievement comparable with Maxwell's unification of electricity and magnetism", is an overstatement. Because however this statement is amply and repeatedly expressed in all journals, and even in the general press, it should be emphasised that the latter theory is not a unified theory on the level of Maxwell's unification. There are two coupling constants G and e of the weak and electromagnetic interactions before the 'unification', and there are still two coupling constants (parameters) after the 'unification', only mixed. Moreover, a large number of new (unobserved) fields are introduced here, but not in Maxwell's case. Two

F. CLOSE REPLIES: In his book 'The First Three Minutes' Weinberg writes: "A field theory which *unifies* [my italics] (weak and electromagnetic) forces was proposed by myself . . . and Salam". It is the continuing accumulation of data supporting this theory that leads me and others to be so optimistic about it. On the other hand I recognise that there are important predictions that are as yet untested, hence my reference to it still as a candidate theory.

If the theory is eventually confirmed to be truly unified (in the sense of involving only one coupling constant,) then I would regard that as an achievement comparable to Maxwell's. That is a personal opinion which Professor Barut might not share. However he is correct to remind us that the *unification* (as defined above) is not yet confirmed

general observations may be made.

(1) Given any two theories, one can always consider them together as part of a larger more symmetric system, if enough new fields (such as gluons, Higgses) are introduced. It is like putting any two broken shapes into a larger more complex or symmetrical figure if enough clay and glue is introduced. (2) A theory is the more renormalisable the more fields are present to cancel the divergences.

Under these circumstances gauge theories can be called unified field theories only if and when all these additional hypothetical gluons and Higgses are found and if there is only one coupling constant. Until then all so-called 'evidence' must remain circumstantial and we should call them simply gauge theories and not unified theories.

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even though no evidence against it has yet been found. The unified theory predicts that charged and neutral weak radiation exist and the corresponding quanta are $W^\pm$ and Z bosons with well defined masses. Just as the prediction of electromagnetic radiation was pivotal in electromagnetic theory so are the predictions of the masses of the weak quanta significant tests for the unified theory.

It is true that the properties of the Higgs fields are as yet unclear but I think that Professor Barut exaggerates by claiming that a "large number" of new unobserved fields are introduced. The inclusion of gluons in his list is particularly misleading. These have nothing to do with the weak-electromagnetic unification but are instead the gauge fields of QCD—the candidate theory of the strong force.

already established has considerably improved.

The original technical divisions of archaeology into Stone, Bronze and Iron Ages have become greatly refined as a result of more subtle studies of the materials manufactured in antiquity. Most studies centre on the interpretation of chemical analytical data, generally obtained by non (or minimally) destructive methods. These are enhanced by such sophisticated techniques as ESR, and Mössbauer spectrometry, from which, for example, information about the firing conditions of pottery, and possibly subsequent weathering on burial, is obtained. Several papers were given on coin analyses, as well as considerations on distributions of coin dies. The study of ancient metallurgy bene-

fits greatly from the information available from metallography. Thus we were able to consider how much the workers of Early Dynastic Egypt knew of the work-hardening properties of their copper tools, and how much Mediaeval armourers understood of the tempering properties of their steels. Modern experiments with reconstructed bows have confirmed theories of armour-piercing, and show that Mediaeval craftsmen, while perhaps not aware of the theory, nevertheless achieved optimum design in practice.

The overall impression left was of an encouraging increase in the quality of work presented. On the whole the archaeology considered was more appropriate and interpretations better judged. □

review article

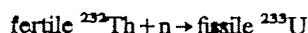
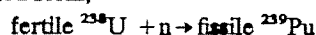
The use of high energy accelerators in the nuclear fuel cycle

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Nuclear power is at present derived from the burning of ^{235}U which constitutes about 0.7% of the natural uranium resource. A long-term supply of nuclear fuel for electrical power generation would require breeders to convert ^{238}U , or ^{232}Th , into fissile material. Accelerator breeders offer an alternative to the fast breeder reactor for this conversion.

THE nuclear-power industry in most of the world is based at present on light-water-cooled thermal reactors (LWRs) either of the pressurised water or the boiling water type. These reactors have proved to be reliable and economical; they supply more than 10% of the electric power generated in the US. In the present nuclear-fuel economy, LWRs burn naturally occurring fissile uranium (^{235}U) concentrated to about 3% of the total fuel volume; the remaining fuel consists of the element ^{238}U . Isotope ^{238}U is called 'fertile' because it can be converted to fissile plutonium (^{239}Pu) which can then be used as a nuclear fuel; thorium (^{232}Th) is also fertile. Conversion from fertile to fissile material is accomplished by neutron capture in fertile nuclei. Thus, in simplified form,



This conversion occurs in every reactor. LWRs have a conversion ratio (fissile material created to fissile material burned) of about 0.6. If this ratio can be made larger than 1, the reactor breeds: it produces more fuel than it burns.

Natural uranium contains only 0.7% fissile ^{235}U , the remainder being ^{238}U . To achieve the ~3% ^{235}U concentration required for LWR fuel, mined uranium is processed in an isotopic separation plant. About 6 tonnes of natural uranium are required to produce one tonne of fuel at 3% concentration. The remaining 5 tonnes, still containing about 0.2% ^{235}U , accumulate at separation plants as waste. The 1 tonne of 3% enriched uranium produces about 2.3×10^6 kWh of electrical energy ($30,000 \text{ MW d}^{-1} \text{ tonne}^{-1}$ (thermal)) in an LWR.

If we assume the useful life of a 1,000 MW(e) nuclear reactor to be 30 yr, its fuel requirements will be about 6,000 tonnes of natural uranium over that period, of which only 1,000 tonnes will go through the reactor burn cycle and end up as spent fuel. Spent fuel still contains ~2% fissile material, about half of which is plutonium and the remainder unburned ^{235}U . Approximately 30% less fuel would be needed were the ^{239}Pu recovered from the spent fuel by chemical reprocessing.

The natural uranium resource in the US has been estimated to be anywhere from 1.5 to 4 M tonnes (refs 1, 2). These estimates include uranium which can be recovered at a cost of less than \$30 per pound of yellow cake (U_3O_8). This resource can only support 250,000–600,000 MW(e) respectively of nuclear power generation capacity based on present once-through LWR fuel cycle. Domestic resources in the US would be sufficient to supply this country's foreseeable power needs well into the twenty-first century using only the once through LWR cycle. However, many other countries, having little or no uranium resources, are not so fortunate. Therefore, the present generation of LWRs, operating on the fuel cycle described here, is not a long-term solution to the world electrical energy problem.

The development and commercialisation of the breeder reactor has been described as the answer to the full use of the world's

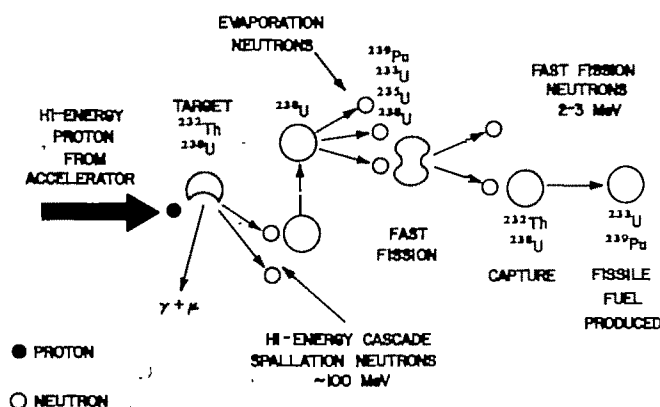


Fig. 1 Nuclear reactions produced in the accelerator breeder target.

uranium resources. As the breeder reactor has a breeding ratio greater than 1, essentially all the naturally occurring fertile ^{238}U in the fuel element could be rendered fissile while at the same time power was produced. Using the breeder reactor, the uranium fuel resource could be extended 100 times, and with higher priced reserves becoming economical, an almost unlimited energy source would become available. However, breeder reactor development has been slower and costlier than first estimated. In addition the concomitant chemical reprocessing facilities, are seen as presenting a serious risk of nuclear weapons proliferation. As such, the desirability of the breeder-reactor fuel cycle is now being seriously questioned. Other reactor types have near-breeding characteristics, such as the CANDU thorium cycle, and the gas-cooled reactor which also uses ^{233}U fuel. These reactors can stretch the fuel resource, but their fuel cycles have not been developed to the same extent as that of the liquid-metal cooled fast breeder reactor.

Accelerator breeding provides an alternative means of greatly extending the natural uranium fuel resource^{3,4}. It can produce the fissile fuel for present-day, well-developed light water reactors, whilst maintaining the high proliferation resistance of the present fuel cycle.

The background to accelerator breeding

The growth of accelerator technology can be attributed wholly to government funding for basic research in nuclear and elementary particle physics over the past 30 yr. Among the many types of accelerators developed, one in particular offers the potential to accelerate large beam currents (many mA)

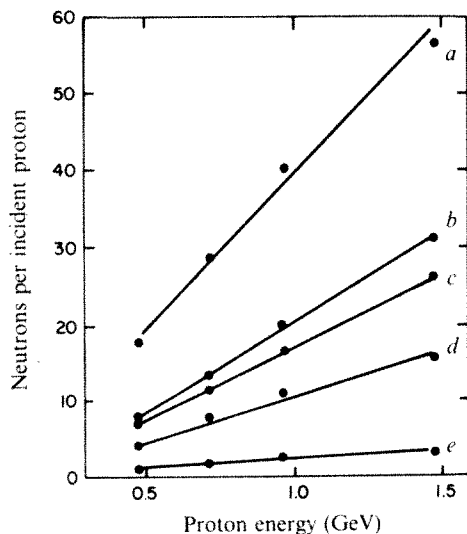


Fig. 2 Measured yield of neutrons by bombardment of a heavy metal target with high energy protons. a, U, 10.2 cm diameter $\times$ 61 cm; b, Pb, 20.4 cm diameter $\times$ 61 cm; c, Pb, 10.2 cm diameter $\times$ 61 cm; d, Gn, 10.2 cm diameter $\times$ 61 cm; e, Be, 10.2 cm $\times$ 10.2 cm $\times$ 91.6 cm.

continuously to very high energies (thousands of MeV)—the linear accelerator^{5,6}. A linear accelerator added to the nuclear fuel cycle makes a different method of producing fissile material possible. This uses the neutrons produced by a nuclear reaction in the target struck by the high energy beam.

At very high energies, charged particles (in this case protons or deuterons generated by the accelerator) interact with the target material, mainly through nuclear inelastic collisions.

When the particle hits a complex nucleus, nucleons are ripped off, or spalled from the nucleus. The energy of the spallation products is sufficient to induce additional spallation in an intra- or inter-nuclear cascade^{7,8}. Ultimately, the energy of the incident particle not carried away by the escaping particles will be shared by the remaining nucleons of the target nuclei leaving the residual nuclei in a highly excited state. This is followed by a statistical evaporation process in which nucleons and γ rays are emitted until the nuclei are de-excited. Thus, neutron multiplication occurs in any target material. Heavy materials, however, will result in larger neutron yield. Additional neutron multiplication will occur in fissile materials due to the fast fission process⁹. Figure 1 shows the processes involved in a uranium or thorium target struck by a high energy proton. Figure 2 shows measured values of neutron multiplication for targets of different materials and given geometry at different proton energies¹⁰.

The earliest exploration into the use of accelerators to produce fissile materials was made by a group at Berkeley in the early 1950s. The device was called the MTA (materials testing accelerator¹¹). The objective was to produce plutonium for the weapons programme from the tails low in ^{235}U accumulating at the Oak Ridge diffusion plant. The feed to the separation plant at the time was mostly uranium from abroad, principally the Belgian Congo and South Africa. If the diffusion-plant tails could have been turned into plutonium, this would have freed the US from dependence on a foreign source of uranium. The project was abandoned when substantial uranium deposits were discovered in the western US. Construction of the accelerator had already begun when the MTA project was ended in 1954, and an accelerator prototype had operated successfully.

In 1952, Lewis, in Canada, had considered the idea for the production of power¹², and in the 1960s, the Canadian concept had evolved into the intense neutron generator (ING) which would have supplied an intense neutron source for research¹³. Although the project was not funded because of financial constraints the Canadians have continued development of the accelerator breeder and its use in the Canadian CANDU reactor fuel cycle¹⁴. Recently, interest has grown because of the success of new linear accelerator designs and the interest in alternative ways of producing nuclear power and nuclear fuels. This led to a 1977 symposium¹⁵ held at Brookhaven National Laboratory¹⁵, which coincided with increased concern over risks of proliferation of nuclear weapons.

Research status and system outline

Present interest in accelerator breeders is mainly concentrated in North America. A sustained programme has been continued for years at Chalk River, Canada. The Canadian team has worked on all aspects of the accelerator breeder as a producer of fissile ^{233}U from thorium to fuel CANDU reactors. Since the CANDU reactor burning ^{233}U , has near-breeding characteristics (conversion ratio ≥ 0.9) a single 0.3 A–1 GeV accelerator breeder could supply fuel for about 10 reactors. This fuel cycle does, however, require chemical reprocessing like that of the breeder reactor, but this fuel cycle also makes perhaps the most efficient use of the accelerator breeder.

The work carried out at Chalk River includes studies, development, and prototyping of new accelerator structures, detailed calculations and experimental validation of target neutronics, and system studies within the context of the Canadian nuclear power programme.

Los Alamos Scientific Laboratory plans to undertake a major experimental programme at the 800 MeV proton accelerator, LAMPF (linear accelerator meson physics facility) to study breeding, target neutron yield, and fissile material production rates. Oak Ridge and Argonne National Laboratories have had small feasibility study programmes dealing mostly with neutronics aspects, systems and economics.

Finally, Brookhaven National Laboratory has a programme to study feasibility of the accelerator breeder and its economic desirability within the context of US policy of a proliferation-resistant fuel cycle. There is also the intention to produce fuel for existing LWRs¹⁶. In this sense the approach is quite different from that taken by the Canadians. The Brookhaven study has yielded two highly proliferation-resistant nuclear fuel cycles showing promise in terms of technical feasibility and economic desirability.

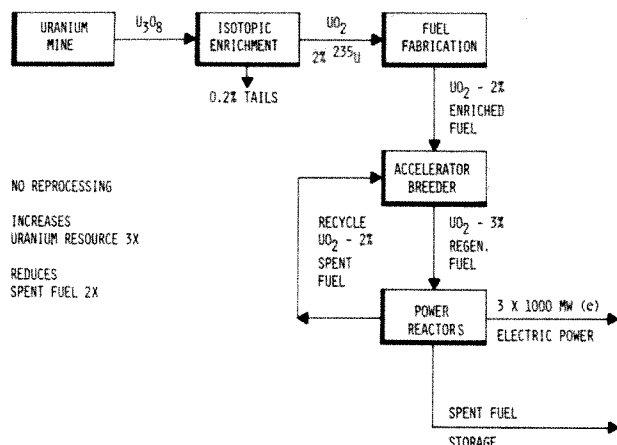


Fig. 3 Linear accelerator fuel regenerator nuclear fuel cycle.

Figure 3 shows the linear accelerator fuel enricher regenerator (LAFER) fuel cycle. It is called an enricher regenerator because its main function is the topping up of the initial fuel enrichment before burning and to rejuvenate the burned or spent fuel elements discharged from the reactor to make possible a second burn cycle. Thus, fuel assemblies that presently are enriched to ~3% ²³⁵U and burned to 30,000 MWD tonne⁻¹ could yield 60,000 MWD tonne⁻¹ with an initial enrichment of only 2%. No reprocessing of fuel is involved. This scenario would require 1 LAFER for each 3 LWRs, the LAFER being a 0.3 A-1.5 GeV linear accelerator with a calculated annual production of 1.1 tonnes of fissile material in conventional LWR fuel assemblies.

In principle, one could consider irradiating fuel assemblies in the LAFER through more than two cycles, with correspondingly better fuel utilisation and better economics. However, uncertainties concerning the effects of fission product buildup, and of radiation and corrosion damage to materials indicate that we should not consider more than about 60,000 MWD tonne⁻¹ burn-up at present.

The natural uranium requirement to fuel an LWR operating in conjunction with a LAFER is calculated to be 1,750 tonnes over its 30-yr life. This is about 3 times less than the 6000 tonnes presently required. Also the rate of generation of spent fuel is only half that of the conventional fuel cycle.

Another proliferation-resistant, accelerator-breeder fuel cycle is shown in Fig. 4. Here, the accelerator re-enriches spent fuel discharged from an LWR back to the fissile concentration required for burning. The fuel assemblies are then dismantled and refabricated without separation of the fissile material component of the fuel. Thus, reprocessing in this case removes the high vapour pressure fission products only. No stream of highly enriched or pure fissile material is ever present. And as the fuel is dilute and radioactive at all times, there would be little or no risk of theft. The fuel is then burned again in the conventional LWR. This approach to the accelerator-breeder fuel cycle has obvious advantages. Like the breeder reactor, it increases the uranium resource availability whilst remaining within the well proved technology of existing light-water cooled

reactors. Here again, one accelerator breeder would be required to support three 1,000 MW(e) power reactors.

The laboratories involved in these studies seem to agree that the linear accelerator technology for this application is well in hand and that the major technical problems reside with the target system. These problems concern the ability to optimise neutron multiplication and capture (the fissile fuel production rate) in the presence of severe engineering constraints such as radiation damage to materials, power dissipation and so on. However, substantial progress has been achieved during the past year and accelerator breeders are not only possible but could be built and demonstrated at a reasonable cost and within a period of around 10 years.

Brookhaven facility

The most advanced facility design is that developed at Brookhaven National Laboratory. This facility would consist of two distinct parts: a 0.3 A-1.5 GeV linear accelerator, or linac, and its associated target-blanket assembly with power generation system.

The head-end of the linear accelerator would consist of an Alvarez drift-tube linac¹⁷, operating at a radio frequency of about 200 MHz up to an energy of 150 MeV. The Alvarez structure would be followed by a so-called $\pi/2$ mode disk-and-washer loaded waveguide structure, up to the final energy. This type of structure has been developed successfully in the USSR¹⁸. An acceleration rate of 1.5 MeV⁻¹ seems close to optimum. The 1.5 GeV linac is about 1000 m long. The design of the accelerator lies well within present capabilities and no fundamental

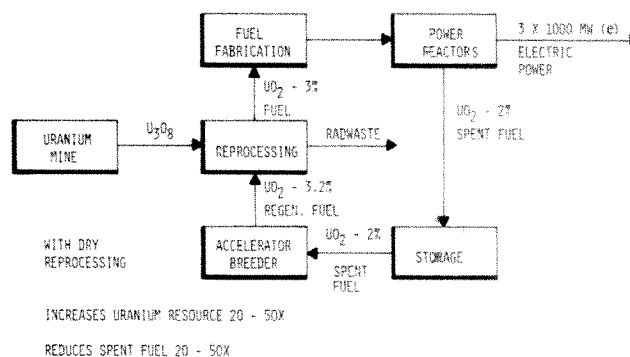
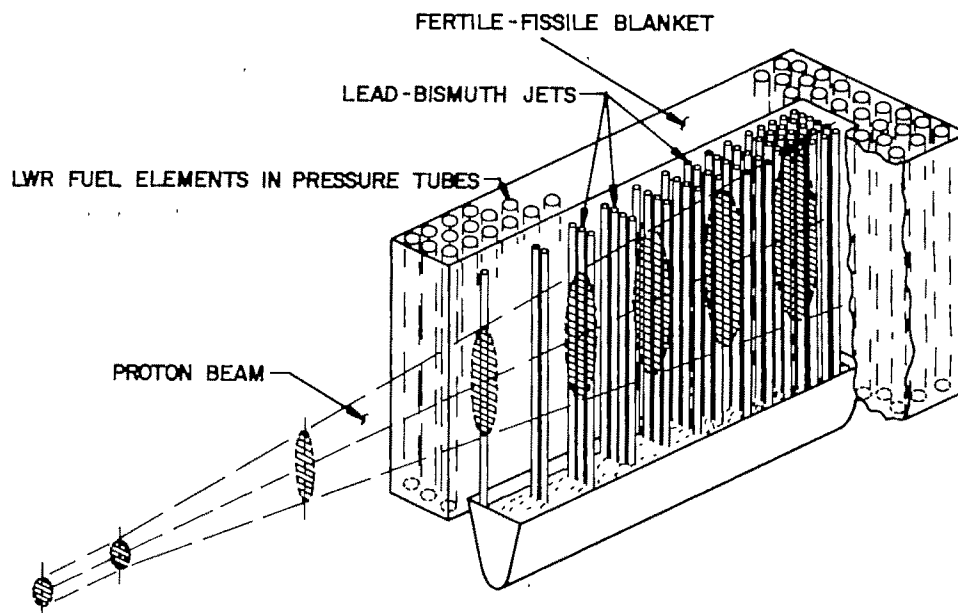


Fig. 4 Linear accelerator fuel producer nuclear fuel cycle (with reprocessing).

problems are anticipated. Experience with linear accelerators indicates that such machines can be operated without appreciable beam loss during acceleration, so that residual radiation will not handicap maintenance of the machine.

The distance between the target system and the accelerator is about 100 m. This drift length, containing isolation valves and instrumentation, will keep the accelerator free of any contamination by the high-radiation target area. Figure 5 shows the design of the target. It consists of a primary lead-bismuth target, stopping the entire proton beam, and of a secondary target (blanket) containing the fertile uranium material. The primary target consists of falling liquid lead-bismuth columns or jets arranged to provide an evenly distributed neutron source. This is accomplished by providing a variable target density with respect to target depth, and by stepping the target to intercept different parts of the proton beam at different points within the neutron source area. The liquid-bismuth jets operate in the

Fig. 5 Accelerator breeder target-blanket concept.



vacuum of the containment vessel, which is directly connected to the accelerator by the beam transport system. No window material is required between the accelerator and the target. The uranium fertile material in the blanket consists of LWR fuel assemblies located in individual pressure-tubes. These surround the primary target neutron source in a manner consistent with minimisation of neutron leakage, parasitic absorption and mechanical design constraints. The assemblies of fuel, coolant and moderator are designed to remain highly subcritical in any circumstance. During an irradiation cycle, fuel shuffling is required to achieve good control of reactivity buildup. The neutron multiplication due to fast fission in the blanket produces thermal energy. This energy, as well as that generated by the proton beam in the lead-bismuth target, is used to generate electrical power by a conventional steam-turbine system. The safety considerations applicable to the target-blanket system are the same as those for an LWR, with the notable exception that the target of the accelerator-breeder is subcritical. However, the entire target-blanket and primary coolant systems are in a containment building similar to that of a conventional power reactor with the same safety features.

Economics and future prospects

Economic analyses carried out at Brookhaven and elsewhere indicate that the cost of fuel produced by an accelerator breeder is high, between \$200 and \$300 per gramme of net fissile fuel produced. This compares with the present cost of $< \$100 \text{ g}^{-1}$ of net ^{235}U burned in LWRs.

A better indication of the accelerator breeder economic prospects is obtained by looking at the entire nuclear fuel cycle cost, or rather, the cost of electric power to the consumer. On that basis, the consumer power cost was calculated using the accelerator breeder in conjunction with LWRs as shown in Figs 3 and 4. It was found that the cost per kWh would increase by

about 25%. At present, therefore, the accelerator breeder cannot compete on a strict economic short-term basis with the other means available to produce the fuel required for power reactors, namely the mining and enrichment of natural uranium. But will things change in the future?

The projected scarcity of uranium had led to the development of the breeder, and the cost of electricity produced by the breeder fuel cycle will be higher than what we are now paying (notwithstanding inflation). The accelerator-breeder-LWR fuel cycle cost may be competitive with the reactor breeder fuel cycle cost. There are also other cost parameters more difficult to assess. For example, what are the cost benefits of the continuing exploitation of the same, and well developed, LWR technology, of the inherent proliferation resistance of the fuel cycle using the accelerator breeder, of the environmental impact of decreased mining activity (especially as we move to lower yield ore bodies) and of the ability to use spent fuel now being stored indefinitely in storage pools?

Also, projections of nuclear fuel cycle cost are clouded with many uncertainties such as: projected cost of U_3O_8 , new licensing requirements for plant safety, spent fuel storage and waste disposal, safety problems specific to the breeder reactor, cost and social desirability of the nuclear fuel cycle of the future, and so on.

The economic picture painted here is one of confusion. It points out the many difficulties at arriving at an objective cost-benefit analysis and projecting the potential of the accelerator breeder in the nuclear fuel cycle, or of any other competing system as well¹⁹. We can conclude however, that accelerator breeding offers a real alternative to the breeder reactor. It does so while retaining the present safety of the existing LWR based nuclear fuel cycle. Accelerator breeders are an extension of existing technologies, and hence can be developed at a reasonable cost and could be used commercially within the next 20 years.

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articles

IUE observations of absorption by hot gas in the nebula NGC6888

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New UV observations of Wolf-Rayet stars obtained with the IUE satellite show narrow absorption components in the highly ionised species of SiIV and CIV. In the case of the WN6 star HD192163 each resonance transition in the above ions exhibits two components, one undisplaced in wavelength and one blueshifted. The latter components are shown to arise in the nebula NGC6888 surrounding HD192163, and these are the first observations of absorption lines arising in a nebula associated with an early-type star. The strengths of the 'interstellar' SiIV and CIV lines in four WR stars and two other early-type stars are compared. There seems to be no correlation in the strengths of these lines with either stellar distance or colour excess, suggesting that they arise not in the general interstellar medium but in material more intimately linked with the stars themselves.

ULTRAVIOLET observations by the Copernicus satellite of several early-type stars revealed sharp absorption lines in the resonance lines of highly ionised species of SiIV, NV and OVI (ref. 1). These lines have usually been attributed to the presence of a hot, low density coronal gas in the interstellar medium resulting from successive supernovae explosions and occupying much of interstellar space². Recent IUE observations of the X-ray binary source HD153919 (ref. 3) have shown the presence of sharp absorptions in SiIV and CIV, whose strengths are more consistent with the presence of an extensive region of photoionised gas associated with the X-ray source.

This article presents further observations of sharp 'interstellar' lines arising in highly ionised species. These are seen in the spectra of several WR stars which are being studied as part of an extensive investigation of this stellar class using the IUE satellite. The sharp absorptions in the SiIV, CIV and AlIII resonance lines of the star HD192163 are split into two

components, one undisplaced in wavelength and one blueshifted. The blueshifted components arise in the nebula NGC6888 surrounding the star, representing the first unambiguous observations of absorption lines in a nebula associated with a hot early-type star. The strengths of the observed sharp SiIV and CIV absorptions in four WR stars and two other early-type stars are compared. We conclude that there is no correlation between the strengths of these lines and either distance or colour excess, which suggests that their origin is not in the general interstellar medium but in material more intimately linked with the stars themselves.

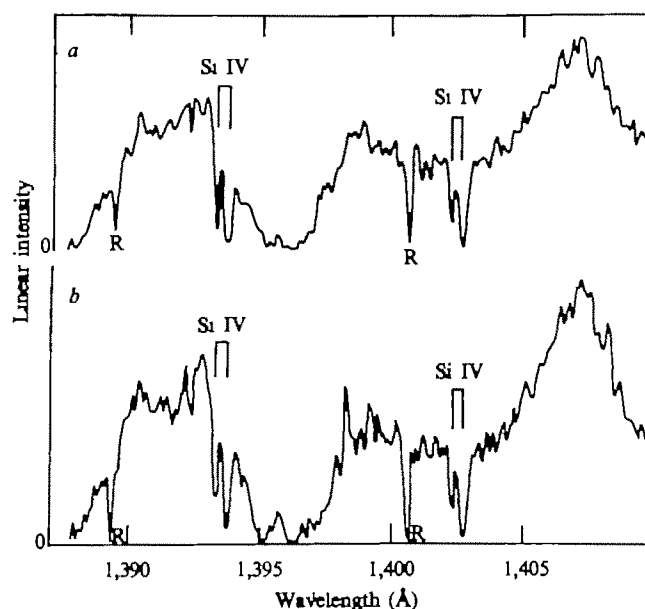


Fig. 1 The IUE spectra of HD192163 in the region of the SiIV 1,393.7 Å, 1,402.8 Å doublet. The two components to each SiIV line are marked R marks the position of a camera resseau. a, SWP 1668; b, SWP 2517.

Table 1 IUE observations of four wolf-rayet stars

Star	Sp	V	E(B - V)	d (kpc)	IUE images*
50896	WN5	6.94	0.12	1.5	SWP 1335
192163	WN6	7.73	0.55	1.4	SWP 2517, SWP 1618, LWR 1580
156385	WC7	7.45	0.25	1.6	SWP 2519, LWR 2304
165763	WC5	8.25	0.21	2.5	SWP 2518, LWR 2303

*SWP, short-wavelength prime camera and image number, LWR, long-wavelength redundant camera and image number.

Observations

The IUE satellite has been described by Boggess *et al.*^{4,5}. The present observations of four WR stars were obtained in the high resolution mode ($\delta\lambda \sim 0.1-0.2$ Å) and cover the full available wavelength from 1,150 to 3,200 Å. Details of the observations and some information about the stars are given in Table 1. The

spectral types and V -magnitudes are taken from ref. 6; the colour excesses $E(B - V)$ from ref. 7 and the distances, d , from these data and the absolute magnitudes given in ref. 8.

Table 2 Equivalent widths, W_λ , velocity shifts, v , and column densities for the displaced SiIV, CIV and AlIII absorption lines observed in the UV spectrum of HD192163

Line	SWP 2517			SWP 1668		
	v (km s ⁻¹)	W_λ (Å)	log N	v (km s ⁻¹)	W_λ (Å)	Log N (cm ⁻²)
SiIV 1,393.7	86	0.21	13.36	97	0.17	13.27
SiIV 1,402.8	86	0.14	13.49	96	0.15	13.52
CIV 1,548.2	82	0.20	13.69	97	0.17	13.61
CIV 1,550.8	68	0.12	13.76	92	0.10	13.68
AlIII 1,854.7	93	0.15	12.96	97	0.12	12.86
AlIII 1,862.8	85	0.07	12.93	97	0.10	13.08

The raw data were reduced by the ESA observatory in Madrid and include corrections for relative intensities, background and echelle grating-blaze effects. The wavelength scales were determined by assigning the vacuum laboratory wavelengths to the narrow interstellar lines arising in low ionisation species (such as CII, 1,334.4 Å, 1,335.7 Å, OI 1,302.2 Å).

Sections of the two spectra of HD192163 covering the lines of SiIV 1,393.7 Å, 1,402.8 Å, CIV 1,548.2 Å, 1,550.8 Å and AlIII 1,854.7 Å, 1,862.8 Å are shown in Figs 1, 2 and 3 respectively. As well as the sharp components to these lines the SiIV and CIV lines are seen as broad P-Cygni profiles formed in the extended expanding atmosphere of the WR star. Each transition displays two sharp components; one, undisplaced at the rest frame of the other interstellar lines, and one, blueshifted. The velocities of the shifted components are given in Table 2 and are seen to be very similar, implying a common origin. The mean value is 90 ± 9 (r.m.s.) km s⁻¹.

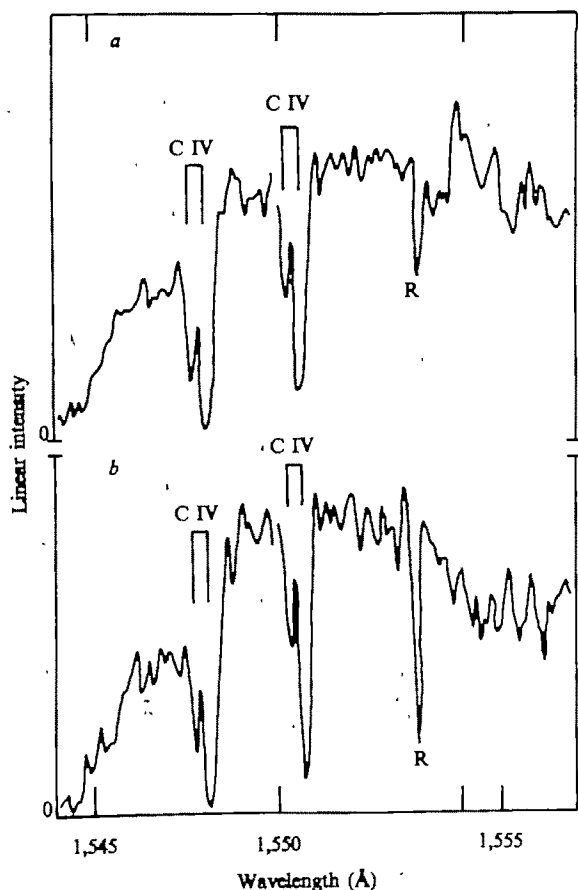


Fig. 2 The IUE spectra of HD192163 in the region of the CIV 1,548.2 Å, 1,550.8 Å doublet. The two components to each CIV line are marked. R marks the position of a camera resseau. a, SWP 1668; b, SWP 2517.

HD192163 is embedded in the nebula NGC6888, which is one of the ring nebula surrounding certain WN stars. Interferometric observations of this nebula in the visible H α , H β , and [NII] lines by Lozinskaya⁹ and Georgin and Monnet¹⁰ have demonstrated that the nebula is expanding from the central WR star with expansion velocities in the range 55–110 km s⁻¹. The infrared mean expansion velocity for NGC6888 is thus very similar to the observed velocity shifts of the sharp SiIV, CIV and AlIII displaced lines, demonstrating that these absorption components arise in NGC6888.

The NV 1,238.8 Å, 1,242.8 Å lines, however, are not seen in either displaced or undisplaced absorptions superimposed on the broad P-Cygni profiles of this doublet seen in the spectrum of HD192163. Their absence is discussed below. The 2,795.5 Å, 2,802.7 Å lines of the ion MgII are observed but are heavily saturated and broadened through normal interstellar absorption, thus masking any nebula component. Indeed the distance and reddening of HD192163 ensures that most of the other interstellar lines observed from common species are saturated, again masking any nebula components.

The measured equivalent widths and column densities for the nebula absorption components to the SiIV, CIV and AlIII doublets are given in Table 2; f -values for these transitions are from ref. 11. The column densities have been determined by assuming that these lines lie on the linear part of the curve of growth, an assumption confirmed by the fact that the doublet ratios for each ion are approximately in their expected ratio of 2:1. The strengths of the undisplaced components to these lines together with those observed in the spectra of other stars are given in Table 3.

Discussion

The electron density, N_e , and electron temperature, T_e , in the region of NGC6888 which gives rise to the observed optical emission have been determined as 400 cm⁻³ (ref. 12) and 19,000 K (ref. 9) respectively. The thickness of the nebula shell giving rise to the optical and radio emission has been determined as 0.01 pc (ref. 13). If it is assumed that the observed SiIV and CIV absorption arises in this region, the corresponding mean number densities of these species would be: $n(\text{CIV}) \sim 1.6 \times 10^{-3}$ cm⁻³ and $n(\text{SiIV}) \sim 8.3 \times 10^{-4}$ cm⁻³. Assuming that hydrogen is fully ionised in the nebula shell, $n(\text{H}) = N_e$, these numbers imply $n(\text{CIV})/n(\text{H}) \sim 4 \times 10^{-6}$ and $n(\text{SiIV})/n(\text{H}) \sim 2 \times 10^{-6}$. The further assumption of a normal cosmic abundance distribution in the nebula ($n(\text{C})/n(\text{H}) = 3.3 \times 10^{-4}$ and $n(\text{Si})/n(\text{H}) = 3.3 \times 10^{-5}$) would imply $n(\text{CIV})/n(\text{C}) \sim 10^{-2}$ and $n(\text{SiIV})/n(\text{Si}) \sim 0.06$. These values are completely at variance with the expected ionisation ratios for this region of the nebula. The ionisation balance calculations of Thuan¹⁴ for HII regions around OB stars indicate that with the effective temperature of the central WN6 star of 30,000 K (refs 7, 15) photoionisation is negligible for producing CIV and SiIV in the nebula, although this process could become significant if the stellar temperature were higher than 35,000 K. With $T_e = 19,000$ K and the ionisation balance calculations of Jordan¹⁶, Nussbaumer and Storey¹⁷ and Landini and Fossi¹⁸ the expected CIV/C and SiIV/Si ratios are both $\ll 10^{-4}$. Only by increasing the abundances of carbon and silicon in the nebula by over two orders of magnitude could the expected ionisation be accommodated by the observed SiIV and CIV column densities and the hydrogen density. Bearing in mind that the central star is a WN rather than a WC, this seems to rule out the optical and radio nebula region as the source of the SiIV and CIV absorption.

Johnson and Hogg¹³ have suggested that the nebula NGC6888 is formed by the sweeping up of the local interstellar material by the stellar wind of the central WR star, a hypothesis supported by Avedisova¹⁹ who showed that the mass of the nebula is consistent in this model with an original interstellar density of 1–2 atom cm⁻³. However, Wendker *et al.*²⁰ suggest that a substantial fraction of the nebula mass comes from the material shed by the WR progenitor during its evolution to the WR phase, a view supported by the recent evolutionary scenario

Table 3 Equivalent widths (Å) of undisplaced interstellar absorption lines in the ultraviolet spectra of four WR stars, ζ Oph and HD153919

Line	Star	HD192163 (SWP 2517)	HD192163 (SWP 1668)	HD50896	HD156385	HD165763	ζ Oph	HD153919
	<i>E(B-V)</i>	0.55	0.55	0.12	0.25	0.21	0.32	0.52
	<i>d</i> (kpc)	1.4	1.4	1.5	1.6	2.5	0.2	1.2
SirV 1,393.7		0.32	0.29	0.24	—	—	0.02	—
SirV 1,402.8		0.34	0.30	0.17	0.10	0.04	0.02	0.10
CrV 1,548.2		0.41	0.39	0.40	0.11	0.12	0.01	0.21
CrV 1,550.8		0.32	0.31	0.32	0.04	0.04	—	0.11
AlIII 1,854.7		0.18	0.19	0.11	0.16	0.12	0.06	—
AlIII 1,862.8		0.13	0.11	0.09	0.05	0.06	0.03	—
CII 1,334.5		0.60	0.70	0.28	0.30	0.50	0.22	—
CII 1,335.7		0.50	0.50	0.20	0.25	0.35	0.13	—
OI 1,302.2		0.60	0.45	0.24	0.40	0.36	0.20	—
SiII 1,526.7		0.50	0.55	0.13	0.24	0.45	0.15	—
SiII 1,304.4		0.50	0.40	0.16	0.17	0.30	0.13	—
SiII 1,260.4		0.70	0.75	0.25	0.60	0.60	0.22	—
SiII 1,259.5		0.34	0.40	0.20	0.30	0.28	0.15	—
SiII 1,253.8		0.37	0.30	0.18	0.22	0.30	0.11	—
SiII 1,250.6		0.26	0.30	0.20	0.18	0.25	0.10	—
MgII 2,802.7*		1.00	—	—	0.70	0.90	0.32	—
MgII 2,795.5*		1.20	—	—	0.80	0.90	0.31	—
MgI 2,852.1*		0.63	—	—	0.39	0.45	0.20	—

*The MgI, II lines in HD192163 measured from the LWR 1580 image.

of Conti²¹ and Willis and Wilson⁷ linking the Of and WR stars, and the very high mass loss rates ($\sim 10^{-4} M_{\odot} \text{ yr}^{-1}$) that are now believed to be appropriate for the WR stars^{21,23}. In either case, as the velocities involved are large, considerable shock heating on the inner boundary of the nebula can be expected, with temperatures in this region perhaps lying in the range $3-6 \times 10^5$ K according to the model of Avedisova¹⁹. Therefore, the observed absorptions in the highly ionised SirV and CrV species are probably formed in this hot region of the nebula. Note that

from the observed column densities of SirV, CrV and AlIII and the ionisation balance calculations of Jordan¹⁶, Nussbaumer and Storey¹⁷ and Landini and Fossi¹⁸ for a low density plasma the infrared ionisation temperature is $\sim 60,000 \pm 8,000$ K, somewhat lower than predicted in the model of Avedisova¹⁹. If this temperature were correct, the expected NV/CrV ratio is $\sim 10^{-4}$ and thus the NV resonance line nebula absorptions would be expected to be negligible, in agreement with the present observations.

A comparison of the strengths of the undisplaced absorption components of AlIII, SirV and CrV seen in the spectra of HD192163 with those observed in the three other WR stars listed in Table 1; in the OfV star ζ Oph^{24,25} and the X-ray binary source HD153919 (ref. 3) is given in Table 3. Also included in Table 3 are the measured strengths of the interstellar lines of the resonance lines of several common ions observed in these stars, the colour excesses $E(B-V)$ and the distances to these stars. The strengths of the low ionisation interstellar lines in HD192163 and ζ Oph are in the ratio $3(\pm 0.4):1$, which is broadly in line with the colour excess ratio of 2:1 bearing in mind the saturation effects which are present in most of these lines. However, the corresponding ratio for the high ionisation SirV and CrV lines is ~ 20 , completely at variance with the colour excess and low ionisation gas column ratio. The corresponding ratio of the strengths of the SirV and CrV lines in HD192163 and HD153919 is $\sim 3:1$, although in this case both the colour excesses and distances are very similar. There thus seems to be little correlation between the strengths of these sharp 'interstellar' lines of SirV and CrV with either distance or colour excess.

Furthermore, a comparison of the strengths of these absorptions in the four WR stars currently observed in our IUE programme, confirms this assertion of a lack of a correlation with either distance or colour excess. Such a comparison for the CrV 1,548.2 Å, 1,550.8 Å doublet observed in the four WR stars listed in Table 1 is shown in Fig. 4. The most distant star, HD165763, exhibits the weakest CrV absorptions, whilst the least reddened star, HD50896, shows these lines with strengths comparable to those observed in HD192163. Although largely saturated the low ionisation interstellar lines listed in Table 3 have strengths in the four WR stars which are broadly in line with the relative stellar distances and colour excesses.

Conclusion

In the present sample of stars there seems to be no correlation between the strengths of sharp interstellar absorptions in the

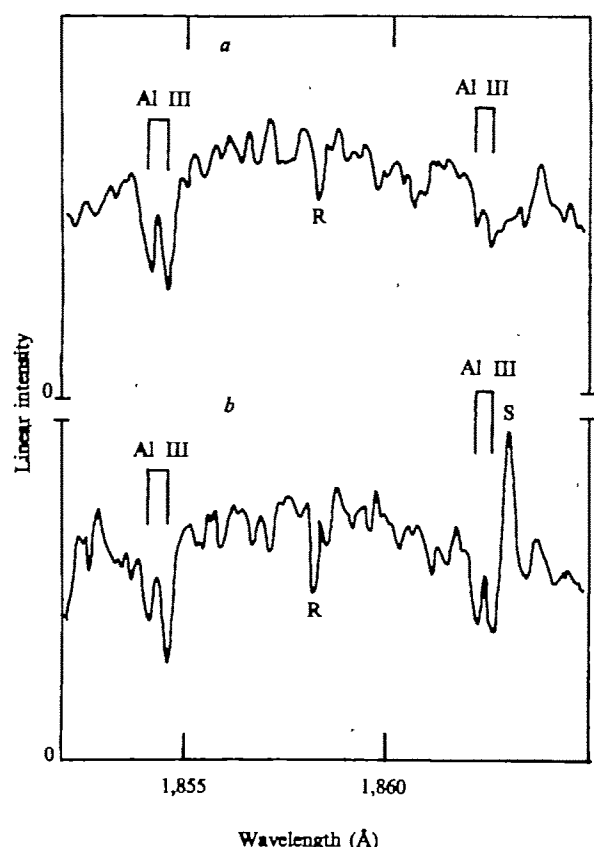


Fig. 3 The IUE spectra of HD192163 in the region of the AlIII 1,854.7 Å, 1,862.8 Å doublet. The two components to each AlIII line are marked. R marks the position of a camera readout and S that of a noise spike in the data. a, SWP 1668; b, SWP 2517.

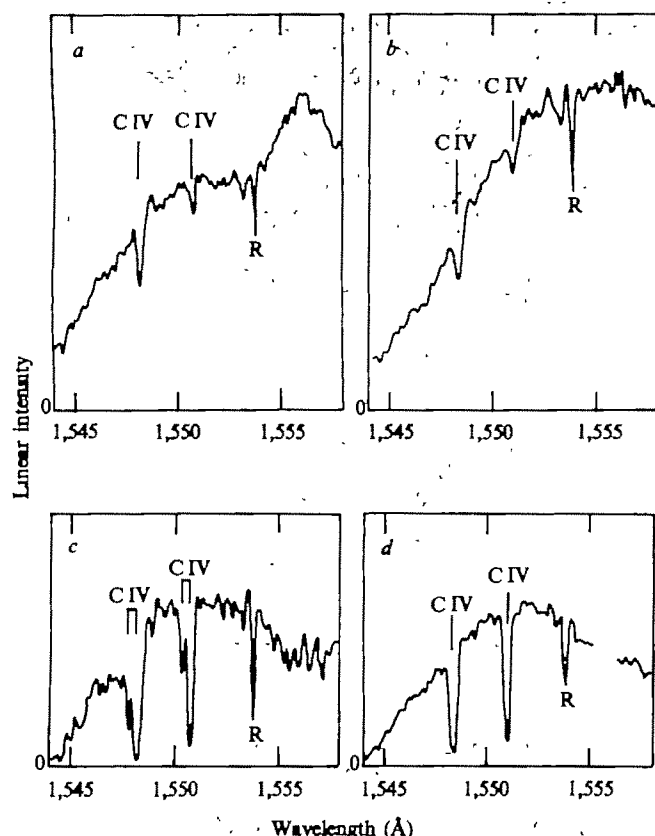


Fig. 4 The IUE spectra of the four WR stars: a, HD156385 (WC7); b, HD165763 (WC5); c, HD192163 (WN6); and d, HD50896 (WN5) in the region of the Crv 1,548.2 Å, 1,550.8 Å doublet. The sharp Crv interstellar components in each star are marked and, in the case of HD192163 the components that arise in the nebula NGC6888.

highly ionised species of SiIV and CIV with distance or colour excess which suggests that these features are not formed in the hot coronal gas permeating interstellar space. It seems more likely that their formation is a local effect closely linked to the WR stars themselves. A 'local' conclusion was also reached by Dupree *et al.*³ to explain the strengths of the highly ionised interstellar lines observed in the X-ray binary HD153919. They argue that the species are produced by photoionisation in the

radiation field of the X-ray source. In the present case it seems more profitable to consider the effects of the interaction of matter ejected by the WR stars and their progenitors with the interstellar medium.

Although there is no correlation between the strengths of the highly ionised interstellar lines in the four WR stars with other interstellar parameters, it is striking that they are much stronger in the two WN stars than in the two WC stars. The sample is too small to establish this as a real effect but the result may be linked to the different physical and chemical characteristics of the two WR sequences or their evolutionary state. Further observations of a larger sample of WR stars are clearly needed to establish this.

In the case of the WN6 star HD192163, it was shown above that absorption components in the SiIV, Crv and AlIII resonance lines also arise in the nebula NGC6888 surrounding the star. More detailed modelling of these lines together with observations of the corresponding nebula emissions in these and other species, should enable the properties of that nebula to be accurately determined, a topic under investigation.

Finally HD192163 has been observed to have a pronounced excess strength in its 2,200 Å band which is believed²⁶ to arise in NGC6888. It may be pertinent to consider whether the great strength of the 2,200 Å band and of the CIV interstellar lines reported here (the strongest yet observed) are in some way linked.

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Short-range structure for amorphous intertransition metal alloys

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Amorphous intertransition metal alloys may be a special class of the glassy state whose short-range structure is random tetrahedral close packing of soft atoms similar to those in the crystalline counterparts. A short-range structure is described based on random stacking of Kasper polyhedra and atomic disorder at the stacking misfit, and has tetrahedral sites, high atomic coordination numbers and asymmetric shapes of atoms.

A STRUCTURAL concept is introduced here for the amorphous intertransition metal alloys by alloying an early transition metal, T_1 , with a late transition metal, T_2 . These are shown in groups in Table 1. Recent investigation of formation, stability and properties of refractory amorphous alloys of W-Fe (ref. 1), Mo-Co (ref. 2), and Mo-Ni (ref. 3) at crystalline μ -phase or δ -phase compositions indicates that these amorphous alloys may have a crystal-like, short-range structure that deviates from the dense random packing of hard spheres (DRPHS)⁴⁻⁶. Consequently, it has been suggested that the amorphous structure of a $\text{Mo}_{46}\text{Co}_{54}$ alloy is a random tetrahedral close packing of atoms resembling

Table 1 A division of transition metals of the Periodic Table

T ₁ element				T ₂ element			
Ti	V	Cr	Mn	Fe	Co	Ni	Cu
Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag
Hf	Ta	W	Re	Os	Ir	Pt	Au

the random interpenetration of Kasper polyhedra in three-dimensional space². This short-range structure for the intertransitional metal alloys is conceived from the concepts of Wang and Merz^{7,8} and Goodman⁹ that mixed atomic bondings of the crystalline counterparts are retained in the amorphous solids and contribute to their thermal stability. We present here evidence and structural features supporting the argument that short-range structure in this type of metallic glass should closely resemble that found in compositionally related crystalline phases.

Evidence for crystal-like short-range structure

Amorphous intertransition metal alloys probably have a crystal-like, short-range structure because of the following unique aspects of formation, structure and properties, which are closely related to those of the intermetallic compounds.

(1) Formation of the amorphous intertransition metal alloys is favoured by compositions that are at, or near, a eutectic point or a complex-structured intermetallic compound. Formation by quenching from the liquid state clearly benefits from an under-cooled liquid with composition near the eutectic point, while at high cooling rates (that is by vapour deposition) formation occurs at compositions of the intermetallic composition^{1-3,10}.

(2) Amorphous intertransition metal alloys generally show a sharp first diffraction peak that corresponds to a coherent diffracting domain size between 13 and 18 Å, depending on the method of preparation, composition and cooling rate^{1,2,11,12}. The sharp diffraction peak indicates a high degree of short-range order which was once considered as evidence of microcrystallinity for liquid-quenched Nb-Ni and Ta-Ni alloys¹². The calculated scattering intensities based on relaxed DRPHS models only qualitatively explain the details of the observed intensities for amorphous Cu-Zr alloys¹³⁻¹⁵. The discrepancy between the observed and calculated scattering intensities also was found in amorphous Nb₃Ge and Nb₃Si¹⁶, due to the asymmetrical nearest-neighbour maximum found in measured dis-

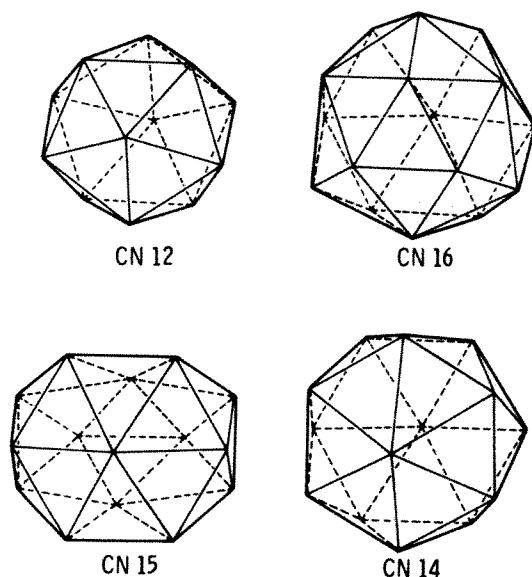


Fig. 1 The four triangulated coordination polyhedra with 5- and 6-fold vertices observed in the intertransition metal compounds (from ref. 27).

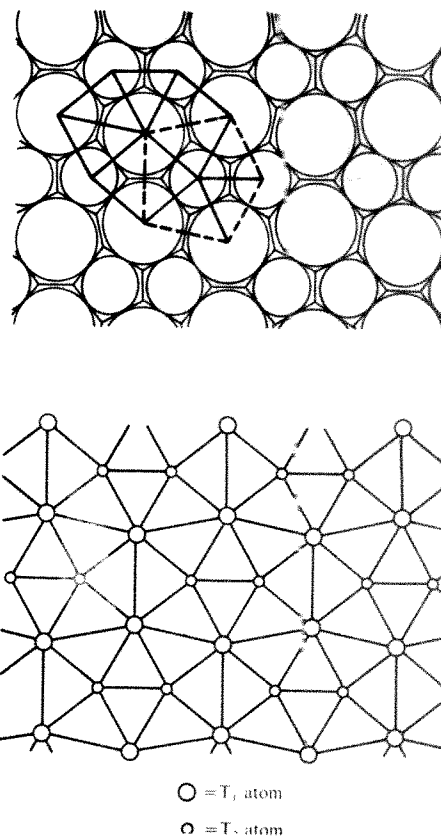


Fig. 2 Two-dimensional analogue of Kasper polyhedra to show irregular triangles that will be irregular tetrahedra in three-dimensional space (from ref. 26).

tribution functions for amorphous Nb₃Ge. Thus, local order in amorphous Cu-Zr has been considered as being related to the crystallised phases¹⁷.

(3) The densities, or packing factors, calculated from the DRPHS models were between 10 and 30% smaller than those observed for the amorphous alloys, depending on the relaxation processes^{16,18,19}. The measured densities of the amorphous intertransition metal alloys are closer to the values of the corresponding crystalline intermetallic compounds^{1,2}.

(4) High thermal stability is usually associated with the amorphous intertransition metal alloys¹⁰, that is, the crystallisation temperatures of Nb₄₀Ni₆₀, Mo₅₀Ni₅₀, and Zr₅₀Cu₅₀ are above 600 °C and those of W₅₀Fe₅₀, and Mo₄₆Co₅₄ alloys are above 800 °C^{1,2}. Several amorphous films of Ta-Re, Mo-Re and Mo-Ru²⁰ at the compositions near the crystalline intermetallic compounds also exhibit similar high thermal stabilities. On the other hand, thin films of single transition metals, such as Zr, Hf, Ta, Mo, Cr, W, and Re, are either microcrystalline or amorphous but stable only below room temperature^{20,21}.

(5) High microhardnesses ranging from 800 to 1,100 kg mm⁻² are typical for the amorphous intertransition metal alloys, even though the differences in electronegativity between the two alloying elements are small. The microhardnesses are comparable to those of the crystalline intermetallic compounds and indicate strong covalent bondings involving d electrons; these bondings also resemble those of the crystalline phases.

These properties suggest that amorphous intertransition metal alloys belong to a special class of glassy state that involves strong atomic interactions among d electrons—similar to those in the intermetallic compounds. Thus, the short-range structure is expected to incline more towards the short-range order of the crystalline phase and to deviate from the random close packing of hard sphere models as far as the metallic glasses and the simple liquids are concerned.

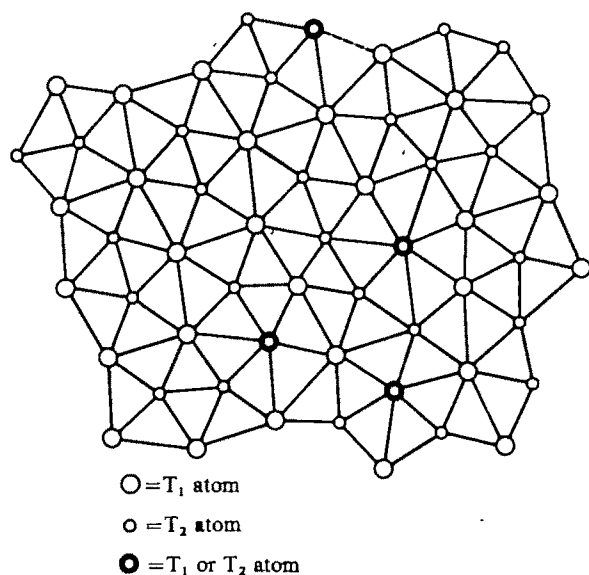


Fig. 3 Proposed structure for amorphous intertransition metal alloys as made by random stacking of irregular tetrahedra shown in a two-dimensional analogue.

Short-range structure

The short-range structure of the amorphous intertransition metal alloys is proposed to be random tetrahedral close packing of soft atoms. This structure is derived from the crystal chemistry of the intertransition metal compounds: the crystal structures are primarily based on tetrahedral close packing of atoms²²⁻²⁵. One can achieve packing with a single type of tetrahedral interstices only by irregularly shaped tetrahedra. Intensive analyses of the known structures of intertransition metal compounds have revealed that there are only four triangulated coordination polyhedra (the so-called Kasper polyhedra²²) that have 5- and 6-fold vertices as shown in Fig. 1. In many crystal structures, these polyhedra interpenetrate in such a way that every vertex atom becomes the centre of a polyhedron. Figure 2 shows the meshing of polyhedra (the 5-fold vertices, for instance). It is taken from Laves' analog²⁶ and is viewed in two-dimensional space. In this structural analogue, the T_1 atom has a CN 7 (seven neighbours close to each T_1 atom), and the T_2

atom has a CN 5 (five neighbours close to each T_2 atom). Crystal structures based on the stacking of these Kasper polyhedra for intermetallic compounds are discussed in detail by Kasper²², Shoemaker *et al.*^{24,25} and Bergman *et al.*²⁷.

Consequently, the proposed short-range structures of the amorphous intertransition metal alloys will also contain interpenetrated Kasper polyhedra similar to those occurring in the intermetallic compounds. The short-range structure of amorphous alloys may be constructed by random stacking of all four types of the Kasper polyhedra, and by introducing atomic disorder at the stacking misfit. A two-dimensional analogue can be used to show the structure of amorphous alloys (Fig. 3) and to compare it with that of the crystalline form (Fig. 2).

The amorphous structure was composed of two sets of stacking configurations based on the 5-fold vertices—the stacking configuration for the crystalline structure (Fig. 4) and the additional stackings uncommon to the crystalline structure (Fig. 5).

Due to long-range order, the stacking configurations for the crystalline structure are limited by three ways able to connect two vertices and one way able to stack four vertices (Fig. 4). However, the amorphous structure contains additional random stackings, as in Fig. 5, which shows both perfect matching of the vertices side by side and the stacking misfit where stacking cannot be matched at one atomic site.

Stacking misfit causes atomic disorder, that is ambiguity of atomic identity at the corner of the 5-fold vertex. For example, the atomic disorder at site 1 occurs because both T_1 and T_2 atoms should occupy this site if the edges of the vertex are made of four T_1 and one T_2 atom. This atomic disorder is common when more than two vertices are stacked randomly. The atomic disorder at site 2 is caused by stacking of three vertices in a specific configuration so that T_1 atoms form a triangle in which one side (connected by dash line) is too short for the normal T_1 - T_1 distance. Either this compresses the T_1 atoms, or one of the T_1 atoms will be replaced by a T_2 atom. In fact, these stacking misfits are also observed in intermetallic compounds at atomic coordination points 13 and 14 (ref. 28).

In constructing the amorphous structure analogue, many 5-fold vertices need to be distorted slightly from ideal shapes in the crystalline structure to lower the stacking misfits in the amorphous structural framework. These distortions were made by hand-adjusting the atomic positions in the vertices after the framework was completed. The atomic position adjustments would not usually exceed 10% of its interatomic distances. The distortion causes the final amorphous structure analogue, shown in Fig. 3, to contain a range of interatomic distances as shown in Fig. 6. As far as short-range atomic interaction is concerned, the two-dimensional amorphous structure analogue is similar to that of the crystalline structure, except a small portion of T_1 and T_2 atoms have the intermediate CN 6.

Discussion of the short-range structure

The structure described here is truly amorphous and contains continuous, random packing of atoms. It should not be considered as a microcrystalline model because there are no apparent line or surface discontinuities such as grain boundaries, and there is no periodicity in any direction. However, short-range atomic interactions, similar to those of crystalline compounds, are retained by stacking various types of polyhedra and atomic disorders at random. The types of polyhedra and the stacking configuration that dominate the amorphous structure would vary according to the composition, alloy system and cooling rate; primarily, however, they would be those of the nearby crystalline compounds, such as the μ -phase polyhedra²⁸ for amorphous $W_{50}Fe_{50}$, $Mo_{46}Co_{54}$, and $Nb_{50}Ni_{50}$.

Several features related to atomic size, coordination number and atomic interactions can be expected for the real amorphous intertransition metal alloys. The atomic size in these amorphous structures will be less specific because the atoms will assume more asymmetric shapes as the atomic coordination number increases from 12 to 16 (those observed in the intermetallic compounds). Consequently, high packing density and atomic

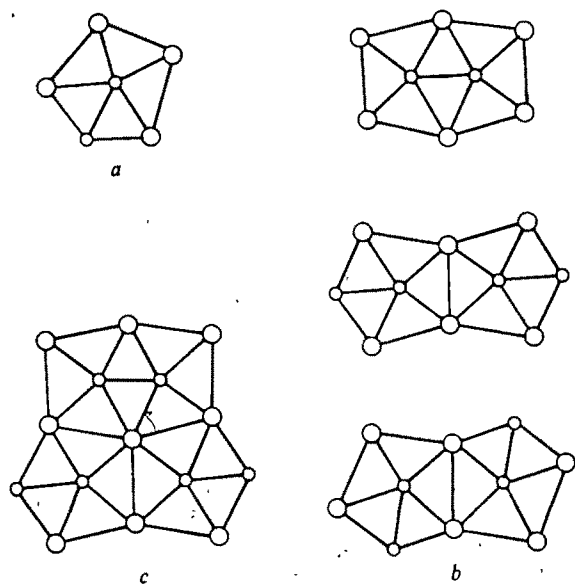


Fig. 4 Stacking configurations of the 5-fold vertices in both crystalline and amorphous structure analogues: a, a single 5-fold vertex; b, connections of two vertices; and c, stacking of four vertices.

interactions among unlike atoms are expected. These can be viewed from the two-dimensional structure analogue of Fig. 3 which consists of 29 T_1 atoms, 25 T_2 atoms and four disordered atoms. A packing density similar to that of the crystalline analogue is expected because T_1 atoms have CN 7 and CN 6, but T_2 atoms have CN 5 and CN 6, and small percentage (7%) of the disordered atoms. The occurrence of different atomic interactions (Fig. 6) shows that with a nearly 1:1 ratio of T_1 and T_2 atoms, there are almost twice as many T_1 - T_2 interactions than T_1 - T_1 interactions, and the T_2 - T_2 interactions are less significant. Diffraction studies have indicated that such strong atomic interactions occur among the unlike atoms for the real amorphous structures, such as Cu-Zr¹⁵, Nb₃Ge¹⁶, and RE-Tm^{18,19}.

Relationship to the formation and thermal stability

The formation and thermal stability of the short-range structure that contains a mixture of all four types of Kasper polyhedra can be understood as follows. The polyhedra represent possible low-energy configurations for atoms to be bonded in the solid, in positions that are separated by small energy differences. During quenching from the liquid or vapour state, the availability of four types of polyhedra allows several alternatives, or saddle-point energies, for atoms to be bonded to their neighbouring atoms in a way that prevents long-range atomic rearrangement. The total energy will not be significantly influenced by those small energy differences as long as the atoms are locally satisfying the requirement for strong d-d electron interactions. This explains the formation and stability of the amorphous inter-transition metal alloys.

For this type of amorphous structure, resembling a short-range ordered version of the crystalline phases, those factors governing the stability of the intermetallic compounds, such as the electron-to-atom ratio (e/a), the electronegativity, and the atomic size difference, would also be important for the forma-

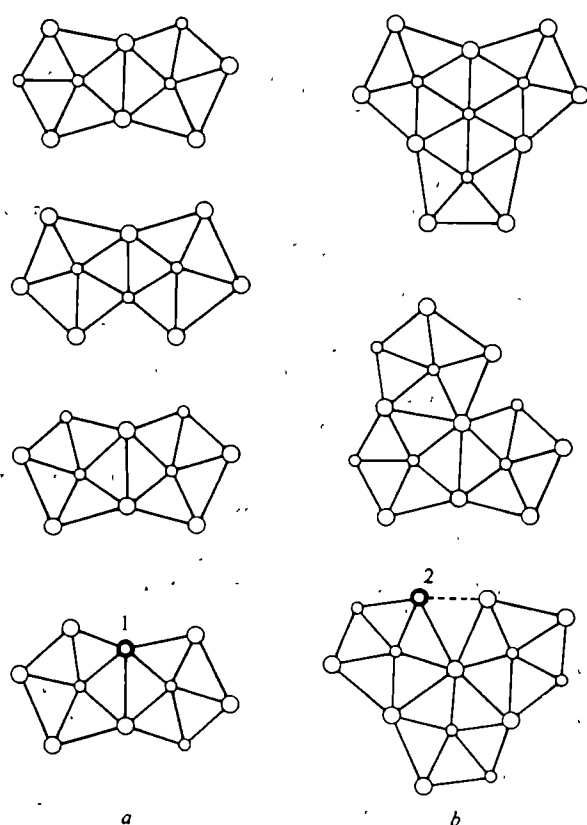


Fig. 5 Additional stackings of the 5-fold vertices with and without stacking misfits in amorphous structure analogue *a*, connections of two vertices; and *b*, stackings of three vertices

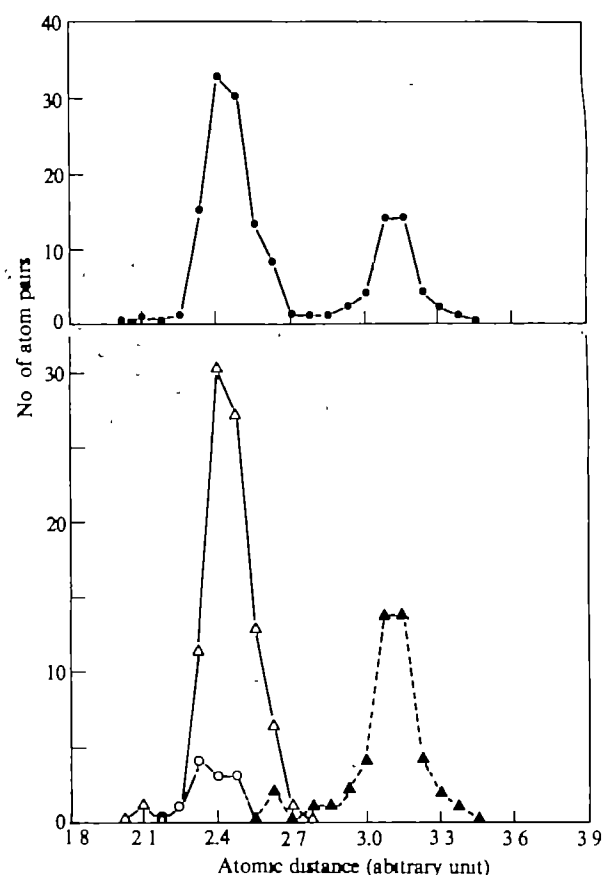


Fig. 6 Number of atom pairs and their distances on an arbitrary scale for the two-dimensional amorphous structure analogue of Fig. 3. Δ - Δ , T_1 - T_2 ; $\blacktriangle$ - $\blacktriangle$, T_1 - T_1 ; $\circ$ - $\circ$, T_2 - T_2 ; $\bullet$, summation of atom pairs.

tion and the stability of the amorphous structure¹⁰. In this regard, the short-range structure will apply to all the amorphous intertransition metal alloys whose crystalline counterparts follow the crystal chemistry principles as electron compounds. It is expected that binary and ternary amorphous alloys will be formed in the composition ranges in which intermediate phases such as σ , μ , δ , P and R phases are present. The above formation criteria and short-range structures can probably be extended to the other amorphous alloys based on Cr_3Si (A15), Laves (C14, C15, and C36) and $CaCu_2$ (D2_d) type phases that are related to the σ phase.

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Evidence of a new isotopic anomaly from titanium isotopic ratios in meteoric materials

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Titanium isotopic ratios in some terrestrial and meteoritic materials have been measured. Most have the same ratios within the experimental precisions. Some inclusion materials from the Allende meteorite have a statistically significant enhancement of the order of 1% in the 50/49 ratio, probably due to a nucleogenic anomaly in ⁵⁰Ti abundance.

SIGNIFICANT variations in the isotopic ratios of certain elements in some meteorites have been well established^{1–11}. Recent results^{10–13} show that some meteoritic materials may be simultaneously 'anomalous' in several elements. Numerous mechanisms^{14–18} have been proposed to explain these observations, and a common engendering phenomenon will probably be found for most, if not all, of the anomalies. To make a correct hypothesis, however, we need to establish the nature and magnitude of the anomalies in a large variety of elements.

A study of the isotopic composition of titanium in terrestrial and meteoritic materials, and especially inclusions in the carbonaceous chondrite Allende, has been undertaken, and some general results are reported here.

Titanium was studied because: (1) the titanium isotopic abundances observed in most materials are thought to be due to a combination of nucleosynthetic mechanisms¹⁹. Therefore it seems unlikely that the same pattern would arise from the phenomenon(a) which caused the anomalies in other elements; (2) most isotopic anomalies have been reported from refractory phases, and titanium is a refractory element in the classical condensation sequence²⁰; (3) carbonaceous chondrites are thought to have undergone less chemical and physical processing since their original formation, and inclusions in Allende have been the prime source of anomaly-bearing materials; (4) although no evidence of titanium isotope anomalies has been presented previously, uncertainties on the only reported study²¹ of similar materials were at a level sufficiently high ($\sim \pm 0.3\%$) for many anomalies observed in other elements to have been obscured.

Experimental techniques

Samples were chemically processed using standard dissolution and cation exchange methods^{22,23}. Blanks ran about 50 ng TiO₂. Samples containing about 50 μ g titanium were loaded onto triple rhenium filaments as aqueous or HF slurries, and were run on a mass spectrometer as described elsewhere²⁴. High side-

filament currents (about 3 A) were required to obtain sufficiently large ion currents ($\sim 10^{-13}$ A) for the less abundant isotopes. All data were taken using Ti⁺ beams, alternating between 47/49 and A/49, where A was 46, 48, or 50. A 'group' consisted of at least four 12 ratio sets for 47/49 and one set each for 46/49, 48/49, and 50/49, a minimum of six groups being obtained in the measurement of most samples. The amplifier zero level was measured at mass 52.5 twice during each group.

For all samples, the observed isotope ratios were found to change with time in a manner consistent with the Rayleigh single stage mass fractionation model, and to an extent comparable with that displayed by other refractory elements (such as Mg) (ref. 13).

To facilitate comparisons, all observed ratios were normalised to the value 1.3360 for 47/49, which is the weighted mean of the observed values obtained for the terrestrial materials and is assumed to be accurate to within $\pm 1\%$ of the true value. The ratio 47/49 was chosen as the basis for normalisation because there are no stable nuclidic isobars at these mass numbers, as there are for the other Ti isotopes. Observed ratios were automatically corrected for contamination from non-Ti nuclidic isobar ions as follows: masses 46 and 48 for Ca using mass 40; mass 50 for V using mass 51 and for Cr using mass 52. Data were not accepted if the isobaric contamination exceeded 0.1%. The entire mass spectrum between 38 and 56 was routinely recorded before the first group and after the last group for a given sample; a record between masses 45 and 53 was taken between groups. No data were accepted if base line irregularity greater than normal electronic noise levels between titanium mass numbers or unusual peak shapes were observed. In a few cases, it was necessary to correct for zero level drift (assumed to be linear with time) during a set. A few ($\sim 2\%$, mostly 48/49, which is more sensitive to transient baseline changes) outlying ratios and set means were rejected by Chauvenet's criterion in computing the means. In Tables 1–4, the uncertainties quoted are three times the sigma of the mean estimated from the observed dispersions of individual results. The three sigma level is preferable here to the more common two sigma level because, on average, one of every 20 replicate means at the latter level, but only one of every 250 replicate means at the former level, will be further from the grand mean than the quoted uncertainty.

Terrestrial materials

Titanium was isolated from the terrestrial materials listed in Table 1. More than 1,000 individual ratio measurements were made for each A/49 on samples prepared from these terrestrial

Table 1 Comparison of terrestrial materials

Material*		Normalised† Ti isotope ratios $\pm 3\sigma(\bar{x})\ddagger$		
		46/49	48/49	50/49
Ti metal	$\bar{x}_w$	1.4618 $\pm$ 0.0014	13.442 $\pm$ 0.004	0.9723 $\pm$ 0.0008
	$\bar{x}_u$	1.4622 $\pm$ 0.0025	13.443 $\pm$ 0.006	0.9726 $\pm$ 0.0010
NBS TiO ₂	$\bar{x}_w$	1.4614 $\pm$ 0.0008	13.441 $\pm$ 0.008	0.9730 $\pm$ 0.0002
	$\bar{x}_u$	1.4612 $\pm$ 0.0009	13.445 $\pm$ 0.012	0.9730 $\pm$ 0.0003
Potassium titanyle oxalate	$\bar{x}_w$	1.4616 $\pm$ 0.0008	13.442 $\pm$ 0.005	0.9728 $\pm$ 0.0005
	$\bar{x}_u$	1.4613 $\pm$ 0.0010	13.445 $\pm$ 0.006	0.9728 $\pm$ 0.0006
Rutile	$\bar{x}_w$	1.4614 $\pm$ 0.0009	13.443 $\pm$ 0.009	0.9723 $\pm$ 0.0006
	$\bar{x}_u$	1.4613 $\pm$ 0.0010	13.442 $\pm$ 0.010	0.9722 $\pm$ 0.0005

* Ti metal: Batch Ti/002, 99.9% Roc-ric Co. NBS TiO₂: SRM 154, US NBS. Potassium titanyle oxalate: BDH Analar grade, 99.93%. Rutile: Beachsand concentrate from south Queensland, supplied by the Bureau of Mineral Resources, Canberra. $\bar{x}_w$, weighted (by inverse variance) mean. $\bar{x}_u$, unweighted mean.

† Normalised to (47/49) = 1.3360.

‡ σ_x Estimated standard deviation of mean based on observed dispersion.

materials. Normalised results for measurements made in the period during which those on the meteoritic materials were also made are presented in Table 1 for both weighted (by inverse variance) and unweighted means, along with the three sigma of the mean uncertainty estimates. No significant differences were found between these recent means and those based on a comparable number of earlier measurements of the same (and other terrestrial) materials.

Because no significant differences among the various terrestrial materials are evident within the precision of the current measurements, all of this terrestrial titanium was assumed to be identical in its isotopic composition regardless of source, and the results were combined through weighted individual sample (six set) means, with results presented in Table 2 as terrestrial. The typical relative uncertainty of the means ($3\sigma_x/\bar{x} \sim 0.03\%$) is comparable to that reported recently by other laboratories for isotopic ratios of other elements in similar materials^{10,13}. Our values agree with most of the reported isotopic analyses of titanium well within the estimated $\pm 1\%$ accuracy. Therefore, our method apparently gives reasonable accuracy and precision for terrestrial materials.

Meteoritic materials

Allende whole rock powder: several samples were prepared directly from Allende powder (TiO₂ content 0.15%) obtained from the US National Museum (3529, S19, P20). The combined (weighted) normalised individual sample mean results of these measurements are given in Table 2.

The Allende whole rock powder titanium isotopic ratio results are not distinguishable from those for terrestrial materials within the experimental precision, which is comparable to that obtained for terrestrial materials. Therefore, our analytical methods seem to apply to Allende as well as to terrestrial materials.

Allende whole rock powder chemical leach fractions: Anders *et al.*^{25,26} have reported that most of the anomalous rare gases are associated with a small amount of the material in Allende, which they concentrated through a sequence of chemical leaches of Allende powder. From its chemical properties, they deduced this carrier to be a sulphide-rich phase. It has been proposed²⁷ that such a phase might be rich in ⁴⁶Ti sulphide derived from a condensing supernova shell. Therefore, a sequence of leaches similar (see Table 2 for details) to that used for the rare gas work^{25,26} was performed on two separate (~ 0.5 g) samples of the Allende powder described above. The titanium isotopic ratios of these various separate acid leach (two HCl, one HF, two HNO₃) samples were found to be the same within experimental precision, and all results were, therefore, combined (as six set means) to produce the values in Table 2. The overall relative precision is again roughly comparable with that observed for terrestrial materials. Included in these means are data from three samples which had been chemically reprocessed for titanium after the usual chemical treatment. The data for the reprocessed samples seemed to disperse in the same manner about common means as those for the one chemical cycle samples.

Table 2 Mean results for terrestrial and Allende materials

Material*	Normalised† Ti isotope ratios $\pm 3\sigma(\bar{x})\ddagger$			Mean no. of measured ratios included
	46/49	48/49	50/49	
Terrestrial	1.4613 $\pm$ 0.0005	13.441 $\pm$ 0.002	0.9728 $\pm$ 0.0005	359
Allende powder whole rock	1.4614 $\pm$ 0.0007	13.439 $\pm$ 0.004	0.9728 $\pm$ 0.0002	245
Acid leaches†	1.4611 $\pm$ 0.0004	13.439 $\pm$ 0.007	0.9733 $\pm$ 0.0002	367
Residue†	1.4613 $\pm$ 0.0010	13.441 $\pm$ 0.005	0.9725 $\pm$ 0.0014	51
Pyroxene	1.4614 $\pm$ 0.0002	13.444 $\pm$ 0.005	0.9737 $\pm$ 0.0007	110
Mean inclusion whole rock	1.4620 $\pm$ 0.0013	13.439 $\pm$ 0.006	0.9733 $\pm$ 0.0004	462
Inclusions with oxygen anomalies	1.4619 $\pm$ 0.0002	13.440 $\pm$ 0.013	0.9738 $\pm$ 0.0003	158

* See Table 1 and text for description of materials.

† See Table 1 for details.

‡ Separate contacts with concentrated HCl, HF, and HNO₃, each for ~ 12 h at about 80°C. Cycle performed twice with each Allende whole rock powder sample, with like fractions from both cycles combined and treated for Ti in usual manner. Acid leaches represents the combined data from two HCl, one HF, and two HNO₃ soluble fractions. Residue represents Ti isolated in the usual manner from the insoluble residue of the above acid leaches.

Allende inclusions: the largest oxygen and magnesium anomalies have been found in inclusions in Allende. Therefore, titanium from a variety of Allende inclusions has also been measured in this work. Most of these samples have been well documented mineralogically and chemically²⁸. We obtained aliquots of powdered whole inclusions which had not been (and were not) further separated before chemical processing. The samples varied between about 0.5 and 2.5% in TiO₂ content and represented all classes of inclusions listed by Taylor and Mason²⁸. These materials were processed using the same chemical procedures as for the terrestrial and whole rock materials described above; however, in some cases, the order of the ion-exchange steps was changed. Due to the small amounts of material available, replicate samples of all materials could not be processed; however, in those cases where replication was possible, agreement was found to be consistent within the precision of the individual measurements involved. The frequency distributions for the measured isotopic ratios of terrestrial and of inclusion material overlap sufficiently that, in the absence of several replicate determinations, none of the individual inclusions can be convincingly demonstrated to be different from terrestrial material in titanium isotopic composition. However, as the results for the various samples seem to be dispersed no more widely than true replicates would be, all data on whole rock inclusions were arbitrarily combined (weighted) as six set means to obtain a single 'mean inclusion' value for each ratio. These results are also shown in Table 2.

Mineral separates from Allende inclusions: mineral separates are the most fertile ground for isotopic anomalies for several elements. A pyroxene separate from an Allende inclusion (USNM3529, 32 described in ref. 29) was run in duplicate samples in the usual manner. The mean results are shown in Table 2. Uncertainties quoted are based on the observed dispersion of these true replicates and are similar to the others quoted.

Allende material with oxygen anomalies: some inclusion whole rock and density separates from Allende in which oxygen anomalies had been found were processed for titanium in the usual manner. As three of the samples (all mostly pyroxene from Al 6S3 and Al 3S4) have virtually identical oxygen anomalies³⁰ and because each of the individual samples seems to be anomalous in titanium (see Table 3) and because the normalised individual sample mean data points fall within the area inferred to exist for replicate results, the results for these samples, including one chemically reprocessed sample, have been combined to provide the mean values shown in Table 2. As usual, the quoted uncertainties are computed from the dispersion of the individual sample values.

Discussion

The data represented by the values shown in Table 2 have been carefully considered and, in particular, all apparent deviations from the terrestrial values have been tested for significance using the *t*-test. If 47/49 is assumed to be the same in all materials measured, the results of this study of titanium isotope ratios show that, within the limits of the relevant experimental precisions: (1) terrestrial materials from four different sources

display the same values with a mean relative three sigma of the mean uncertainty of about 0.03%; (2) Allende whole rock is not distinguishable from mean terrestrial material; (3) the chemical leaches from Allende whole rock, while showing some variations, do not differ significantly (three sigma level) from mean terrestrial material in their means; (4) results on inclusions and on a mineral separate extracted from an inclusion scatter in a manner consistent with the precision observed for the replication of other materials and with mean values within 0.1% (and not different from at the three sigma level) the mean terrestrial values for all ratios. However, note that arbitrary combination of all inclusion values would tend to dilute the effect of any anomalous material which might be present in only one or some of the inclusions or fractions thereof. Therefore, (5) if only inclusion material with documented oxygen anomalies is considered, the frequency distribution of individual 50/49 set means is found to be displaced from that for terrestrial materials, as shown in Table 4, and the mean 50/49 is found to be significantly (greater than three sigma of the mean) higher than for the mean terrestrial material. The observed *t* is 3.95, which for eight degrees of freedom represents about four chances in 1,000 that both the terrestrial and oxygen-anomalous sample means represent individual results drawn from a single normal frequency distribution. This is highly unlikely and we conclude that these meteoritic materials are different from terrestrial materials in their titanium isotopic composition to an extent comparable with recently reported anomalies in other elements in Allende^{8,11}.

Although the statistical analyses described above confirm a significant difference between the isotopic ratios in question, they cannot be used to exclude the presence of any of several possible systematic errors which might cause such a difference. We have carefully considered all such possibilities. For example, the origin of this observed enhancement of the 50/49 ratio in these inclusions cannot be single-stage Rayleigh mass fractionation because the process of normalisation would, of course, eliminate or at least obscure any pre-laboratory physicochemical mass fractionation such as has been observed for other elements, as well as that introduced by the mass-spectrometric measurement procedures. However, any normalisation can also distort the nature and extent of any nucleogenic anomalies present. The effects of such alternate normalisations have been extensively investigated and found to provide results consistent with expectations as to their nature and magnitude but of poorer precision due to the additional extrapolations and interpolations required vis-a-vis the 47/49 normalisation. Therefore, in the absence of evidence to the contrary, we prefer to use the latter.

Thus, either a depletion of ⁴⁹Ti or an enhancement in ⁵⁰Ti abundance is required. Ockham's Razor can be applied to argue against the former in that simultaneous appropriate depletions in the other three titanium isotope abundances would be required to maintain the agreement with terrestrial values obtained for the other ratios. Therefore, an enhancement at mass 50 is indicated. As described above, mass scans allowed the rejection of data where questionable peak shapes or baselines were present and corrections for known nuclidic isobars present during each set were made, with no systematic trends evident.

Table 3 Magnitudes of isotopic ratio anomalies

Allende inclusion*	Relative magnitudes (p.p.t.) of anomaly in isotopic ratio					
	¹⁷ O/ ¹⁶ O*	¹⁸ O/ ¹⁶ O*	²⁶ Mg/ ²⁴ Mg†	²⁹ Si/ ²⁸ Si‡	³⁰ Si/ ²⁸ Si‡	⁵⁰ Ti/ ⁴⁹ Ti§
Al 3S4	-39.3	-38.0	0.3 to 135	-0.3	1.8	1.2 ± 0.9
Al 6S3	-38.8	-36.6	—	—	—	1.2 ± 0.9
						2.0 ± 1.2
						Average = 1.4 ± 0.7

* Ref. 30. † Ref. 35. ‡ Ref. 36.

§ Values are individual sample means and mean (weighted) of combined results for both samples. Uncertainties quoted are three sigma of the mean estimated from dispersion (see text).

|| Chemically reprocessed.

Table 4 Frequency distributions of $^{50}\text{Ti}/^{49}\text{Ti}$ set means

	Relative no. of normalised† individual set means with 50/49 value						
Material*	<0.9716	0.9716–24	0.9724–32	0.9732–40	0.9740–48	0.9748–56	>0.9756
Terrestrial	0.03	0.26	0.42	0.29	0	0	0
Inclusions with O-anomalies	0	0.05	0.23	0.32	0.23	0.14	0.05

* See Table 1 and text for descriptions of materials.

† Normalised to (47/49) = 1.3360.

Therefore, in the absence of any evidence of unusual conditions during the measurement of any of the three anomalous samples, we conclude that the ^{50}Ti abundance is higher than normal in these oxygen anomaly-bearing inclusions.

The coincidence of anomalies in different elements in the same material suggests a common origin in a nucleosynthetic history for some fraction of the material that is distinct from the history for most of the materials studied. In attempting to account for the normal nuclide abundance pattern, various workers have modelled both hydrostatic and explosive thermonuclear processes. Despite great uncertainties in both the nuclear physical and the astrophysical input information, considerable success has been achieved. Such calculations^{31–33} indicate that explosive carbon burning can result in matter which is simultaneously enriched in ^{16}O , ^{26}Mg , and ^{50}Ti , just the coincidences reported in this work and previously^{30,35}, as shown in Table 3.

The picture concerning the magnitude and variety of isotopic anomalies is far from complete³⁷. Therefore, it seems that

additional studies of the correlation of anomalies in different elements must be undertaken. In particular, in the case of titanium, the existence and locus of a true anomaly (rather than a highly improbable statistical fluctuation or an undetected systematic error) must be independently confirmed, and the anomaly-carrying phase(s) must be identified. These are tasks for which the high resolution ion microprobe may be particularly well suited.

In conclusion, the results of this study indicate that a nucleogenic anomaly in titanium, most probably an enrichment in ^{50}Ti abundance, exists in some oxygen-anomaly bearing Allende inclusions.

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Volcanic eruption through a geothermal borehole at Námafjall, Iceland

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During the present rifting episode in the Krafla fault swarm in North Iceland, large scale horizontal movement of basaltic magma is taking place underground. On 8 September 1977 magma reached the depth range of boreholes in the Námafjall geothermal field. In 20 min 3 ton of magma erupted through an 1,138 m borehole forming a small scoria sheet.

THE eruption on 8 September 1977 in the Námafjall geothermal field was a part of a rifting event that took place during that day, and which itself is a part of a major rifting episode which has been in progress on the Krafla fault swarm in North Iceland since 20 December 1975^{1–4}. The eruption through the borehole provides further evidence for a large scale magma movement along the fault swarm initially postulated from ground deformation observations⁵.

The Krafla fault swarm is ~100 km long, and about 70 km

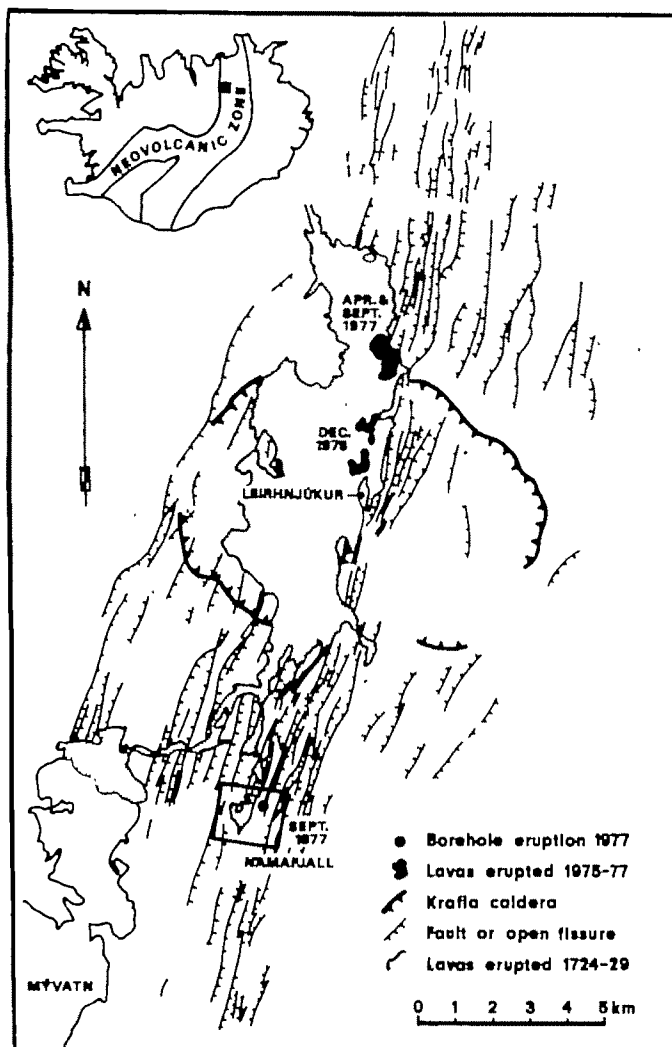


Fig. 1 The main geological features of the Krafla area and location of the eruptions 1975-77. The Námafjall area is shown in greater detail in Fig. 2. Adapted from a map by Kristján Saemundsson¹.

have been activated with horizontal widening of 2-3 m in places. Magma is being continuously fed (about $5 \text{ m}^3 \text{ s}^{-1}$) into magma reservoirs below the Krafla caldera (Fig. 1) at a depth of about 3 km, as indicated by seismic and ground deformation evidence¹.

The rifting activity takes place in short events where 5-15 km long segments of the fault swarm are activated with movements on faults, earthquakes and the formation of new steam fields. This is accompanied by subsidence of a large area with a centre within the Krafla caldera caused by magma movement into the fault swarm.

Three small lava eruptions have accompanied the eight rifting events that have taken place, but all three eruptions were in the vicinity of the magma reservoirs and so far only the eruption through the borehole has provided samples of the magma actually moved into the fault swarm.

The total volume of magma moved into the fault swarm is about $3.9 \times 10^8 \text{ m}^3$, while the volume of the lavas is about $2.4 \times 10^6 \text{ m}^3$ or 0.6%.

The first eruption, on 20 December 1975, took place near the centre of the caldera (Fig. 1) although the main rifting occurred 45 km to the north. The second eruption, on 27 April 1977, took place near the northern rim of the caldera (Fig. 1), while the main rifting occurred about 10 km to the south of the caldera centre.

In the third eruption, on 8 September 1977, the main outflow of lava was again near the northern rim of the caldera, while the

main rifting took place south of the caldera, just north of the Námafjall geothermal field. We give here a short account of this event and the borehole eruption.

8 September deflation/rifting event

The deflation and rifting of 8 September 1977 started with a volcanic tremor recorded on seismographs at 15.47 h and about the same time the centre of the caldera started deflating. Just before 18.00 h a lava eruption started near the northern rim of the Krafla caldera (Fig. 1). According to the pilots of an overflying aeroplane, its first sign was a white column of steam darkening at the base after a few minutes. Shortly afterwards lava spattering started from a short segment of the fissure, which rapidly extended to a total length of 800 m. The lava output increased quickly, reaching maximum in less than about 30 min, and by 19.30 h most of the lava was already erupted. By 22.30 h the activity had died out. Assuming that the magma started to ascend when volcanic tremor appeared on the seismometers and deflation on the tiltmeter, the rate of ascent is about 0.5 m s^{-1} assuming a depth of 3 km for the magma reservoirs.

The deflation rate soon decreased and stopped temporarily at about 17.20 h. Deflation started again at about 18.20 h. This was the main deflation and most likely related to the magma movement to the south. The centre of the rifting segment in this event was just north of the Námafjall geothermal field (Fig. 1). Some overlap exists between this rifting segment and that rifted in the previous event on 27 April 1977, as reference lines across the rift zone that already had increased in length by 2 m extended a further 1 m on 8 September⁴.

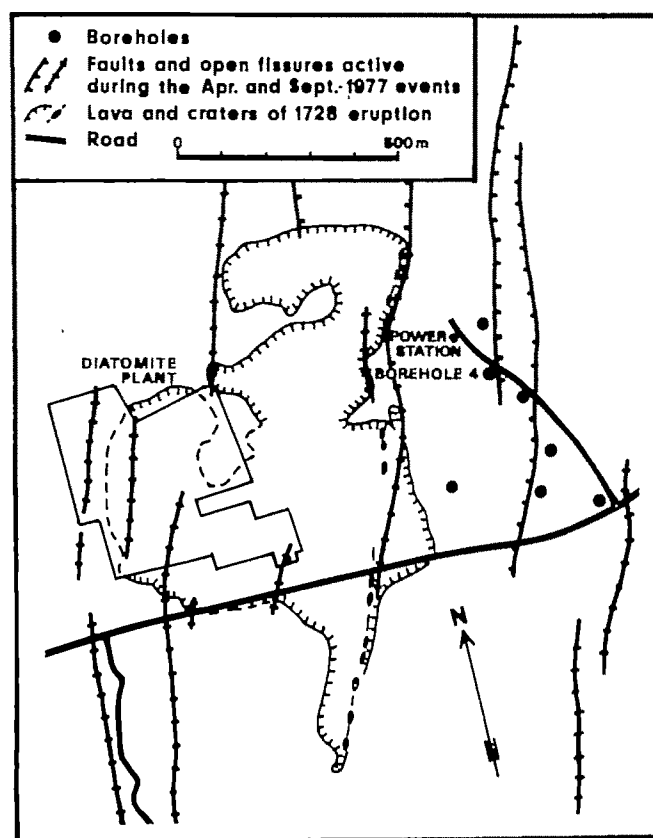


Fig. 2 Sketch map of the Námafjall area from an aerial photograph of 9 September 1977. The location of boreholes is shown by dots. The faults shown on the map have all been active in the recent events. For location see Fig. 1.

At Námafjall the first observed sign of the rifting event was movement of faults crossing a road (Fig. 2) at about 22.40 h. Assuming that the main magma movement to the south started at 18.20 h and with the distance of 9 km from Leirhnjúkur at the centre of the caldera, the rate of horizontal movement is about 0.6 m s^{-1} . A similar velocity was derived for magma movement in the April 1977 deflation/rifting event³. The figures for vertical and horizontal movement of the magma are strikingly similar to the figures (0.6 m s^{-1}) arrived at for the maximum rate of magma ascent in the Askja eruption 1961 and the Lakagígur eruption of 1783⁵, and there are indications that the magma ascended with similar velocity during the Eldfell eruption 1973 (ref. 6, p 30).

The eruption through the borehole began about one hour later, at 23.45 h. The rate of ground deflation decreased rapidly, and at about 05.00 h on 9 September deflation had stopped and the centre of the caldera was inflating again.

The borehole eruption

The Námafjall geothermal field lies on the Krafla fault swarm 9 km south of the centre of the Krafla caldera. The five boreholes in use for steam extraction are spaced with the distance of 80–200 m (Fig. 2) and extend down to 650–1,800 m with casing extending down to 500–600 m. The deepest hole has an additional slotted liner down to the bottom at 1,800 m. The boreholes are situated in a highly faulted area and deliberately placed to intersect faults at favourable depths.

The eruption took place through borehole number 4, drilled in 1968. It is 1,138 m deep, has 7" casing down to 380 m and 5" casing down to 625 m. The diameter from 625 to the bottom is 6.25" and this part has no casing or liner. The main aquifers are reported at 638 and 1,038 m (ref. 8). The average temperature of the inflowing water is estimated at 251–256 °C (ref. 7). The total discharge of the borehole at the time of eruption is not known accurately, but from measurements in 1971⁸ and the estimated inflow temperature, the total discharge of water-steam can be estimated at 30 kg s^{-1} at about 7 bar absolute well head pressure. This assumes that the inflow is water only and

that steam is generated in the borehole during its ascent. (If the inflow is a steam-water mixture a lower value results.) The superstructure of the borehole suffered minor damage due to the eruption and the borehole continued to produce steam at a similar rate as before. Following the eruption there was a marked increase in fumarolic activity in the area around the boreholes, which later decreased markedly.

Movements on faults in the Námafjall area were first noted at about 22.40 h, when the main road through the area was cut through and became impassable. By that time the eruption that had taken place 12 km to the north was finished and further activity to the south was anticipated. It was already dark, and with the steam from the boreholes drastically affecting visibility, conditions at Námafjall were confusing.

An examination of the earthquake activity shows a southward migration from the caldera along the fault swarm. The earthquake activity reached the Námafjall area shortly before 22.00 h (ref. 9).

The description below is compiled from accounts of 10 people, each of whom saw only a part of the event.

The whole event lasted between 15–25 min. It can be divided into three stages. The first phase was best observed from the main road about 400 m south of borehole 4. At approximately 23.45 h an explosion in the borehole field was heard and a thin incandescent column, about 15–25 m in height, was seen. The column widened slightly upwards and sparks and cinders were continuously shot from it. This phase of the eruption, accompanied by a continuous roar, lasted no longer than 1 min.

During the second phase, lasting the next 10–20 min, there was little or no activity at the eruption site. Occasional red flashes may have occurred during the latter part of this phase, according to some of the observers, but could also belong to the final phase.

The final phase consisted of a series of very rapid explosions or shots of glowing scoria. A few groups of explosions were observed each consisting of several individual shots (Fig. 3a). The total length of the final phase is estimated at about 1 min. During this phase the exact location of the eruption was established, when it was discovered that the pipe directly above the hole conducting the steam-water mixture had been ruptured by the eruption (Fig. 3b). It is not known whether the steam-water

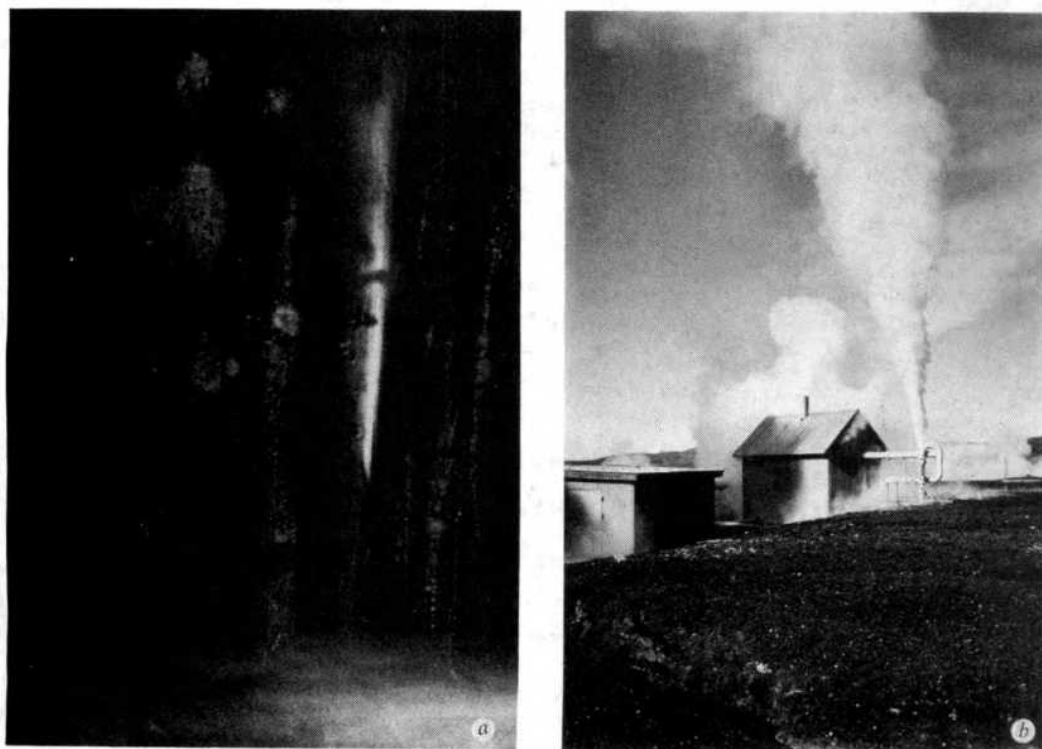


Fig. 3 a, The eruption through borehole 4 at Námafjall on 8 September 1977. The picture was taken just about midnight at the distance of 50–70 m. The explosion lasted about 1 s. By comparison with the tool shed on the left the height of the eruptive column is estimated at 13–15 m. Photo Hörður Vilhjálmsson. b, Borehole 4 at Námafjall, on the morning of 9 September 1977. The steam jet is emitted through the rupture in the pipe created by the volcanic eruption. The steam-water separators are housed in the shed to the left. Practically the same scale as in Fig. 3a. Photo Sigurdur Thorarinnsson.

output was cut off at any time during the eruption, but during the second phase red flashes were seen in the steam–water mixture emitted through the ruptured knee on the pipe.

The magma was apparently injected into the hole through a steeply dipping fault intersecting the borehole above the aquifer at 1,038 m and most probably below the casing at 625 m as steam production continued after repairs of the superstructure. At the surface substantial movements on faults near the borehole were observed.

Products of the eruption

The eruption produced a tiny sheet of tephra (scoria and cinders). An area about 50 m long and of 25 m maximal width was covered by continuous layer, but single grains could be traced to at least 150 m north of the borehole. The tephra was very loosely packed and unevenly distributed and thickness estimates are uncertain, especially away from the borehole. Maximum thickness was 8–10 cm at a distance of 15–20 m.

The volume of the total output is estimated at 26 m³. The density of the deposit was roughly calculated at 0.12 g m⁻³ and the total weight of the deposit at 3,100 kg. The volume of the magma erupted is therefore 1.2 m³ using 2.7 g m⁻³ as the density of the magma.

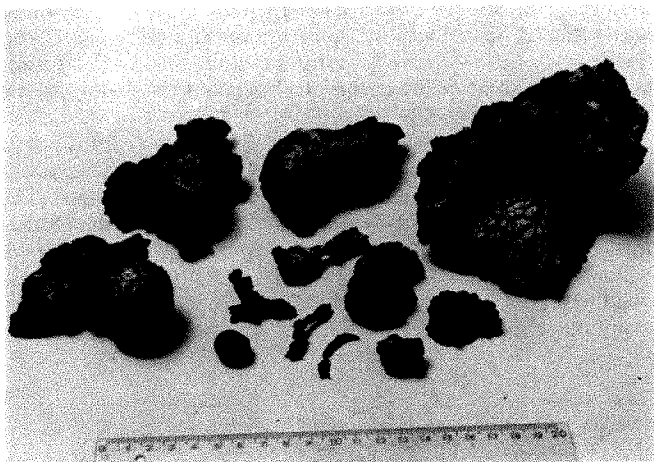


Fig. 4 The borehole scoria. From the right: a large scoria fragment (type 1) showing a chilled, crusted surface. The edges curled up when the original piece broke and show frothy glass formed from the molten interior. Fragments (type 2) formed from the interior of larger pieces, deformed to various degrees. Some show relatively little deformation while others form spindles and small spheres. Two scoria pieces with a protrusion (type 3). The bread crust part is formed by the initial chilling and the spherical protrusion later from the still molten interior.

The tephra is greyish-black in colour and coarse grained. Median (ϕ 50) grain size in a sample collected 20 m from the 'vent' is -4.2ϕ (18.4 mm). As the largest clasts break very easily, this is likely to be an underestimate. Grains smaller than 1 mm are scarce.

The clasts (Fig. 4) can be divided into three types: (1) clasts with 2–4 mm thick, cracked, black, glassy crust ('bread crust') on one side and glass froth on the other. (2) Grains made entirely of torn glass froth of various shapes and sizes. (3) Crusted clasts of various sizes and shapes, each with a neat frothy sphere protruding from one end.

There are gradations between the different types of clasts making it possible to establish how they were formed. On injection into the borehole, the magma seems to have formed

Table 1 Microprobe analysis of the products from the eruption of borehole 4 at Námafjall, 8 September 1977

Composition of the glass										
SiO ₂	TiO ₂	Al ₂ O ₃	FeO ¹	MnO	MgO	CaO	Na ₂ O	K ₂ O	P ₂ O ₅	
50.0	2.24	12.7	14.9	0.24	5.16	10.3	2.34	0.37	0.23	
Plagioclase										
SiO ₂	Al ₂ O ₃	FeO ¹	CaO	Na ₂ O	K ₂ O	Total	An			
52.9	29.0	1.20	13.2	4.03	0.09	100.4	75.9			
Olivine										
SiO ₂	FeO	MnO	MgO	CaO	NiO	Total	Fo			
37.7	25.7	0.35	35.7	0.29	0.13	99.9	71.2			
Pyroxene										
SiO ₂	TiO ₂	Al ₂ O ₃	FeO	MnO	MgO	CaO	Na ₂ O	Cr ₂ O ₃	Total	
49.8	1.09	3.76	11.5	0.34	16.1	16.2	0.22	0.28	99.3	

lumps of various shapes and sizes. Some of the smaller lumps survived all the way to the surface where they ripped open, and small, rounded protrusions formed from the molten interior. The larger lumps seem to have broken at various depths. The incandescence of the column indicates that most of them disintegrated within the upper levels of the borehole. Clasts with glassy crust on one side were formed from the cooled exterior of the lumps, while the molten interior disintegrated into fragments of twisted glass froth.

Assuming that the bulk of the magma was injected into the hole during the two 1-min periods, the intrusion rate is about 25 kg s⁻¹, which is of the same order as the amount of steam–water production of the borehole. Using the heights between 20 and 40 m velocities between 20 and 30 m s⁻¹ are obtained. This means that if the magma was injected at 1,000 m depth it took 0.5–1 min for it to reach the surface.

Chemistry and petrology of the magma

The scoria produced in the borehole eruption is extremely vesicular and composed almost entirely of glass. There are very occasional micropheocrysts of plagioclase, olivine and augite, always less than 0.2 mm in length. Average chemical analysis of the glass and the mineral phases are given in Table 1. The minerals seem to be in equilibrium with the liquid at the time of cooling¹⁰.

Temperature estimates at the time of chilling give 1,153 °C from the olivine–glass composition¹¹ and, 1,158 °C for the plagioclase–glass composition¹².

The chemical composition of the borehole products differs significantly from that erupted a few hours earlier 12 km to the north, but has similar composition as the lavas erupted at the beginning of the rifting on 20 December 1975¹⁰.

Observations made by the following were used in compiling this report: Ágúst Hilmarsson, Árni Ingólfsson, Baldur Thorsteinsson, Benedikt Sveinbjörnsson, Fridrik Steingrímsson, Gestur Gíslason, Hjörtur Tryggvason, Hrefna Kristmannsdóttir, Ómar Sigurdsson, Thorfinnur Finnlaugsson.

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Thrombin-stimulated cell division involves proteolysis of its cell surface receptor

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A cell-surface component of molecular weight 43,000 is cleaved by thrombin on cells that divide after thrombin treatment, but is not cleaved on cells that are unresponsive to its mitogenic action. Studies with a photoreactive derivative of thrombin showed that its cell surface receptor has a molecular weight of 43,000. This indicates that thrombin must cleave its receptor to stimulate cell division.

STUDIES on the mechanism of the mitogenic action of thrombin have been facilitated by its ability to bring about cell division in serum-free cultures of non-dividing chick embryo (CE), mouse embryo (ME) or human foreskin (HF) cells¹⁻³. This allows experiments to be conducted in chemically defined culture conditions. Also, thrombin possesses a well characterized enzymatic activity which is necessary for stimulation of cell division^{2,4}. This makes it feasible to look for specific biochemical events that it must produce so as to bring about cell proliferation.

The first step was to establish the cellular site of thrombin action. Although thrombin is internalised by CE cells in mitogenic conditions^{5,6}, we recently demonstrated that internalisation is not necessary and that its action at the cell surface is sufficient to cause CE cells to divide⁷. This led us to examine the cell surface for events that are involved in thrombin-stimulated cell division. One such event is the binding of ¹²⁵I-thrombin to a specific cell surface receptor⁸. Scatchard analysis of the binding data to ME cells indicated a single class of high affinity receptors. It seems that thrombin must bind to its receptor to cause cell division, as small amounts of serum inhibited both the binding of thrombin to its receptor and its ability to stimulate cell division without reducing its proteolytic activity or its nonspecific association with the cells⁸.

In the present study, we radiolabelled the thrombin receptor and obtained evidence that thrombin must cleave it to stimulate cell division. The receptor was labelled using a photoreactive cross-linking derivative of ¹²⁵I-thrombin. The amount of cross-linked complex formed was proportional to the amount of specific binding, indicating that the labelled component was the cell surface receptor for thrombin. The molecular weight of the receptor was 43,000, equal to that of a cell surface component which other experiments indicated thrombin must cleave to stimulate cell division. In those experiments, we labelled cell surface proteins with lactoperoxidase and ¹²⁵I⁻ and found that a 43,000-dalton cell surface component (43 K) was present on CE cells that were responsive to the mitogenic action of thrombin and also on four separately isolated populations of CE cells that did not divide after thrombin treatment. However, 43 K was cleaved by thrombin only on the responsive cells; it was thrombin resistant on the four unresponsive populations. Thus, cleavage of 43 K by thrombin seems to be necessary for thrombin-stimulated cell division. These separate approaches suggest that a 43,000-dalton cell surface protein initiates a mitogenic signal by binding thrombin and undergoing thrombin-mediated proteolysis.

CE cells that are unresponsive to the mitogenic action of thrombin

To determine the events which cause thrombin-stimulated cell division, it was important to compare the effects of thrombin on both cells that divide and those that do not divide after thrombin treatment. An event which occurs in the responsive but not the unresponsive cells can be further studied as a possible necessary step in thrombin mitogenesis. The procedure for obtaining unresponsive cells was based on our finding that during serial subculture of CE cells, they lose virtually all of their response to the mitogenic action of thrombin or trypsin, but retain responsiveness to serum³. By the 25th population doubling, we found in four separate isolates a loss of about 80% or 90% of the mitogenic response to thrombin or trypsin, respectively (Fig. 1). This loss was not dependent on the method used to subculture the cells during the 25 population doublings, as unresponsive population I (Fig. 1b) was subcultured using 0.01% chymotrypsin whereas unresponsive population II (Fig. 1c) was sub-

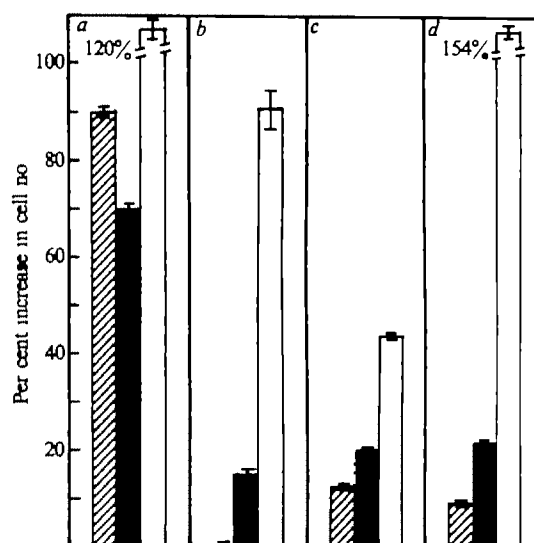


Fig. 1 Comparison of cell number increases of responsive cells with two different unresponsive cell isolates. Cultures of nondividing CE cells in serum-free medium were set up as previously described³. The indicated mitogen was added and cell number was measured 24 h (a-c) or 48 h (d) later. The data shown are means of duplicate determinations obtained with a Coulter electronic particle counter. Error bars show one standard deviation from the mean. Hatched bars, trypsin ($3 \times$ recrystallised, 247 units mg^{-1} (Worthington)) was added to $0.2 \mu\text{g ml}^{-1}$. Solid bars, highly purified human thrombin (about 3,000 NIH units mg^{-1})¹⁷ was added to $3.0 \mu\text{g ml}^{-1}$. Open bars, chicken serum (Irvine) was added to 5.0%. a, secondary CE cells; b, CE cells at population doubling 26 (passage 13) which had been subcultured using chymotrypsin (0.01%) in phosphate-buffered saline (PBS) (unresponsive population I); c and d, CE cells at population doubling 28 (passage 14) which had been subcultured using trypsin (0.05%) and EDTA (0.02%) in PBS (unresponsive population II).

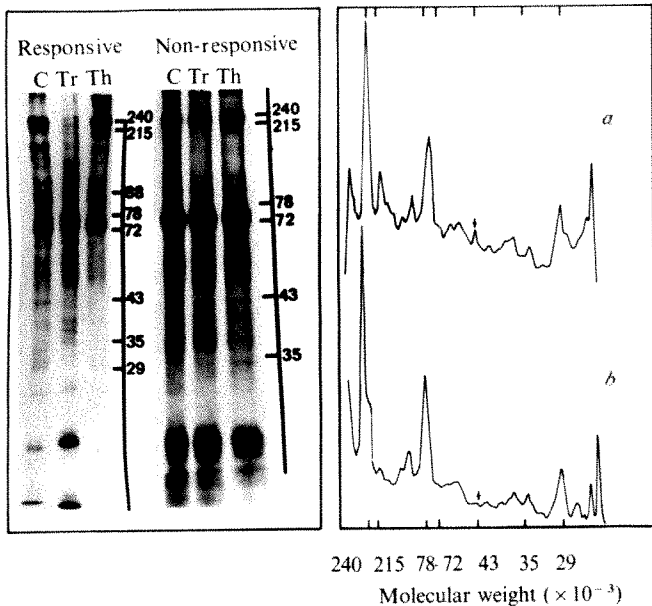


Fig. 2 Analysis of cell surface of responsive cells with or without exposure to thrombin or trypsin. Cell surface proteins were labelled by lactoperoxidase-catalysed iodination with $^{125}\text{I}^-$ (ref. 9). Cell monolayers were rinsed five times with Dulbecco's PBS and incubated for 5 min at 22 °C with 6 IU ml^{-1} lactoperoxidase (Calbiochem), 6 U ml^{-1} glucose oxidase (Calbiochem), 0.5 mCi ml^{-1} carrier free $^{125}\text{I}^-$ and 10 mM glucose in Dulbecco's PBS. The cells were then rinsed five times with ice cold calcium and magnesium-free PBS and solubilised and boiled for 3 min in 0.1 M Tris-HCl (pH 6.8) containing 1.0% SDS, 1.0% 2-mercaptoethanol, 5.0% glycerol and bromophenol blue. The samples were electrophoresed using 7.5–15% linear gradient SDS-polyacrylamide slab gels¹⁰. Molecular weights were determined by electrophoresing human red blood cell membrane samples²² in slab gel lanes adjacent to the ^{125}I -labelled samples. Autoradiography was performed by exposing Kodak XR-5 X-ray film to dried Coomassie blue-stained gels. Left, autoradiograms of cell surface proteins from responsive and unresponsive (population II of Fig. 1) cells that were untreated (lanes C), treated for 24 h with 3.0 $\mu\text{g ml}^{-1}$ thrombin (lanes Th) or treated for 24 h with 0.2 $\mu\text{g ml}^{-1}$ trypsin (lanes Tr) before iodination of cell surface proteins. Right, densitometric scans of autoradiograms from responsive untreated cells (a) and from responsive thrombin-treated cells (b). The arrows indicate the position of the 43,000 MW marker.

cultured with 0.05% trypsin in 0.02% EDTA. The loss of responsiveness did not result simply from a slower response to protease stimulation, as doubling the length of exposure to thrombin or trypsin from 24 h to 48 h did not increase the amount of cell division (Fig. 1c, d). Also, the loss of responsiveness was not due to a shift in the effective mitogenic dosage of thrombin on the CE cells, as they were serially subcultured. Figure 3 shows that the cells at population doubling 25 remained unresponsive even after exposures to high thrombin concentrations.

In contrast, CE cells at population doubling 25 remained responsive to the mitogenic action of serum. Although the response was reduced if cell number was measured 24 h after serum addition, there was more than a cell doubling by 48 h (Fig. 1d). ^3H -thymidine autoradiography showed that by 48 h all of the cells had synthesised DNA, demonstrating that the cell number increase did not represent multiple divisions of only a small subpopulation of cells (unpublished results). Together, these results show that by population doubling 25, CE cells lost most of their mitogenic response to thrombin or trypsin. However, they retained a full but delayed response to the mitogenic action of serum.

Cleavage by thrombin of a 43,000-dalton cell surface component from responsive but not unresponsive CE cells

These results made it possible to look for cell surface components that were cleaved by thrombin on responsive CE cells but which were thrombin resistant or absent on the unresponsive cells. Cell surface proteins were examined on thrombin-treated and untreated cells by lactoperoxidase-catalysed iodination with $^{125}\text{I}^-$ (ref. 9) followed by SDS-polyacrylamide gel electrophoresis¹⁰ and autoradiography. We also examined trypsin-treated and untreated cells, as trypsin is a potent mitogen for CE cells^{3,11–14} and can produce cell division as a result of action at the cell surface¹⁵. Figure 2 shows that 43 K was present on responsive secondary CE cells and also on CE cells at population doubling 25 that were unresponsive to the mitogenic action of thrombin or trypsin. As shown, 43 K was removed from the responsive cells by mitogenic treatments with either thrombin or trypsin. However, in four separately isolated populations of unresponsive CE cells, 43 K was not removed by these thrombin or trypsin treatments. Figure 2 shows that this could be determined by either visual examination of autoradiograms of the gels or densitometric scans of the autoradiograms. Stimulation of division of non-dividing secondary CE cells by insulin or serum did not remove 43 K from the cell surface (unpublished results). Thus, its removal was not simply a consequence of stimulating cell proliferation. DFP (di-isopropylfluorophosphate)-inactivated thrombin is non-mitogenic⁴ and does not remove 43 K from responsive cells (unpublished results). Together, the above results indicate that cleavage of 43 K is necessary for thrombin-stimulated cell division and that its cleavage is due to thrombin rather than a cellular protease.

It was important to ensure that 43 K was not a serum protein or an intracellular component. It is unlikely to be a serum protein as the cells were extensively rinsed free of serum and placed in serum-free medium for 48 h before labelling with $^{125}\text{I}^-$.

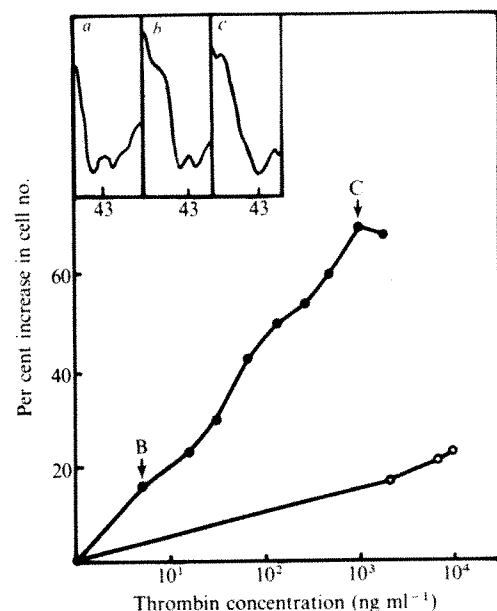


Fig. 3 Effect of thrombin concentration on cleavage of 43 K and initiation of cell division. The main figure shows the per cent increase in cell number for responsive secondary CE cells (●) and unresponsive cell population II (○) following a 24-h exposure to the indicated concentrations of thrombin. The insets show densitometric scans of autoradiograms of ^{125}I -labelled cell surface proteins of responsive cells electrophoresed as in Fig. 2. The 30,000–60,000 molecular weight regions of the scans are shown with the position of the 43,000 molecular weight marker. Responsive CE cells were either untreated (inset a), or treated with 5.0 ng ml^{-1} thrombin (inset b) or 1.0 $\mu\text{g ml}^{-1}$ thrombin (inset c) for 24 h before labelling cell surface proteins as in Fig. 2.

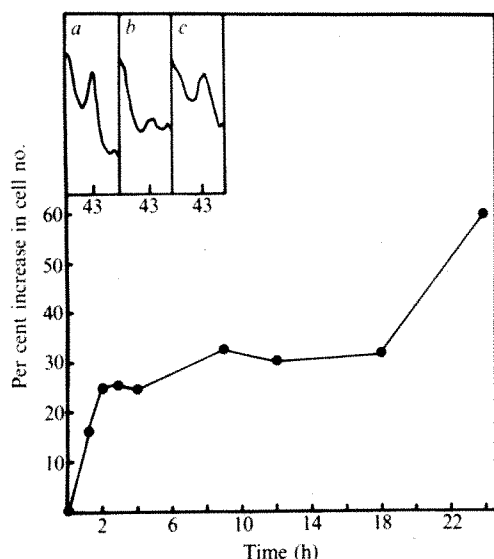


Fig. 4 Effect of length of thrombin treatment on stimulation of cell division and cleavage of 43 K in responsive CE cells. The main figure shows the cell number increases after treating non-dividing cultures with $3.0 \mu\text{g ml}^{-1}$ thrombin for the indicated times, rinsing the cultures and continuing incubation in thrombin-free medium from parallel cultures. Cell number was measured 24 h after the initial exposure to thrombin. Measurements at 72 h showed that the observed low responses after short thrombin treatments were not a result of delayed increases in cell number (unpublished results). The insets show densitometric scans of 43 K as in Fig. 3. Inset a, control cells not treated with thrombin; inset b, cells were labelled after a 7-h treatment with $3.0 \mu\text{g ml}^{-1}$ thrombin; inset c, like inset b, except the cells were incubated for 3 h in thrombin-free medium following the 7-h thrombin treatment but before surface labelling.

and lactoperoxidase. More important, if it were a serum protein, it would have to be one that is sensitive to thrombin and trypsin after binding to responsive cells, but resistant to cleavage by these proteases after binding to the unresponsive cells. Also, 43 K does not seem to be an intracellular protein. It was not labelled by ^{125}I in the absence of lactoperoxidase, and it was removed by thrombin treatments as short as 1 h. Although some thrombin would be internalised by that time, it would probably be in endocytic vesicles and would not have access to cytoplasmic proteins.

Removal of 43 K from responsive cells by mitogenic but not non-mitogenic thrombin treatments

To further test the relationship between cleavage of 43 K and the stimulation of cell division by thrombin, we attempted to dissociate the two events by varying the concentration of thrombin. The condition most likely to dissociate cell division from removal of 43 K by thrombin is to expose cells to the highest concentration of thrombin which is not mitogenic. Figure 3 shows that the highest concentration of thrombin that did not produce significant cell division was 5 ng ml^{-1} . As shown in Fig. 3, inset b, this concentration did not bring about a detectable change in 43 K. However, inset c shows that a maximally mitogenic concentration of thrombin removed 43 K. Intermediate concentrations gave incomplete removal of 43 K.

Replacements of 43 K after thrombin treatment seems to prevent cell division

Figure 4 shows that maximal stimulation of CE cell division by thrombin required continued exposure during the 24-h period; shorter exposures resulted in smaller increases in cell number measured either at 24 h or 72 h. An apparent dissociation between removal of 43 K and stimulation of cell division by

thrombin is shown in Fig. 4 where a 7-h treatment with $3 \mu\text{g ml}^{-1}$ thrombin removed 43 K (inset b) but produced only a $\sim 30\%$ increase in cell number by 24 h. This suggested that cleavage of 43 K by thrombin was not sufficient to achieve maximal thrombin-stimulated cell division. However, inset c shows that 43 K was replaced in the cell membrane within 3 h if thrombin was removed after the 7-h treatment. This suggests that replacement of 43 K stops the events leading to cell division and that maximal stimulation of cell division requires continuous removal of 43 K. An alternative possibility is that thrombin releases a specific fragment of 43 K that is a positive effector, the continuous production of which is required to produce maximal cell division. By studying the effects of non-mitogenic proteases on 43 K, it might be possible to determine whether 43 K is a positive or a negative effector molecule.

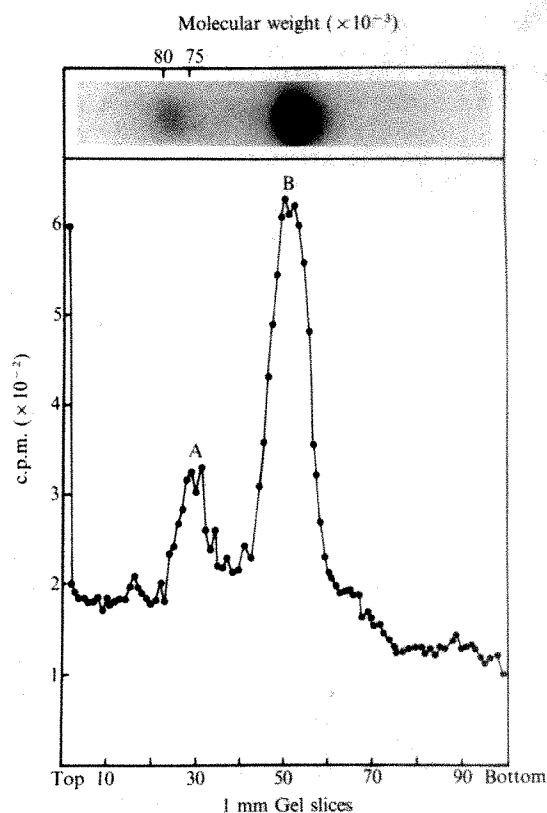


Fig. 5 Labelling of the thrombin receptor with a photoreactive derivative of ^{125}I -thrombin. Details of the preparation of NAPEDE- ^{125}I -thrombin and the methods to photoaffinity label the thrombin receptor will be described elsewhere¹⁶. The general protocol for this experiment is described in the text and was based on previous studies with the epidermal growth factor receptor²³. After cross-linking the ^{125}I -thrombin to its receptor, the cells were solubilised in 1.0% SDS and 1.0% 2-mercaptoethanol at 60°C for 30 min and cellular proteins were electrophoresed in 7.5% polyacrylamide tube gels containing 0.1% SDS. The line drawing shows the radioactivity in 1.0-mm slices of the gel. The inset autoradiogram shows the radioactivity in a 7.5–15% linear gradient slab gel following electrophoresis of a similarly labelled sample. The position of 80,000 and 75,000 molecular weight marker proteins are shown for this autoradiogram.

Evidence that 43 K is the thrombin receptor

As stimulation of cell division by thrombin seems to require both binding to its receptor⁸ and cleavage of 43 K, it was important to determine the relationship, if any, between the thrombin receptor and 43 K. Indeed, a relationship was suggested by the following experiments which showed that the thrombin receptor has the same molecular weight as 43 K.

As described elsewhere¹⁶, a photoactivable cross-linking derivative of ^{125}I -thrombin was prepared by covalently linking it

to *N*-(4-azido-2-nitrophenyl)ethylenediamine (NAPDE). The NAPDE-¹²⁵I-thrombin bound to cells like ¹²⁵I-thrombin¹⁶, with 60% of the total binding to specific receptors at a ligand concentration of 50 ng ml⁻¹. We then incubated CE cells with 50 ng ml⁻¹ NAPDE-¹²⁵I-thrombin for 2 h at 22 °C, rinsed away unbound reagent, treated the cells for 5 min with UV light and solubilised and electrophoresed the cellular proteins in SDS-polyacrylamide gels. Figure 5 shows the radioactivity in the gel slices from such an experiment. Peak B co-migrated with NAPDE-¹²⁵I-thrombin that had not been reacted with the cells. Peak A represents a high molecular weight cross-linked complex. Two lines of evidence showed that this complex was formed between NAPDE-¹²⁵I-thrombin and the cell surface receptor for thrombin. First, the complex was not formed in the absence of cells; thus, it was not simply intermolecular complexes of NAPDE-¹²⁵I-thrombin. Second, addition of unlabelled thrombin caused parallel declines in the amount of specific binding and the amount of cross-linked complex formed, indicating a direct relationship between the thrombin receptor and the cross-linked cellular component (unpublished results).

We estimated that the molecular weight of the cross-linked complex was 79,000 (Fig. 5). The NAPDE-¹²⁵I-thrombin had an apparent molecular weight of 36,000. Subtracting this from the molecular weight of the complex leaves a cellular receptor of 43,000 daltons. Thus, on the basis of their apparent molecular weights, the cell surface receptor for thrombin seems to be identical to 43 K described above that was identified by labelling cell surface proteins with ¹²⁵I- and lactoperoxidase.

Other cell surface events involving thrombin

We recently found that a significant fraction of ¹²⁵I-thrombin that is specifically bound to the surface of HF cells becomes linked to the thrombin receptor by a linkage that is apparently covalent¹⁸. This occurs in the absence of a cross-linking reagent. More linkage occurs with NAPDE-¹²⁵I-thrombin, although this increment depends on the cell type (unpublished results). By studying the occurrence of the direct linkage of thrombin to its receptor in responsive and unresponsive cells, it should be possible to evaluate its role in thrombin-stimulated cell division.

The treatment of mouse splenocytes with thrombin removes a cell surface component of 45,000 daltons that was suggested to be the histocompatibility antigen¹⁹. Although its role in the stimulation of splenocytes by thrombin was not examined, it might correspond to 43 K examined here. Another cell surface component that is sensitive to thrombin is one with a molecular

weight of 205,000 (205 K) on CE cells²⁰. This component was removed by both mitogenic and non-mitogenic proteases²¹. Thus, it is not simply a negative effector molecule whose removal from the cell surface is sufficient to stimulate cell division. On the other hand, the above results are consistent with the possibility that proteolysis of 205 K by thrombin produces a specific peptide that is a positive signal in or on the cell. In our experiments, 205 K was not labelled or resolved clearly enough to allow a meaningful examination of it. In future studies, it will be important to use other techniques to label cell surface proteins and to separate them on two-dimensional gels. This should allow us to study the possible involvement of 205 K in thrombin-mediated cell division and to determine if it or other cell surface components are thrombin sensitive in responsive cells but either absent or thrombin resistant in cells that do not divide after thrombin treatment. Such an attempt seems worthwhile as the requirement for at least several hours' exposure to thrombin to produce a detectable increase in cell number might reflect the need for several perhaps interdependent cell surface interactions with thrombin to produce cell division.

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Distribution of polymorphic forms of troponin components and tropomyosin in skeletal muscle

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Specific antibodies have been used to show that in adult skeletal muscle the slow and fast forms of the components of the troponin complex are located in type I and type II fibres respectively. α -Tropomyosin is restricted to type II cells. During development, as a result of changes in innervation and in certain diseased stages, both the slow and fast polymorphic forms of the troponin components are present in the same cell.

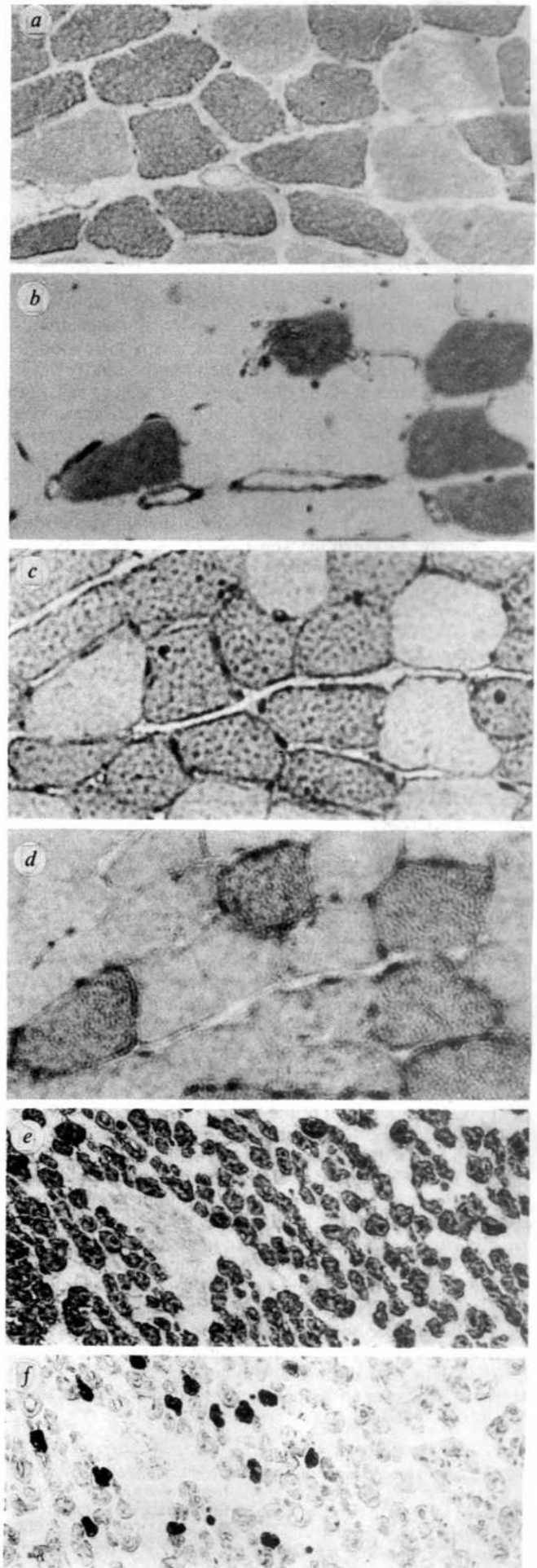
MYOFIBRILLAR proteins are known to exist in several different forms, and the complement of these proteins present in striated muscle depends on the activity pattern of the tissue. For example, it has long been appreciated that myosins of differing ATPase activities and light chain composition are associated with fast skeletal, slow skeletal and cardiac muscles. The differential staining for myosin ATPase at different pH values implies that different types of myosin are located in each of the main fibre types of which skeletal muscle is composed¹. Electrophoretic studies of the myosin light chain components in

samples obtained from single fibres² and investigations with fluorescent antibodies^{3,4} have now confirmed that in adult skeletal muscle the fast and slow forms of myosin exist in different cells. The situation with myosin is, however, complicated, as study of its light chain composition suggests that the fast and slow isoenzymic forms of this protein may each also exist in at least two forms. For example, the alkali light chains A1 and A2 of fast skeletal muscle myosin are both present in type II fibres²⁻⁶. Therefore, if the myosin exists as homodimers (that is, each head is identical) there will be two isoenzymic forms in each cell type and three if the heterodimer possessing different alkali light chains in each head is present in addition to the homodimers. Further, there is evidence to suggest that these forms may also possess different actin-activated ATPase activities^{7,8}.

Although there is a good correlation between the type of myosin present and the speed of contraction of skeletal muscle⁹, the characteristics of the overall response to stimulation must also depend on several other factors. One of these is the nature of the regulatory proteins of the myofibril—troponin C, troponin I, troponin T and tropomyosin. The function of these proteins is to determine the way in which the myofibrillar ATPase responds to the rise in intracellular Ca^{2+} concentration that occurs during stimulation. As judged by criteria such as amino acid composition, electrophoretic mobility, complex formation and sequence studies, troponin I^{10,11}, troponin T¹²⁻¹⁴, troponin C¹⁷⁻¹⁹ and tropomyosin^{21,22} exist in striated muscles in polymorphic forms with different properties and structure. Different forms of troponin I are present in fast skeletal, slow skeletal and cardiac muscles. Although it has not been as intensively studied as troponin I the evidence available suggests that troponin T probably also exists in different forms in each type of striated muscle. Two types of troponin C have been isolated from human skeletal muscle²³ and cardiac troponin C has been reported to differ from the form present in fast skeletal muscle¹⁹. Most adult skeletal muscles are of mixed type and contain the two forms of each of the troponin components that are characteristic for the two types of skeletal muscle. Likewise the proportions of α and β tropomyosin in skeletal muscle vary according to the speed of the muscle²². The proportions of the two forms of each protein of the regulatory system present in the whole muscle are, in general, roughly that which would be expected from the ratio of the numbers of type I and type II fibres present, but not always so²⁴.

Thus the presumptive skeletal muscle cell has the capacity to synthesise several different forms of each of the myofibrillar proteins. From the information currently available about the polypeptide compositions of actin, myosin, tropomyosin and the troponin complex of rabbit skeletal muscle, about 22 structural genes are involved in the synthesis of these proteins. At any one time, in a given cell type, only 8 genes from this pool need to be expressed to produce a single form of each of these myofibrillar proteins (see ref. 25).

Fig. 1 Distribution of fast and slow skeletal forms of troponin I in sections of adult and 2-day-old mouse muscle. Unfixed tissue sections (6 μ m thick) were stained with $\frac{1}{20}$ dilution of the antiserum and the antibody localised with sheep anti guinea pig IgG labelled with type VI horseradish peroxidase^{32,37,38}. Stained for ATPase according to the method of Padykula and Herman¹ as described by Dubowitz and Brooke³⁹. The granular appearance and distribution of the stain (also see Figs 2 and 3) is a consequence of the conditions selected to obtain optimal staining for study by optical microscopy. The distribution of granules therefore does not accurately indicate the location of the myofibrils to which the antibodies are attached. *a* To *d* are serial sections of adult and *e* and *f* serial sections of 2-day-old mouse gastrocnemius muscles. *a*, ATPase at pH 9.4; *b*, ATPase at pH 4.2; *c*, immunoperoxidase with anti-fast skeletal troponin I; *d*, immunoperoxidase with anti-slow skeletal troponin I; *e*, immunoperoxidase with anti-fast skeletal troponin I (2-day-old); *f*, immunoperoxidase with anti-slow skeletal troponin I (2-day-old); $\times 200$.



As a first approach to understanding the mechanisms that regulate the complex gene pool responsible for myofibrillar protein synthesis in skeletal muscle, we have attempted to answer the following questions: (1) Are each of the two polymorphic forms of the components of the regulatory protein system associated with a particular cell type? (2) Is there a particular association of each of the polymorphic forms of the regulatory protein, that is, are the regulatory proteins laid down as a 'kit' in a given muscle cell?

The localisation of the polymorphic forms of regulatory proteins has been determined in individual cells by staining serial cross sections of skeletal muscle by the immunoperoxidase technique with antibodies to each of the purified proteins in turn. As the polymorphic forms of troponin I, troponin T, troponin C and tropomyosin are single polypeptide chains and therefore presumably single gene products, specific antibodies prepared against each of them can be used to identify unambiguously the form of each protein present in a given cell. Such studies with the regulatory proteins therefore have advantages over similar studies with myosin, each polymorphic form of which is the product of at least three structural genes. For this reason localisation studies using antibodies prepared against the whole myosin molecule, part of the molecule or one of its polypeptide subunits may not provide unambiguous information about the nature of the whole myosin molecule in the cell. This may explain why some groups report that slow myosin is present in developing skeletal muscle^{26,27} whereas other investigators conclude that the fast skeletal myosin is the major form in this tissue (ref. 27 and N. Rubinstein, personal communication). There are still other reports suggesting that myosin present in fetal muscle differs from the fast and slow forms of adult muscle^{30,31}.

Preparation and specificities of the antibodies

The fast and slow skeletal muscle forms of troponin I were isolated from rabbit white and red skeletal muscle as described by Dhoot *et al.*³² and the corresponding polymorphs of troponin T isolated from the same sources by the methods of Perry and Cole¹³ and Margossian and Cohen³³. Troponin C was isolated from combined chicken breast and leg muscles by the method of Perry and Cole¹³. All proteins were examined for homogeneity by electrophoresis of 20–30 μ g on polyacrylamide gels in 0.1% SDS, 85 mM-Tris, 400 mM borate buffer, pH 7.0. Only preparations that migrated as a single band were used as antigens. The purity of the two forms of troponin I was confirmed by comparing the mobilities of the complexes formed by the polymorphs with rabbit fast skeletal muscle troponin C^{10,32}. Although isolated from mixed breast and leg muscles of the chicken, troponin C behaved as a single antigen and its antibody does not react with the cardiac form of the protein³⁴. Further evidence for its homogeneity is given by the fact that no heterogeneity has been observed in sequence studies of chicken skeletal troponin C prepared from the same source³⁵.

Antibodies to the different forms of each of the regulatory proteins of skeletal muscle of the rabbit were raised in guinea pigs in the manner described elsewhere for antibodies to troponin I^{24,32}. The specificities of the antibodies in all cases were established by the Ouchterlony immunodiffusion test³², by the demonstration of specific staining of isolated myofibrils and by

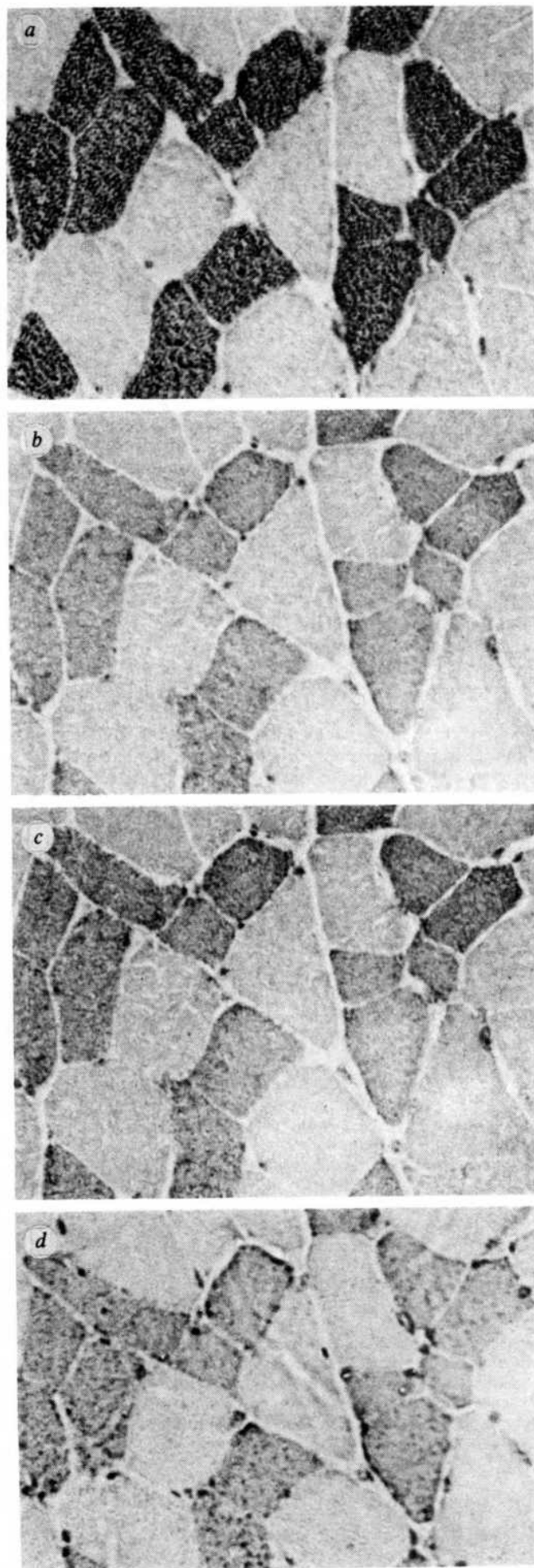


Fig. 2 Serial sections of human semispinalis capitis muscle stained for the troponin components and α -tropomyosin. Sections were stained by the indirect immunoperoxidase staining technique as described in the legend to Fig. 1. *a*, Fast skeletal troponin I; *b*, fast skeletal troponin T; *c*, fast skeletal troponin C; *d*, α -tropomyosin; $\times 200$.

the use of adsorption techniques involving antigen linked to Sepharose or cellulose carbonate³⁶. The specificity of each antibody was further confirmed by the staining of muscle sections using the immunoperoxidase technique³². If the antibody was specific for a single polymorphic form only one fibre type was stained (Fig. 1c, d and Fig. 2). If it also contained antibody to the other form, all cells in a section of adult skeletal muscle were stained.

Although usually specific for the polymorphic form of the protein used as antigen, the antibodies to the myofibrillar regulatory proteins are not species specific^{22,32,34}. The only exception reported to date is the antibody to rabbit β -tropomyosin raised in the guinea pig. This antiserum seemed to be specific for rabbit β -tropomyosin as judged by immunodiffusion²².

Cell types and the different forms of the troponin components and tropomyosin

Immunoperoxidase studies in muscle cross-sections showed that fast skeletal troponin I was localised in type II cells (those staining dark for myosin ATPase measured at pH 9.4 (ref. 1) and slow skeletal troponin I in type I cells (those staining dark for myosin ATPase measured after preincubation at pH 4.2 (Fig. 1a, b). This localisation was a feature of all muscles from the human, rabbit, rat, mouse, hamster, rhesus monkey and baboon that were examined³². In normal adult muscle, virtually all the cells studied appeared to contain either one or other of the two forms of skeletal troponin I. In Fig. 1c, d this distribution is illustrated for mouse gastrocnemius muscle. Only on very rare occasions were intermediate type cells seen that stained for both fast and slow forms of troponin I. In these cases the intensity of staining for each polymorphic form of skeletal troponin I was less than that observed in cells that stained for one form only.

In view of the precise and clear differentiation of adult skeletal muscle cells into two types based on the immunoperoxidase staining method for fast and slow skeletal muscle forms of troponin I, we have suggested that this procedure presents a rapid, convenient new method for typing muscle cells³².

Study of serial sections showed that the antibodies to the fast skeletal muscle forms of troponin T, troponin C and α -tropomyosin stained the same cells as those stained by fast skeletal muscle troponin I. This distribution is demonstrated with human semispinalis capitis muscle in Fig. 2, but has been observed in all muscles studied to date. As far as it could be judged from the intensity of staining observed in the immunoperoxidase test, the fast skeletal muscle forms of the components of the troponin complex and α -tropomyosin were absent from the type I cells. Slow skeletal muscle troponin T was localised in the type I cells. The specificities of the antibodies to the fast and slow forms of troponin T and the differential staining of the type I and type II cells with those antibodies is the first unambiguous demonstration that the slow and fast skeletal muscle forms of troponin T are different. Preliminary studies with antibody raised against rabbit cardiac troponin C indicate that this antibody reacts with type I cells only. In Ouchterlony diffusion tests the antibody gave a precipitin line with troponin C isolated from rabbit slow skeletal and cardiac muscles but not with fast skeletal muscle troponin C. Our results do not permit us to decide whether troponin C in the type I cells is identical with cardiac troponin C or merely cross-reacts with antibody to this protein. The primary sequences of troponin C from rabbit slow skeletal muscle and bovine cardiac muscle are very similar (J. M. Wilkinson, personal communication).

Extrapolation from the general pattern of results obtained with antibody to α -tropomyosin and the fact that all the muscles studied contain β -tropomyosin suggest that the latter polymorph is located in type I cells in mammalian muscle. Preliminary studies with antibody to β -tropomyosin indicate that this is the case (G. K. D., N. Frearson and S. V. P., unpublished

results). Our data do not yet permit us to conclude with certainty whether β -tropomyosin is absent or present in type II cells. As the results indicate that α -tropomyosin is absent, or present in such small amounts that it cannot be identified by the immunoperoxidase technique in type I cells, tropomyosin must be present as the β_2 dimer in these cells. Direct evidence for the

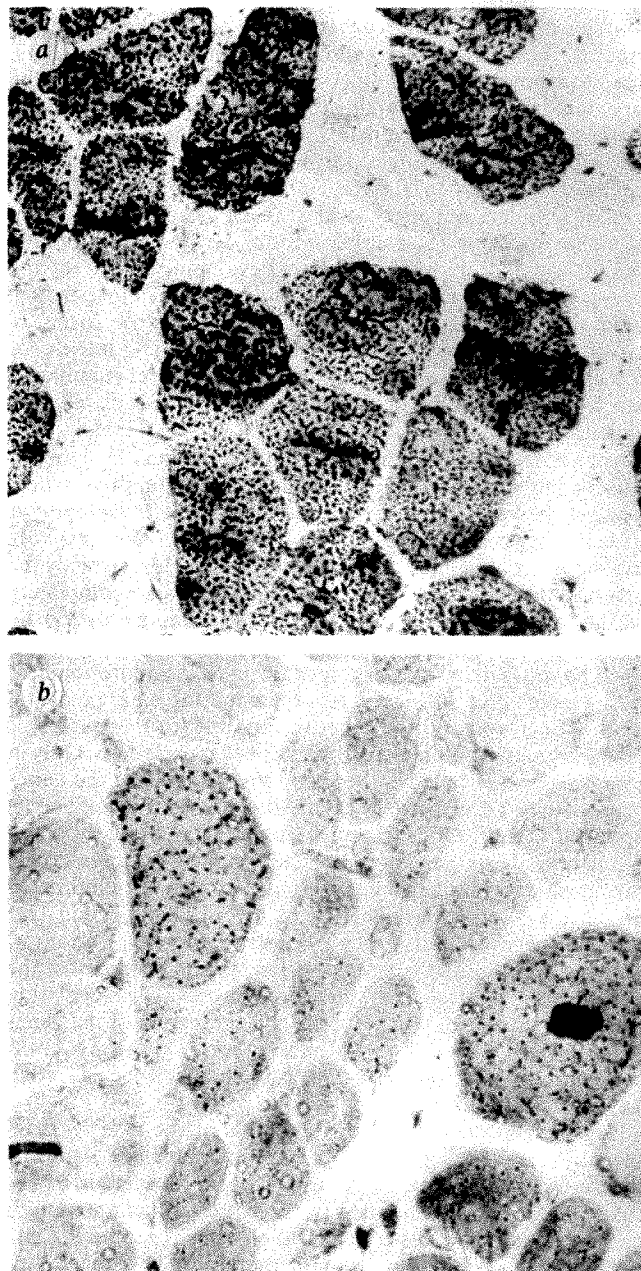


Fig. 3 Comparison of localisation of fast skeletal troponin I in normal and dystrophic human muscle. Sections stained for fast skeletal troponin I by the indirect immunoperoxidase technique as described in Fig. 1. *a*, Normal human quadriceps muscle; *b*, quadriceps muscle from a patient with Duchenne muscular dystrophy; $\times 200$.

existence of the β_2 form of tropomyosin in adult muscle has not previously been obtained, although it must exist in rabbit fetal muscle, the tropomyosin of which consists of 80% of the β form⁴¹. Table 1 summarises the conclusions we can make so far on the localisation of the polymorphic forms of the regulatory proteins in the two types of adult skeletal muscle.

Factors affecting the expression of genes controlling synthesis of regulatory proteins

Our studies suggest that there is a very precise association of the different polymorphic forms of the regulatory proteins in normal adult skeletal muscle. The existence of two isoenzymic forms of fast and slow myosin in type II and type I cells respectively, however, indicates that there may be more flexibility as to the form of the myosin molecule incorporated into the myofibrillar structure of a given fibre type than there is with the regulatory proteins. Such a rigid association of the different forms of the regulatory proteins in adult skeletal muscle must be an important factor in determining the contractile properties of the myofibril that are characteristic for the cell type.

The presence of a single polymorphic form of each of the regulatory proteins in a given cell type is a feature of adult muscle. This does not apply at all stages of muscle development for we have observed fast and slow forms of troponin I, troponin T and troponin C in the same cells in developing rabbit, rat and mouse leg muscles. Gauthier *et al.*²⁷ and N. Rubinstein (personal communication) have reported the fast and slow forms of myosin to be present in a single cell in developing muscle, as have Pette and Schnez⁵ in adult muscle after direct stimulation with frequencies different from that normally associated with the muscle.

In newborn animals all the cells from skeletal muscle of the leg stain for fast troponin I. A few, which are presumptive type I fibres, stain weakly for slow troponin I and as development proceeds those cells containing slow troponin I progressively stain more strongly for the slow polymorph and less strongly for the fast form (Fig. 1e, f). Finally the latter disappears completely and the cells appear as normal type I cells staining only for the slow forms of troponin I. A similar pattern of localisation is shown by the fast and slow forms of troponin T and troponin C. Thus it seems that during the late fetal and the early postnatal stages of development, the genes responsible for the fast forms of the troponin components are active in all muscle cells. Only in those cells programmed to become type I fibres are the genes responsible for the slow forms of the troponin components expressed as well. As development and specialisation progress, all but one of the genes responsible for the syntheses of the polymorphic forms of a given regulatory protein are suppressed until finally a predetermined kit of myofibrillar protein (see Table 1) is synthesised, probably under some type of control involving coordinated gene expression, possibly similar to the operon type of control observed in prokaryotes. The elucidation of the mechanisms of the control of the expression of the genes responsible for synthesis of the different forms of the myofibrillar proteins presents a fascinating problem of cell specialisation in response to innervation and activity pattern.

Thus the rigid control of the synthesis of the various polymorphic forms of the myofibrillar proteins is a feature of normal adult muscle. Any change from the normal function of the muscle caused by an alteration in the pattern of stimulation or changes induced by cross re-innervation or by regeneration as a result of disease or injury would, therefore, be expected to change the pattern of localisation of regulatory proteins and possibly also other myofibrillar proteins. It was mentioned above that fibres from the skeletal muscle of adult animals which stained for both fast and slow forms of skeletal troponin I were observed very infrequently. These cases could be explained on the basis that the muscle was not normal or that the animal had been treated in some way that could have promoted some change in its muscle. This view has been reinforced by the fact that examination of samples of muscles from patients with some known primary and secondary myopathies has usually shown that a large number of cells contain both fast and slow polymorphic forms of troponin I and troponin T. This is illustrated for troponin I in Fig. 3 which shows sections through muscle from a normal adult and a patient suffering from Duchenne muscular dystrophy. The study of the distribution of the different forms of the regulatory and probably other myofibrillar

Table 1 Distribution of polymorphic forms of the myofibrillar proteins in skeletal and cardiac muscle of mammals

	Skeletal		Cardiac
	Type I	Type II	
Myosin	Slow A1 Slow A2 ² α^{40}	Fast A1 Fast A2 ^{2,3} ?	Cardiac
Actin	β	α	Cardiac ⁴¹
Tropomyosin	Slow (= cardiac?) [*]	Fast	α †
Troponin C	Slow	Fast	Cardiac
Troponin I	Slow	Fast	Cardiac
Troponin T	Slow	Fast	Cardiac

* Only type I fibres stained when sections were treated with anti-cardiac troponin C serum. The primary sequences of troponin C from rabbit slow skeletal muscle and bovine heart are very similar (J. M. Wilkinson, personal communication).

† Although the α -tropomyosin present in cardiac muscle is very similar to the α form present in skeletal muscle it will be necessary to determine the primary sequence to establish whether the identity is complete.

proteins in muscle cells shows considerable promise for the diagnosis and study of the origin of human muscle disease.

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letters

SS433—a massive black hole?

SPECTROPHOTOMETRIC observations by Margon *et al.*¹ have drawn attention to some remarkable spectroscopic activity in the object Stephenson–Sanduleak 433. In addition to broad, intense Balmer, He I and He II emission lines, Margon *et al.*¹ report the presence of broad, strong anomalous emission lines which move rapidly across the spectrum. For example, their Fig. 3 shows the behaviour of two such lines (one at $\sim 6,000$ Å and the other at $\sim 7,400$ Å with mean value just redwards of $H\alpha$) on three nights in a four-day interval. The IR line moved redwards by ~ 150 Å and the red line bluewards by ~ 70 Å. If interpreted as Doppler shifts these movements imply velocity changes of $6,000 \text{ km s}^{-1}$ and $-3,500 \text{ km s}^{-1}$ respectively. We propose here that these anomalous emission lines are produced by an emitting ring of gas in a circular orbit around a central, massive black hole.

The line profile of emission from such an orbiting ring (see ref. 2) consists of two emission peaks, one on either side of the rest wavelength λ_0 , at wavelengths $\lambda_{\pm}$ corresponding roughly to $\pm V_{\text{orb}}$, the orbital velocity of the ring. That is, roughly, $\lambda_{\pm} = \lambda_0 (1 \pm V_{\text{orb}}/c)$. There is also a certain amount of emission between the two peaks, which in this case, we suggest, is masked by the underlying continuum. For SS433, however, the orbital velocity must be so high ($V_{\text{orb}}/c \approx 0.15$) that the orbit is sufficiently close to the central object that the net gravitational redshift of the orbit must also be taken into account. This has the effect of shifting the mean wavelength of the two emission peaks to the red of the rest wavelength as is indeed observed.

For a thin ring in a circular orbit at radius $R = r GM/c^2$ around a Schwarzschild black hole of mass M , the two emission peaks will be at wavelengths given roughly by

$$\lambda_{\pm} = \lambda_0 \left\{ 1 \pm \left(1 - \frac{2}{r} \right)^{-1/2} \frac{\sin i}{r^{1/2}} \right\} / \left(1 - \frac{3}{r} \right)^{1/2}$$

where i is the inclination of the ring's orbit to the line of sight³. These wavelengths are shown for $H\alpha$ and $H\beta$ in Fig. 1, for various values of r and i . Also shown are the wavelengths observed by Margon *et al.*¹, which can be fitted by a ring of given inclination whose radius steadily decreases with time. Note also that as the ring's radius decreases, we would expect the blueward peak to reverse direction, having got as far as the region around He I at $5,876$ Å, the precise value depending sensitively on the inclination (see ref. 4). At the same time the red peak from $H\beta$ appears in the same region. Evidence for such behaviour is reported by Ciatti *et al.*⁵ and by Margon *et al.*¹. Furthermore, from symmetry considerations we would expect the profiles of the emission peaks at any given time to be mirror images of each other (at least on timescales longer than the orbital time). The data presented by Margon *et al.* lend some credence to this. Although we have considered mainly the strong Balmer lines, there is evidence that other lines (such as, He I, He II) display similar behaviour.

We now consider the physical properties of the emitting region. The width of the emission peaks indicates that the thickness of the ring, ΔR , is small, say $\Delta R \leq 0.1R$. Taking $R \approx 50 GM/c^2$ (see Fig. 1) we find that for the emitting region to be large enough that its brightness temperature in the lines does not exceed $\sim 10^4$ K, we require $M \geq 3 \times 10^5 M_{\odot}$. Hence we conclude that the central object is a massive black hole. To obtain the observed (dereddened) anomalous line luminosity, we find that the particle density in the emitting region is $n_{\text{em}} \sim$

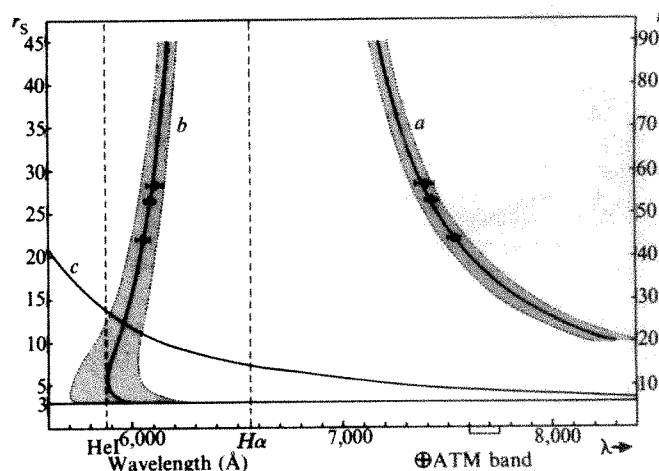


Fig. 1 The predicted wavelengths λ of the red (a) and blue (b) wings of $H\alpha$ and the red (c) wing of $H\beta$ are shown as functions of the radius of the emitting ring, r in mass units or r_s in Schwarzschild radius units, for inclination $i = 45^\circ$. The shaded region corresponds to $40^\circ \leq i \leq 50^\circ$. The filled circles and bars indicate the positions and widths of the lines observed by Margon *et al.* on 23, 24 and 26 October 1978. Thus the observations are consistent with a steadily decreasing radius. Also shown are the rest wavelengths of He I, $H\alpha$ and of the atmospheric A band.

10^{12} cm^{-3} and the total emitting mass to be $m_{\text{em}} \sim 10^{24} \text{ g}$. Note that the rate at which such a mass releases energy as it falls from $r = 28$ to $r = 22$ in a time of three days (Fig. 1) is $\sim 10^{36} \text{ erg s}^{-1}$, comparable to the dereddened anomalous emission line luminosity.

Ipser and Price⁶ have considered accretion on to massive black holes within the galaxy. They find that a hole of $\sim 3 \times 10^5 M_{\odot}$ passing through the plane of the galaxy would accrete sufficiently to radiate a total luminosity of $\approx 10^{38} \text{ erg s}^{-1}$. They also predict that, if a large part of the mass of our galaxy is in the form of such objects, the nearest such object should be at a distance of a few kpc and have a velocity of $\sim 300 \text{ km s}^{-1}$. These findings are in accord with the observations of SS433. We suggest therefore that the optical continuum radiation, broad emission lines, weak radio and X-ray emission from SS433 are produced by the accretion process.

One problem with our model is the explanation of why the emitting ring remains so narrow. The effect of viscosity on an accreting ring spreads the ring out as it accretes⁷. The emission observed may come predominantly from the densest part of the accreting ring; or we may be witnessing the accretion of a solid body of about planet size (say of mass $\sim 10^{28} \text{ g}$) as it spirals inwards because of friction within a more slowly moving accretion disk. The anomalous emission would then come from a thin ring of steadily decreasing radius in the neighbourhood of the planetoid. In this case the orbital period of $\sim 1 \text{ h}$ might be detectable.

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Neutrinos from neutron stars

SEVERAL schemes to detect ultra-high energy (UHE: $\geq 10^{12}$ eV) muons and neutrinos produced by cosmic ray interactions in the atmosphere have been recently studied¹, and the applicability of the resulting designs to extraterrestrial sources is being discussed². We present here the results of a calculation to determine the flux of UHE neutrinos from galactic neutron stars and the consequent number of point sources detectable at the sensitivity threshold of the proposed deep underwater muon and neutrino detector¹ (DUMAND) array.

Pulsars, with their huge magnetic and electric fields and their ready supply of (rotational) energy, are obvious candidates as sources of UHE particles and radiation³. The Crab pulsar emits pulsed γ -rays at energies exceeding 10^{12} eV, and the neutron star in the binary X-ray system Cyg X-3 has been detected as a UHE γ -ray source modulated at its binary orbital period of 4.8 h (ref. 4). In addition, pulsars are considered to be a likely site of cosmic-ray particle acceleration, and the highly relativistic wind from young sources probably dominates the energetics of some supernova remnants.

The requirements for the production of celestial UHE neutrinos are simply a source of UHE particles, and sufficient target material to produce a significant probability that these particles will convert their energy into neutrinos by a kaon/pion-producing collision followed by the charged meson's decay. For example, to convert ~50% of its energy into neutrinos, a 10^{12} eV proton must traverse ≥ 50 g cm⁻² of material or a column density of $\sim 10^{28}$ high energy photons cm⁻² (ref. 3). The high photon densities are probably not relevant to neutron stars in a galactic setting and will not be considered further here. Table 1 lists common pulsar environments and the resulting column densities of matter encountered by a UHE proton on its journey from the neutron star's surface to Earth. In general, for single pulsars, it seems that the amount of material present is 3–5 orders of magnitude too small; even for a source on the far side of the Galaxy with a line of sight passing through several giant molecular clouds, <1% of the material needed by a proton for the deposition of half its energy in neutrinos is encountered.

Only in the case of a neutron star in a close binary system with a companion undergoing rapid mass loss does the amount of matter become significant. In systems where massive accretion disks or circumstellar shells develop, or where the neutron star actually buries itself within the envelope of its companion⁵, the

Table 1 Location of the target material

Location	Density (cm ⁻³)	Path length (pc)	Amount (g cm ⁻²)
Supernova remnants (such as Crab Nebula)	10	1	10^{-4}
General interstellar medium	1	5000	10^{-2}
Giant molecular clouds			
(a) Core	10^6	0.1	10^{-1}
(b) Halo	10^3	10	10^{-2}
Binary star systems with semi-major axes $R \sim 10 R_{\odot}$ and mass loss rates $\dot{M}$ of:			
(a) $\dot{M} = 10^{-6} M_{\odot} \text{ yr}^{-1}$	3×10^{11}	10^{-5}	3
(b) $\dot{M} = 10^{-9} M_{\odot} \text{ yr}^{-1}$	3×10^8	10^{-5}	3×10^{-3}

Table 2 Pulsar energy loss rates

Pulsar age (yr)	No. observed with $\tau \leq 10^6$ yr	$\dot{E}$ (10^{34} erg s ⁻¹)	Estimated number in Galaxy	
			Isolated	In accreting binaries
0– 1.0×10^3	1	47,000	150	1
1.0×10^4 – 5.0×10^4	2	400	7,000	7
5.0×10^4 – 1.0×10^5	3	2.7	7,500	7
1.0×10^5 – 3.3×10^5	7	3.0	35,000	35
3.3×10^5 – 6.7×10^5	9	1.3	50,000	50
6.7×10^5 – 1.0×10^6	3	0.5	50,000	50
Total	25		~150,000	150

column densities can easily exceed the required 50 g cm⁻². In the estimates of neutrino fluxes derived below, we adopt a conversion efficiency $\eta_T = 10^{-1}$ for the energy available in UHE particles from neutron stars in such binaries, and $\eta_T = 10^{-4}$ for the remaining objects.

The ultimate power source for relativistic particles and radiation from neutron stars is their rotational kinetic energy. All of the radio pulsars for which measurements have been made are observed to be slowing down. Their energy loss rate $\dot{E}$ is given by

$$\dot{E} = \frac{1}{(2\pi)^2} I \nu \dot{\nu} = 4 \times 10^{46} \nu \dot{\nu} \text{ erg s}^{-1}$$

where ν and $\dot{\nu}$ are the pulsar rotation frequency and its first derivative, and the value of the star's moment of inertia I is taken to be 10^{45} g cm². The potential UHE particle luminosity (L_p) from the Crab pulsar, then, is $\sim 5 \times 10^{38}$ erg s⁻¹, while from a typical 1 Myr old source, L_p is $\sim 5 \times 10^{33}$ erg s⁻¹ (see Table 2); these numbers represent an absolute, model-independent upper limit to the energy available in UHE particles from pulsars.

Michel⁶ offers an alternative to this naive assumption of a rotational energy-to-particle conversion efficiency of unity. Working from the basic assumption that the plasma being accelerated from the neutron star's polar cap is described as space charge limited flow, he has performed a dimensionless analysis which indicates that most of the star's energy loss goes into electromagnetic fields rather than particles, regardless of the physical parameters of the source. In particular, one can estimate L_p and the average particle energy ϵ_p solely as a function of the observed energy loss rate and the charge and mass of the accelerated nuclei. The particle energies are just those required for UHE neutrino production: for the range $10^{32} \leq \dot{E} \leq 10^{39}$, we obtain 2.5×10^{12} eV $\leq \epsilon_p \leq 1.5 \times 10^{14}$ eV for iron nuclei and values a factor of 2–4 less for α particles. However, the conversion efficiency for rotational energy to particles, η_B , is typically $\leq 1\%$; we find

$$L_p = 6 \times 10^{35} (\dot{E} \times 10^{-38})^{3/4} \text{ erg s}^{-1}$$

implying a Crab pulsar particle luminosity of $L_p \sim 2.4 \times 10^{36}$ erg s⁻¹ (for iron ions) which decreases to $L_p \sim 5 \times 10^{32}$ erg s⁻¹ for a 1 Myr old source (these values are lower by a factor of ~ 4 for α particles). For those neutron stars in accreting binaries, the infalling plasma may hinder the particle acceleration mechanism and reduce the UHE particle flux still further.

Having obtained estimates of the relevant efficiencies for the energy transformation rotational kinetic $\rightarrow$ relativistic particle $\rightarrow$ UHE neutrino, we need only determine the number of active neutron stars in the Galaxy to establish limits on the number of detectable neutrino sources. X-ray catalogues^{7,8} (which are complete for the galactic sources with luminosities $\geq 10^{36}$ erg s⁻¹) list ~ 100 objects thought to contain heavily accreting neutron stars in binaries (type (a) binary of Table 1), while a review of data on X-ray transients⁹ suggests perhaps 10–50 times this number of systems may exist with lower and/or sporadic accretion rates. Many authors^{10–14} have analysed the

Table 3 Neutrino luminosities and the number of detectable sources*

E (erg s ⁻¹)	Isolated stars ($\eta_T = 10^{-4}$)				Close binary systems ($\eta_T = 10^{-1}$)			
	$\eta_E = 1^\dagger$	$\eta_E = \text{SCLF}^\ddagger$	$\eta_E = 1^\dagger$	$\eta_E = \text{SCLF}^\ddagger$	$\eta_E = 1^\dagger$	$\eta_E = \text{SCLF}^\ddagger$	$\eta_E = 1^\dagger$	$\eta_E = \text{SCLF}^\ddagger$
	L_ν (10 ³⁰ erg s ⁻¹)	No. detectable	L_ν (10 ³⁰ erg s ⁻¹)	No. detectable	L_ν (10 ³⁰ erg s ⁻¹)	No. detectable	L_ν (10 ³⁰ erg s ⁻¹)	No. detectable
10 ³⁸	10 ⁴	1	60	0	10 ⁷	1	60,000	0.2
10 ³⁶	10 ²	1	2	0	10 ⁵	0.5	2,000	0.1
10 ³⁴	1	0.1	0.06	0	10 ³	0	60	0
10 ³²	10 ⁻²	0	0.002	0	10	0	2	0

* Assuming a DUMAND sensitivity threshold of 10² eV (cm² s)⁻¹. Note that no sources are detectable at the threshold of current detectors of 10⁴ eV (cm² s)⁻¹.

† Model independent upper limits.

‡ Based on the space charge limited flow (SCLF) assumption of Michel⁶.

available pulsar data and have estimated the total number of active radio pulsars in the Galaxy to be between 10⁵ and 10⁶. However, due to their monotonic decrease in energy loss rate, only the youngest of these pulsars will contribute significantly to the flux of UHE particles available for neutrino production. Although it is generally accepted that pulsar characteristic ages ($\tau = P/2\dot{P}$) are poor indicators of the true source ages^{10,11,15}, the values of τ for the two youngest objects (the pulsars associated with the Crab and Vela supernova remnants) agree well with the independent age estimates available, and most authors agree that up to about 1×10^6 y, τ may provide a reasonable chronological age. Of the ~110 sources with measured period derivatives, 25 have characteristic ages of <1 Myr^{16,17}. The numbers of these pulsars in each of several age brackets, their observed energy loss rates and the extrapolated total population of each group in the Galaxy, are shown in Table 2. The estimates for single pulsars are based on a steady-state birthrate of one source per 6 yr^{10,14}. The numbers for neutron stars in binary systems are crude estimates based on a constant (with age) fraction of 0.1% of the total population. This value is consistent with the X-ray data, but it does not include evolutionary considerations which might either selectively enhance (such as, by the production of relativistic wind-induced envelopes around young objects¹⁸) or deplete (for example, due to long evolutionary time required for the companion to reach the mass-losing stage¹⁹) the number of young objects in accreting systems.

The proposed DUMAND array¹ has a point source detection threshold of ~10² eV cm⁻² s⁻¹. The results presented in Tables 1 and 2, with our adopted efficiencies η_T and η_E , allow us to estimate the number of neutron stars that we can expect to observe at this level of sensitivity. We assume that the young pulsars enumerated in Table 2 are uniformly distributed throughout the galactic disk within a galactocentric radius of 12 kpc (this ignores the radial and Z-height dependences of the pulsar distribution¹⁰, however, this will not appreciably affect the results). Table 3 presents the neutrino luminosities for various energy loss rates and the estimated number of detectable sources based on our derivations of η_E and η_T . To typical astrophysical accuracy, we may conclude that there will be no source confusion problem for DUMAND (resolution $\leq 1^\circ$) in observing galactic neutron stars, but, with a little luck, a few detections are possible.

Note that any such source observed in the UHE neutrinos will not be invisible to all other types of detectors. These objects will produce a copious flux of γ rays, and in the case of the close binary systems, an accretion-powered X-ray source is also expected. In fact, among the 15 or so localised 100 MeV γ -ray sources in the galactic plane²⁰, five are associated with known or suspected neutron stars: four radio pulsars including the one in the Crab Nebula, and the binary X-ray source Cyg X-3. The latter two objects have already been observed by Cerenkov detectors in the UHE regime ($E_\gamma \approx 10^{12}$ eV)⁴. In the case of the radio pulsars, these high energy photons may be produced through curvature radiation or some other processes associated with the pulsar mechanism rather than through the meson decay

which would require an accompanying flux of neutrinos. However, the case of Cyg X-3 is promising. Bignami, Maraschi and Treves²¹ and others^{22,23} have suggested a model for this source involving a young neutron star embedded in the dense stellar wind of its red dwarf companion. The X-ray emission is powered not by accretion, but by interaction of the relativistic outflow (in dipole radiation, particles, and so on) from the pulsar with the plasma surrounding the system. The observed UHE γ -rays show an orbital phase distribution which peaks when the X-ray source (the neutron star) is near eclipse⁴; that is, when our line of sight passes through the maximum path length of circumstellar target material. No other similar systems have been optically identified, and it is difficult to resist the speculation that this source is one of the handful of binary neutrino sources predicted in Table 3.

We have attempted to estimate the number of neutrino point sources powered by galactic neutron stars which will be detectable with the DUMAND array. While the prospects for such observations are far from sanguine, the totally new view of the objects discussed here offered by a UHE neutrino telescope can only add to the many potentially fundamental physical insights that the study of galactic neutron stars is sure to provide.

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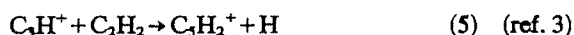
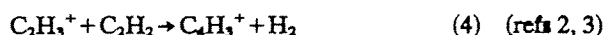
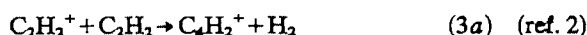
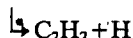
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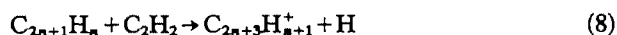
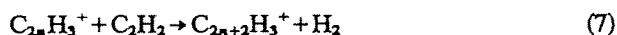
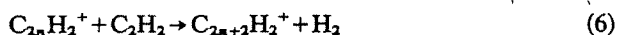
Long chain carbon molecules and diffuse interstellar lines

THE diffuse interstellar lines, seen in the optical spectra of stars which lie behind fairly low density interstellar clouds, have remained unidentified for many years. It has recently been suggested¹ that the diffuse interstellar lines are the absorption bands of long chain carbon molecules, C_n , where n may be in the range 5–15. As one possible source for these molecules, Douglas¹ proposes that long chain hydrocarbons are synthesised in dense interstellar clouds. After cloud disruption, the hydrocarbons may lose their hydrogen atoms by photodissociation and exist as pure carbon chains. While the shortest of the chains (C_2 , C_3 , C_4) will themselves be photodissociated, Douglas¹ argues that chains beyond a certain length are stable against photodissociation and will persist in the low density clouds in which the diffuse interstellar lines are formed. As a first step in evaluating the feasibility of this route, we estimate here the abundances of chain hydrocarbon molecules which would be expected in dense interstellar clouds as a result of gas-phase chemistry alone. The rather high abundances of hydrocarbons which we predict support the identification of carbon chains as the source of the diffuse interstellar spectral features.

Rate coefficients for ion-molecule reactions leading to hydrocarbon-chain molecules with up to five carbon atoms have been measured in the laboratory^{2,3}. The most important of these measured reactions are



Further acetylene additions can occur by reactions of the form³



for $n = 2, 3, 4, \dots$

We have argued⁴ that methane, which is required if the synthetic reactions (1–5) are to proceed, should be abundant in dense clouds. We found, for a fractional ionisation of $e/n = 3 \times 10^{-8}$ (refs 5, 6), and a rate coefficient of $4 \times 10^{-13} \text{ cm}^3 \text{ s}^{-1}$ for the radiative association reaction of CH_3^+ with H_2 (ref. 7), that $[CH_4]/n \approx 10^{-6}$, where $n = n(H) + 2n(H_2)$. The recent detection of methane in Orion A⁸, although not leading to a reliable column density because of non-thermal excitation, is compatible with a high methane abundance. Acetylene is produced by reaction (1a) and is destroyed by reaction (2) so that the relative abundance of acetylene is

$$C_2H_2/n \approx \frac{k_{1a}(C^+/n)(CH_4/n)}{k_2(C^+/n)}$$

where we use the notation $X/n = [X]/n$. Since $k_{1a} \approx k_2$, we find that $C_2H_2/n \approx CH_4/n \approx 10^{-6}$.

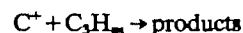
At each step in the sequence of reactions (1–8), recombination with electrons leads to neutral hydrocarbons. The destruction rate of the neutral molecule is required for a determination of its abundance. The assumption that destruction by reaction with C^+ provides an estimate of the actual destruction

rate for a complex molecule⁴. With this assumption, the equilibrium abundances of the neutral molecules are readily obtained. In view of the uncertainty in the number of hydrogen atoms ejected after recombination, we use the notation C_nH_m for the neutral molecule containing n carbon atoms. For example, C_4H_m will represent all of the likely products for the recombination of both $C_4H_3^+$ and $C_4H_2^+$, namely C_4 , C_4H , C_4H_2 and C_4H_3 .

To illustrate the method of calculation we will obtain the equilibrium abundances of C_3H_m and C_2H_m . C_3H_m is formed by reaction (2) followed by recombination, and is destroyed by reactions with C^+ (by assumption), so that

$$C_3H_m/n = \frac{k_2(C^+/n)(C_2H_2/n)}{k_{C^+}(C^+/n)}$$

where k_{C^+} is the rate coefficient of the reaction



If $k_{C^+} \approx k_2$, we find that $C_3H_m/n \approx C_2H_2/n \approx 10^{-6}$. Similarly,

$$C_3H_m/n = \frac{k_3(C_3H^+/n)(C_2H_2/n)}{k_{C^+}(C^+/n)}$$

Since C_3H^+ is formed by reaction (2) and destroyed by recombination with electrons,

$$C_3H^+/n = \frac{k_2(C^+/n)(C_2H_2/n)}{k_e(e/n)}$$

where $k_e (\approx 3 \times 10^{-7})$ is the recombination rate coefficient. Using $C_2H_2/n \approx 10^{-6}$, $k_{C^+} = 2 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$, $e/n = 3 \times 10^{-8}$, and the measured values³ for k_2 and k_3 , we find $C_3H_m/n \approx 2 \times 10^{-7}$.

The equilibrium abundances of molecules with up to 12 carbon atoms, obtained with the assumption that reactions (6–8) have a rate coefficient of $2 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$, are given in Table 1. It can be seen from Table 1 that the calculated abundances of C_n molecules remain quite high even for n as large as 11. By way of comparison, a fractional abundance of $10^{-9} \leq CH/n \leq 10^{-8}$ produces the strong observed interstellar lines of CH. The decline in abundance towards larger molecules is slower than might have been expected: $C_{2n+1}H_m/C_{2n+3}H_m \approx 5 \approx C_{2n}H_m/C_{2n+2}H_m$ for $n = 1, 2, 3$ and 4. The predicted ratio for hydrocarbons is close to the observed ratio for cyanopolynes of $HC_{2n}CN/HC_{2n+2}CN \approx 4$ for $n = 1, 2$ and 3 (ref. 9).

The predicted abundances of hydrocarbons with an odd number of carbon atoms are higher than the abundances of hydrocarbons with an even number of carbon atoms (Table 1). This occurs because C_3 hydrocarbons are formed by reaction of C^+ with C_2H_2 while C_4 hydrocarbons are produced by the reaction of $C_2H_2^+$ with C_2H_2 . Since C^+ is more abundant than $C_2H_2^+$, C_3H_m will be more abundant than C_4H_m . Subsequent acetylene additions preserve this relation: that is $C_{2n+1}H_m/n > C_{2n+2}H_m/n$.

The fractional ionisation which we have assumed, $e/n \approx 3 \times 10^{-8}$, is an upper limit deduced from observed abundance ratios of isotopic forms of various molecules. It is unlikely that e/n can be much less than 10^{-8} because the observed abundance of HCO^+ is often $HCO^+/n \approx 10^{-8}$, providing a lower limit to e/n if the regions are electrically neutral. Furthermore, model calculations show that, even with extreme heavy element depletion, the fractional electron abundance remains near 10^{-8} (ref. 10). On the other hand, the fractional ionisation in less

Table 1 Predicted relative abundances of hydrocarbons in dense clouds

$C_3H_m/n \approx 1 \times 10^{-6}$	$C_4H_m/n \approx 8 \times 10^{-8}$
$C_5H_m/n \approx 2 \times 10^{-7}$	$C_6H_m/n \approx 2 \times 10^{-8}$
$C_7H_m/n \approx 3 \times 10^{-8}$	$C_8H_m/n \approx 3 \times 10^{-9}$
$C_9H_m/n \approx 7 \times 10^{-9}$	$C_{10}H_m/n \approx 7 \times 10^{-10}$
$C_{11}H_m/n \approx 1 \times 10^{-9}$	$C_{12}H_m/n \approx 1 \times 10^{-10}$

dense and/or warmer regions may be as high as 3×10^{-7} . A fractional electron abundance of 3×10^{-7} would not change the methane abundance⁴, the acetylene abundance, or the abundance of C_2H_m . All other hydrocarbons, C_n/H_m with $n > 4$, would decrease in abundance by a factor of 10 because their precursor ions, $C_nH_m^+$, would decrease in abundance by this factor.

The high abundances we find here for dense clouds ($\Sigma_{m>5} C_m/n \approx 10^{-7}$) lend support to the idea that C_n molecules may be the source of the diffuse interstellar lines.

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γ and X rays from the zodiacal dust

γ -RAY lines can be produced by MeV protons in interactions with interstellar¹⁻⁴ and solar coronal matter⁵⁻⁷. Here I show that, in favourable conditions, solar flare protons can produce both observable γ -ray lines and characteristic X-ray lines, when interacting with the material of the zodiacal dust cloud. Of particular interest are narrow γ -ray lines without kinematical broadening ($\Delta E < 1$ keV), which are produced by long-lived

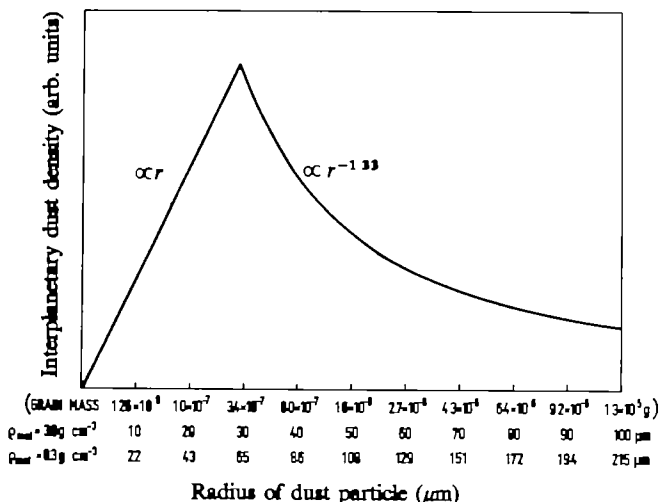


Fig. 1 Idealised mass density as a function of grain size of the zodiacal dust. This was adapted from Giese and Grün's⁶ maximum model. The abscissa shows particle radii for two different assumed grain material densities; also indicated is the corresponding grain mass. This graph shows that the bulk of the zodiacal dust matter is contained in grains with radii $10 \mu m \leq r \leq 100 \mu m$.

Table 1 The most intense nuclear γ -ray lines expected from zodiacal dust matter

Element	Photon energy (MeV)	Mean life (10^{-12} s)	4 August 1972 expected line flux at Earth ($10^{-6} \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$)
¹⁶ O	6.23	24.0	1.0
²⁴ Mg	1.37	1.75	1.3
²⁸ Si	1.78	0.68	0.5
⁵⁶ Fe	0.847	9.7	1.2

The line flux expected for the 4 August 1972 event from zodiacal dust oxygen is estimated in the text, the other three values were scaled for differing abundance ratios from ref. 4.

nuclear excited states ($\tau \geq 10^{-12}$ s) in solids. A typical zodiacal dust grain is sufficiently large for such narrow line production, and modern germanium detectors can detect and identify these lines. Observation of X rays and γ rays together would yield information on the average interplanetary dust density, grain size, material density, and chemical composition. This might help to determine whether the dust is of Solar System (for example, cometary) or of interstellar origin. Protons ≥ 10 MeV form a probe for which the dust grains are transparent, so that the measured quantity does not depend on grain size and surface properties, in contrast to the zodiacal light.

The properties of the zodiacal dust are not too well known. The grain size spectrum shows a knee^{8,9} around particle size 3×10^{-7} g, which translates into grain radii (spherical shape assumed) of $r \approx 30 \mu m$ to $r \approx 60 \mu m$, for assumed material densities of $\rho_{\text{mat}} = 3 \text{ g cm}^{-3}$ to $\rho_{\text{mat}} = 0.3 \text{ g cm}^{-3}$, respectively. The bulk of the matter is contained in grain sizes between about 10 and $100 \mu m$ (see Fig. 1). The range of 10 MeV protons is $\geq 0.1 \text{ g cm}^{-2}$, so the grains are transparent up to $r \approx 1,000 \mu m$. The total mass density at 1 AU is somewhere between $4 \times 10^{-23} \text{ g cm}^{-3}$ (refs 8, 9) and $4 \times 10^{-22} \text{ g cm}^{-3}$ (refs 10, 11); in the following, ρ is taken as $1.2 \times 10^{-22} \text{ g cm}^{-3}$. The number density dependence on solar distance R is estimated to be $n(R) \propto R^{-\nu}$ with ν between 1.0 and 1.7 (ref. 12). Recent space probe observations imply that ν from 1.3 (ref. 13) to 1.5 (ref. 14). Around the Sun there seems to be a dust-free zone extending to a few solar radii¹⁵. Infrared observations¹⁶ and orbital calculations¹⁷ indicate that the dust cloud indeed extends down to a few solar radii. The chemical composition of the grains seems to be similar to chondrites^{10,18} or even to carbonaceous chondrites^{19,20}. The material density of the dust particles is often assumed to be between that of water and iron²¹, typically $\rho_{\text{mat}} \approx 3 \text{ g cm}^{-3}$. On the other hand, fluffy particles, which consist of a mosaic of smaller particles and which are probably much less dense, have been recorded^{10,20,22,23}. Other estimates are as low as $\rho_{\text{mat}} = 0.18$ (ref. 11).

Nuclei that are excited by 10–20 MeV protons (where the excitation cross-sections exhibit a broad maximum) receive recoil energies in the 10–100 keV/AMU range⁶. Lines from short-lived states or gaseous material would be of the order of 100 keV wide^{2-4,6}. However, in solids such nuclei have projected ranges of several $100 \mu g \times \text{cm}^{-2}$ (refs 24–26), and their stopping time is of the order of 10^{-12} s, so that lines from longer-lived nuclear states from grains with $r \gg 1 \mu m$ would have a significant component that is very narrow due to lack of kinematical broadening^{3,4,6}. Table 1 lists the most important long-lived γ -ray lines that can be expected from extraterrestrial matter²⁴. These are probably the only lines that will be observable. The carbon 4.44 MeV line is not included because it is short-lived and shows full Doppler broadening in solids, as can be demonstrated in the laboratory²⁷, while the states listed produce a significant narrow component. If the dust were mostly cometary debris, the carbon abundance would be too low to produce an observable line flux anyway.

The expected line flux of the oxygen 6.13 MeV line can be estimated using a grain composition similar to carbonaceous chondrites (see ref. 28), that is $\approx 40\%$ O, $\approx 20\%$ Fe, $\approx 10\%$ Si, $\approx 10\%$ Mg by weight, which for $\rho = 1.2 \times 10^{-22} \text{ g cm}^{-3}$ yields 1.8

O-atoms, 0.26 Fe-atoms, 0.26 Si-atoms, and 0.3 Mg-atoms per cm^3 . This is about 300–1,000 times larger than in the interplanetary gas (see ref. 30).

The emissivity per unit volume is $P_\gamma = \int_0^\infty n \cdot \sigma(E_i, E) \cdot F_p(E) dE \text{ cm}^{-3} \text{ s}^{-1} \text{ sr}^{-1}$, where $n \text{ cm}^{-3}$ is the number density of the nucleus under consideration, $\sigma(E_i, E)$ is the total cross-section for production of a photon of line energy E_i by a proton of energy E , and $F_p(E) \text{ cm}^{-2} \text{ s}^{-1} \text{ MeV}^{-1}$ is the flux of energetic protons. Oxygen cross-sections by Ramaty *et al.*³⁰ are used in an example of the 4 August 1972 particle event with a proton spectrum $F_p(E) = 1.7 \times 10^6 (E/\text{MeV})^{-1.7}$. To determine this spectrum the spectral shape and α/p ratio ($\approx 3\%$) before and after the maximum phase of the event were adapted from ref. 31. Then the maximum phase α -particle flux from ref. 32 was multiplied by 30, because the proton flux measurement suffered from detector saturation. Although the proton flux thus derived is larger than reported elsewhere, it is a realistic estimate because most of the proton detectors in space were probably saturated during the flux maximum. With these values $P_\gamma \approx 7 \times 10^{-20} \text{ cm}^{-3} \text{ s}^{-1} \text{ sr}^{-1}$. Assuming the lateral extent near Earth of this event to be about 1 AU, the total flux at Earth becomes $F_{6.13} \approx 10^{-6} \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ (see also Table 1). This is about 10–20 times smaller than the sensitivity threshold of currently planned γ -ray detectors in space (aperture of $\approx 1 \text{ sr}$ assumed).

Larger γ -ray fluxes may be observed, for the following reasons: (1) the largest estimates of the dust density are three times the value used here^{10,11}, (2) Conservative extrapolation of particle event statistics³³ indicate the possibility during maximum solar activity of several events per year of the August 1972 size, and ≥ 1 event yr^{-1} of ≥ 10 times that size, occurring all over the visible hemisphere of the Sun. On Earth only those events originating near the preferred solar longitude³³ will be seen as strong particle events, while all events can be seen in γ rays. (3) The detector could view the Sun directly as after the short flash phase of the flare the Sun will be quiet again³⁴, causing no background. Assuming that the proton density (or flux) rises as R^{-2} towards the Sun, and the dust density as $R^{-1.3}$ to $R^{-1.5}$, the γ ray line flux at Earth from the solar direction can be 15–30 times larger than estimated above. Thus, despite the first low estimate above, there is a good chance that these γ -ray lines can be observed in the future.

Protons of this energy also ionise with fairly large probability the K -shells of atoms, while at the same time they generate little continuum X-ray background. The product of proton flux and cross-section peaks at less than 1,000 U_K (where U_K is the K -absorption edge energy), which is as low as 0.5 MeV for oxygen. Grains are not transparent to these protons or to the corresponding K_α -photons. Yet, the characteristic K_α lines should be observable.

K_α line fluxes will be estimated in a way similar to that used for γ -ray line fluxes, the main difference being that the grains are not transparent except for the 6.42 keV K_α line of iron. For simplicity I assume that half the photons that are produced in the outer layer of the grain of thickness $1/(\mu/\rho) \text{ g cm}^{-2}$ leave the grain and can be observed (μ/ρ is the mass absorption coefficient). This 'active' fraction of the total mass of the dust depends on grain material density and composition, and therefore estimates are given for $\rho_{\text{mat}} = 3 \text{ g cm}^{-3}$ and $\rho_{\text{mat}} = 0.3 \text{ g cm}^{-3}$.

Table 2 The most intense K_α lines expected from the zodiacal dust during a solar proton event

Element	K -shell ionisation energy (U_K) keV	K_α -mass absorption coefficient (μ/ρ) $\text{cm}^2 \text{ g}^{-1}$	K_α -line energy keV	4 August 1972 expected K_α -line flux at Earth for grain density $\text{cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$	
				$\rho_{\text{mat}} = 3$ g cm^{-3}	$\rho_{\text{mat}} = 0.3$ g cm^{-3}
O	0.53	6.5×10^3	0.52	1.9×10^{-3}	6.6×10^{-3}
Mg	1.31	2.5×10^3	1.26	5.0×10^{-4}	1.5×10^{-3}
Si	1.85	1.3×10^3	1.74	6.8×10^{-4}	1.8×10^{-3}
Fe	7.14	1.0×10^2	6.42	1.5×10^{-3}	1.7×10^{-3}

Fluffy particles probably behave like low density material.

To compute the mass absorption coefficient I have added 20% Ca to the chemical composition assumed above. This should account approximately for the rest of the material which is a mixture of elements ranging from carbon to at least nickel²⁰. I have used experimental and computed mass absorption coefficients^{35,36} and K -shell ionisation cross-sections³⁷, and published quantum yield values³⁸ to derive Table 2. The last two columns of Table 2 contain flux values expected for the 4 August 1972 event.

The main problem for registering these fluxes is caused by the diffuse X-ray background of the sky, which at 6.4 keV is about 0.6 photons $\text{cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ keV}^{-1}$ (refs 39, 40). Taking this into account, the expected Fe- K_α flux is about a factor of 3 below the sensitivity threshold of large orbiting X-ray detectors (on HEAO 1). In this case, only a three times larger dust density, or a three times larger particle event would be required for positive detection.

The moon should emit γ -ray lines and X-ray lines by the same mechanisms. In case of the 4 August 1972 event, the oxygen 6.13 MeV line flux at Earth should have been $>10^{-3}$ photons $\text{cm}^{-2} \text{ s}^{-1}$ (40% O by weight assumed), and the 6.42 keV Fe characteristic X-ray line flux should have been >0.5 photons $\text{cm}^{-2} \text{ s}^{-1}$ (10% Fe assumed). Proton-stimulated characteristic X-ray emission as compared to X-ray fluorescence from the lunar surface could be of interest to a possible lunar orbiter mission. The solar X-ray spectra are very soft so that not much intensity is expected in energetic fluorescence lines like the 6.4 keV Fe line, while the MeV protons could stimulate these lines in measurable quantities.

Finally, after large solar flare particle events, it is worthwhile to look at γ - and X-ray lines caused by interactions of MeV protons with the zodiacal dust. For MeV protons and γ rays the dust grains are transparent, so that some data can be derived without complicating grain size and surface effects: the mean composition can be derived in a model independent way, and together with *in situ* measurements of the proton flux it can be used to calculate the average spatial density along the line of sight. X-ray observations with some existing knowledge of the grain size distribution and its dependence on solar distance, can be used to give data on the size and material grain density. On the other hand, if the dust properties were sufficiently well known, large particle events could be monitored at other than the preferred heliolongitude.

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The influence of dynamic loading on sliding friction

THERE have been many studies of the rheology of thin polymeric films in sliding contacts¹⁻⁴, and many of the results have been obtained with a particular experimental arrangement which involves studying the motion of a glass hemisphere traversing a thin film deposited on a smooth glass flat³⁻⁵ (Fig. 1). The development of this subject has concentrated on an important quantity of practical interest, τ , the interfacial shear strength, which may be considered to be the energy dissipated per unit distance per unit contact area. These dissipative processes occurring in a particular film are not generally known. The interfacial shear strength has been found, for a wide range of organic polymers, to depend on many parameters, such as

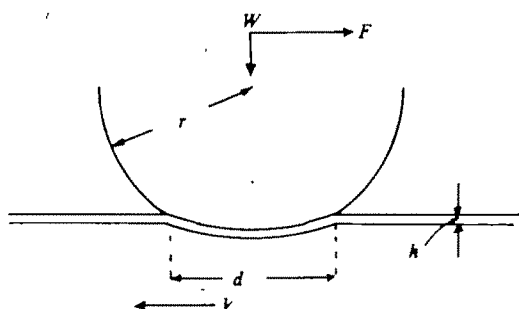


Fig. 1 Schematic diagram of the experimental arrangement. A glass sphere of radius r is loaded against a glass plate on which is deposited a thin organic film of thickness h (typically 100 nm). The surfaces are set in relative motion with a velocity v by the application of frictional force, F , orthogonal to the normal load, W . To a good approximation the area of contact and also the area of film sheared, A , is given by $\pi(W/Kr)^{2/3}$ where K is $\frac{2}{3}(1-\nu^2)/E$ where E and ν are respectively the Young's modulus and Poisson's ratio of the glass⁶. The contact diameter, d , is $2(A/\pi)^{1/2}$ and the intrinsic contact time, t_0 , is d/v . The interfacial shear strength, τ , and mean contact pressure, P_m , are respectively F/A and W/A . The effective rate of strain, $\dot{\epsilon}$, in the film is v/h . Because the mechanical properties of the glasses are invariant with respect to temperature and time, changes in τ in these variables can be attributed wholly to the response of the organic layer. Typically P_m is of the order of 10^8 - 10^9 Pa; $t_0 \sim 0.1$ s and $\dot{\epsilon}$ exceeds 10^3 s⁻¹. Classical calculations suggest that adiabatic heating is trivial in these experiments⁷.

contact pressure, temperature and strain rate, for which simple empirical relations have been found⁵. Providing the real area of contact can be estimated, these relationships are of value in predicting the friction of certain lubricated contacts. These relationships are broadly similar to those obtained from the study of the variation of the bulk yield stress of polymers and in certain cases the magnitude of the fitting coefficients (for example, τ_0 and α below) are similar for a given polymer, in bulk and interfacial shear tests. Direct comparison is difficult because interfacial shear measurements generally correspond to much higher pressures and effective strains and rates of strain. However, it seems that τ may be considered a measure of the bulk shear yield or rupture stress of the organic layer in relatively intense deformation conditions. Using a modification of the basic experimental method we have now obtained detailed information on the effect of velocity or contact time on τ .

For the present note it is only necessary to recall that τ is generally an increasing linear function of contact pressure and

$$\tau = \tau_0 + \alpha P_m \quad (1)$$

where τ_0 and α are constants and P_m is the mean effective contact stress (Fig. 1). For organic polymers α is large (0.1-0.6) and for metals and inorganic solids, it is a factor of between 10 and 100 smaller. More recently, further dependent variables have appeared, for example, film preparation conditions⁸ and with reference to this note, contact time, t_0 (ref. 9). In this context, the intrinsic contact time is approximately the contact length divided by the sliding velocity (Fig. 1).

Previous studies of the velocity dependence of shear strength for a variety of polymers⁵ showed that generally this quantity increases with velocity or effective rate of strain. The variation of τ seems to be a linear function of $\ln \dot{\epsilon}$ consistent with the response of bulk polymers at lower rates of strain. Although the evidence is not conclusive, it seems that τ_0 , but not α , is an increasing function of $\dot{\epsilon}$. One exception was found, polymethylmethacrylate (PMMA), which when studied at room temperature, showed a decrease. PMMA at 160°C ($T_g = 110^\circ\text{C}$) shows an increase of τ with increasing velocity.

Apart from the generation of an effective strain rate, the nature of such a sliding experiment involves the transient application of a normal stress on the film due to the load in the contact. Thus an infinitely thin element of film (Fig. 1) is subjected to a progressive increase in P up to $3/2 P_m$ followed by a decrease to zero as it leaves the contact. The form of this pressure cycle is given by the hertzian pressure distribution in the contact. It has been hypothesized that the effective (or fictive) contact stress in a viscoelastic material will be somewhat less than that which is applied, due to the inability of the film to respond to the rapid variation during sliding¹. In this way the shear strength may be considered to be attenuated through the pressure term in equation (1), where P_m becomes a fictive pressure and a function of t_0 .

Direct experimental confirmation of this hypothesis is difficult, because in practice only small changes in t_0 through d can be effected and the variation of v increases $\dot{\epsilon}$. We have introduced a modification to the basic experimental technique which uniquely elucidates the effect of contact time. A periodic loading was superimposed on the hemisphere by means of a steel core coil fed by an a.c. signal resulting in a signal of amplitude about one-tenth of the 'dead' load. In this way a second (extrinsic) contact time is introduced. To compare polymers a loading frequency of 1.5-30 Hz was used. The frictional force responds with a superimposed ripple which may be extracted from dead load friction using a phase-sensitive detector. The ripple amplitude may then be measured as a function of frequency. As a measure of the attenuation of the sinusoidal signal as a function of frequency, a relative attenuation factor, R , was defined as

$$R = \frac{\Delta \text{Ripple amplitude}}{(\text{Ripple amplitude})_1} \frac{f_1}{f}$$

Table 1 gives values of R for a variety of polymers as well as graphite, MoS₂ and stearic acid for an intrinsic frequency of

Table 1

R	Material
0.45	Molybdenum disulphide
0.45	Stearic acid
0.46	Graphite
0.52	Polyvinyl acetate
0.49	Polycarbonate
0.48	High density polyethylene
0.50	Polystyrene
0.75	Polymethylmethacrylate (room temperature)
0.50	Polymethylmethacrylate (temperature 160 °C)

5 Hz. PMMA at room temperature is the only polymer with high R , confirming that the response of the film is highly contact time sensitive. All other polymers including PMMA at 160 °C show $R = 0.48-0.52$. It thus seems that the dynamic loading technique discerns the influence of contact time and the results are consistent with the velocity behaviour previously obtained. Note that the values of R for non-polymeric solids are slightly lower, indicating that all the polymers studied show some attenuation with contact frequency increase. It would be expected that the non-polymers would not show any retardation in response, and thus $R = 0.45$ seems to be the 'zero' of the technique. Ideally, R should be zero for these films but the apparatus probably has viscous or elastic damping associated with it, although no direct mechanical damping was used.

We have also studied the influence of dynamic contact frequency for a range of intrinsic contact frequencies (generated by maintaining P_m constant and changing v and d). Both the form and magnitude of the attenuation were found to be a function of intrinsic contact frequency. Thus the two loading processes seem to interact for all polymers but the nature of this type of interaction is conceptually difficult to rationalise.

This attenuation of response with contact time decrease has been termed viscoelastic retardation in compression. Previous studies^{10,11} have put forward evidence for its occurrence in oil film shear, although this interpretation was subsequently questioned¹². Recently Johnson and Tevaarwerk¹³ found some evidence that was consistent with a delay in response of oil films. They also showed a general similarity in behaviour between thin oil films in elastohydrodynamic contacts and thin polymeric film shear. There is also some evidence that the low strain properties of bulk polymers show some retardation in response following the application of a hydrostatic stress^{14,15}. These data are complicated because of adiabatic heating. In this context it is worth noting that PMMA has a major bulk relaxation (β transition) at ~ 1 Hz at 20 °C (ref. 16), and it may be this dissipative process which is responsible for the retardation in sliding.

This result has implications for the study of stick-slip or intermittent sliding motion. This type of motion may occur when there are viscoelastic interactions between the machine and the sliding process^{17,18}. Simple analysis shows that it is present when a machine is subcritically damped and when either the static frictional force is greater than the dynamic or when the frictional force decreases with increasing sliding velocity. Because the frictional force is made up, in part, from a product of τ and the real area of contact, the retardation process may be an important factor in controlling its occurrence. In addition the experimental method seems to be useful for the study of the time-dependent properties of organic polymers.

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IR studies of long-range surface effects—excess proton mobility in water in quartz pores

LONG-RANGE ordered elements of water structure of micro-metre dimensions have long been suspected to exist near certain aqueous/solid interfaces. However, the evidence has been inconclusive¹ and it has also been argued that such ordered elements of water structure exist near a glass surface from a comparison of the diffusion coefficients of the hydrogen ion from polarography and the sintered glass diaphragm cell method^{2-4,10}. The pore size of the sintered glass varied between 4 and 15 μm . The mobility of the hydrogen ion is known to be very sensitive to the structure of water^{5,6}. We present here convincing evidence from an IR spectroscopic study of 1M HCl in porous silica that such long-range ordering effects exist near a quartz surface and that as a consequence the excess proton mobility is reduced. Such long-range effects have not been conclusively demonstrated previously. With acid solutions the interaction between the easily polarisable hydrogen bonds of H_2O_2^+ groupings may favour such a long-range order and as the absorbance of the polarisable hydrogen bonds is sensitively influenced by all interaction effects this order may be indicated by changes of the IR continua.

The method of detecting long-range surface effects consists of measuring the absorbance of the continuous absorption observed in acid and base solutions beginning at 3,200 cm^{-1} and extending to smaller wave numbers. It has been shown⁷ that the

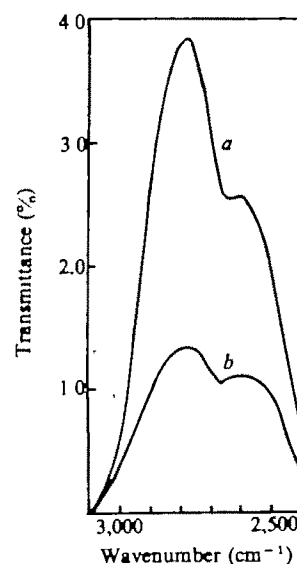


Fig. 1 Spectra of water (a) and 1M HCl (b), effective thickness $31.8 \pm 0.5 \mu\text{m}$, absorbed in porous quartz (pore size 4-15 μm).

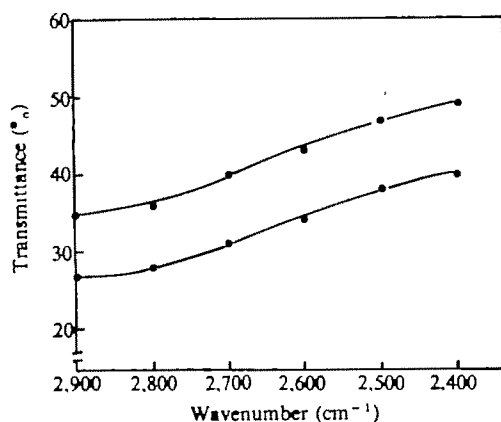
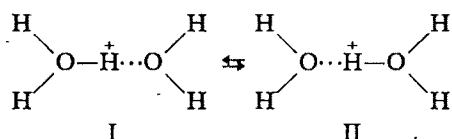


Fig. 2 Calculated difference spectra, that is the spectra of continuum for 1M HCl, effective thickness $31.8 \pm 0.5 \mu\text{m}$, absorbed in quartz pores (●); and for film of 1M HCl, thickness $32.0 \pm 0.2 \mu\text{m}$ between parallel silicon plates (○).

continuum is due to the H_3O_2^+ groupings in the case of acids and the H_3O_2^- grouping in the case of bases. The phenomenon is not confined to acids and bases and has been observed whenever there is a grouping with a hydrogen bond in which a potential well with a double minimum or a flat broad potential well is present. Such cases have been observed in aqueous and non-aqueous solutions⁷. Furthermore, it has been demonstrated that such H-bonds are extremely easily polarisable. The polarisability of such H-bonds is about two orders of magnitude greater than the usual polarisabilities and hence they interact strongly with the environment, for example, by induced dipole interactions with ions and with dipole fields of the solvate and solvent molecules and particularly with each other. The energy surfaces of the easily polarisable hydrogen bond in H_3O_2^+ becomes strongly deformed by these interactions. As the strength of these interactions shows a broad distribution, a continuity of energy level differences occurs which is observed as continuum in the IR spectra. On the other hand, crystals containing H_3O_2^+ , for example, give broad bands⁸ in the IR spectrum instead of a continuous absorption because the H_3O_2^+ ions are held in rigid crystalline lattice and do not experience the fluctuating random electric fields of a solution. The presence of the continuum with aqueous acids indicates that protons are fluctuating between two boundary structures I and II



If the continuum decreases due to some external field this means that the hydrogen bond becomes polarised, the proton lingering time is greater at one of the two water molecules in the H_3O_2^+ grouping and the proton fluctuation frequency has decreased⁷. Consequently any influence which modifies the normal fluctuating structure of water containing, for example, H_3O_2^+ grouping should also influence the continuous IR absorption. If the influence structures the water causing an asymmetrical environment of the H_3O_2^+ groupings then the continuum should decrease and if, on the other hand, the influence renders the normal bulk structure of water containing the H_3O_2^+ groupings more random, then the continuum should increase⁹. Such effects have been observed on adding electrolytes to aqueous solutions of strong acids⁶.

Disks of sintered quartz 30 mm diameter and 4 mm thick (Heraeus) were each sliced into two disks of the same diameter as the original disk and the resulting disks were ground with a diamond wheel to a thickness between 130 and 100 μm . Only water without any additives was used in the cutting and grinding processes and the disks were held in a special holder without any

adhesive. After grinding, the disks were placed in water and treated with an ultrasonic vibrator to remove any loose particles. The ground disks were chosen for the IR study on the basis of the uniform appearance under the microscope. Water and 1M HCl were absorbed in the disks and the difference in weight between the wet disk, which was carefully blotted between filter papers, and the dry disk was determined. Using also the diameter of the disk, 30 mm, the effective thickness of the liquid could be calculated. For the IR measurements the porous quartz disk was held between thin clear optically flat quartz plates in a special holder to avoid evaporation.

The transmittance of the disks was $<4\%$ in the region 3,000–2,400 cm^{-1} and hence it was not possible to obtain reliable spectra with an optical-null IR spectrophotometer. All spectra were obtained with an electrical-null, ratio-recording Perkin-Elmer 580 at an ordinate expansion of 20 $\times$. On the expanded scale the transmittance was reliable to 0.4%. The disk was placed in the sample beam and the transmittance recorded.

Figure 1 shows the spectra for water and 1M HCl absorbed in a porous quartz disk at an effective thickness of $31.8 \pm 0.5 \mu\text{m}$. Reproducibility was excellent. Figure 2 shows the calculated difference spectra (the spectra for the continuum) between 1M HCl and water absorbed in porous quartz ($31.8 \pm 0.5 \mu\text{m}$ thickness), and 1M HCl and water as thin films between silicon plates ($32.0 \pm 0.2 \mu\text{m}$ thickness). The two spectra are identical in shape but that for porous quartz shows a higher transmittance, that is the absorbance of the continuum for 1M HCl is less when

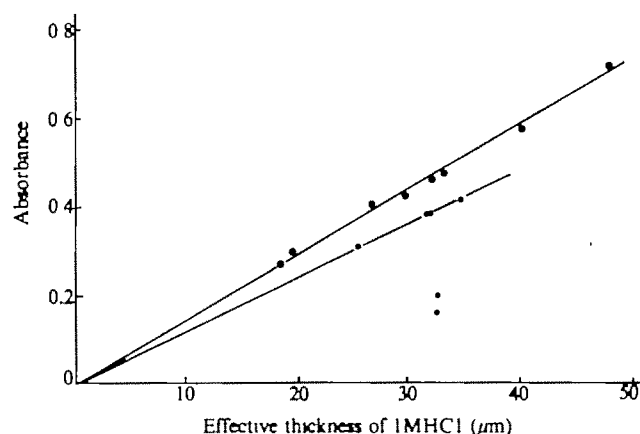


Fig. 3 Dependence of absorbance of continuum on effective thickness of 1M HCl at $2,560 \text{ cm}^{-1}$ absorbed in porous quartz (pore size 4–15 μm) (○) and as film between silicon plates (●).

absorbed in porous quartz of 4–15 μm pore size range. The identical shapes of the spectra seem to eliminate optical effects as a cause of lower absorbance in porous quartz, as such effects are strongly wavelength dependent and would affect the shape of the spectrum differently at different wavelengths.

The absorbance of the thin films of 1M HCl and water between parallel silicon plates was determined with a special holder described previously⁶. Figure 3 shows the absorbance of the continuum of 1M HCl at $2,560 \text{ cm}^{-1}$ plotted against the effective layer thickness for 1M HCl as a film and absorbed in porous quartz. At all thicknesses studied, the absorbance of the continuum for 1M HCl absorbed in quartz was less than that for the film.

The large depression of the continuous absorption must be caused by a polarisation of the H_3O_2^+ groupings by their local environments. The fluctuation frequency of the excess proton between structures I and II is decreased. The only source of asymmetry of the local environments at the H_3O_2^+ groupings can be a long-range order of the liquid structure by the surface of the pores. Thus, the decrease in intensity of the continuum of H_3O_2^+ in quartz pores indicates the order which may be favoured by interactions of the easily polarisable hydrogen bonds with the environment and especially with each other.

In principle this method of observing long-range surface forces could be extended to non-aqueous systems containing a solute with a high polarisable H-bond. Zundel has discussed such systems elsewhere⁷.

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Radiation-induced shrinkage of voids in molybdenum and TZM

SINCE the discovery of voids in neutron-irradiated stainless steels by Cawthorne and Fulton¹ considerable effort has been made to obtain experimental data on the nucleation and growth of voids and to understand the processes involved. The phenomenon has been of interest to the designers of fast reactors and could also be an important factor in material development for fusion reactor requirements. From both experiment and theory it is generally expected that for irradiations in constant temperature conditions, the voids will grow and the total void swelling increase with increasing displacement damage. Results are presented here which are contrary to this behaviour and show that in molybdenum and its alloy TZM (Mo, 0.5% Ti, 0.1% Zr), conditions exist where voids initially grow but then shrink as the irradiation proceeds.

High purity zone refined molybdenum² and TZM, both in recrystallised condition, were irradiated in the Dounreay fast reactor to total neutron doses of 1.5, 3.5 and $8 \times 10^{26} \text{ m}^{-2}$ giving calculated damage levels of 7.5, 17.5 and 40 displacements per atom respectively. The highest dose specimens were obtained by re-irradiating some of the specimens after the 3.5×10^{26} radiation. All the specimens discussed here were irradiated in

instrumented rigs at temperatures of $450 \pm 25^\circ \text{C}$. They were in the form of 3 mm disks and were examined after irradiation and after suitable electro-polishing by transmission electron microscopy in a Philips EM400 microscope. This system enabled voids with diameters $>0.8 \text{ nm}$ to be resolved.

The main results from the microscopy study are given in Table 1. The general result of a high density of voids and the appearance of loops agreed with previous high-dose neutron radiation studies of molybdenum and TZM near 450°C (refs 4–8). Similarly, the observation of void and loop lattices was not unusual. The unexpected result was the absence of visible voids in the high-dose TZM specimens. As it was known that these specimens contained voids before their second irradiation, it was at first thought that some aberration, such as a high temperature excursion (the most obvious way to remove voids) must have occurred. This was ruled out by two factors, namely the rig temperature records and the presence of voids in commercial molybdenum packing disks irradiated adjacent to the TZM specimens. However, when all the results are examined it becomes clear that the TZM result is the end product of a general irradiation process involving, first, the formation and growth of voids and, finally, their radiation-induced shrinkage. In the highest dose molybdenum specimen the large reduction in void swelling is clear but Table 1 also shows, when the void concentrations are examined, that the void shrinkage processes were already in operation during the medium dose irradiation. This is supported by evidence from void density histograms⁹ where the differences between the low and medium doses clearly show the simultaneous shrinkage of small voids and the continued growth of larger voids.

Some aspects of the dislocation loop structure are also of interest. The high loop density is evident in all the TZM specimens and also in the high-dose molybdenum shown in Table 1. The diameter of these loops, close to 5 nm, was surprisingly uniform. An important point was that the loops were indirectly established to be vacancy in nature; loop analysis procedures were carried out in annealed specimens where the fine loop structure had coarsened sufficiently to allow unambiguous identification. The loop lattice was apparent only in the presence of a void lattice. Since the results on molybdenum show the existence of a void lattice before loop growth, it implies that vacancy loop growth takes place preferentially on vacant void lattice sites.

The normal situation where voids acquire vacancies during the displacement damage process has been adequately explained elsewhere. The dislocations in the system have a preference, or bias, for interstitials leaving a supersaturation of vacancies to be captured by less biased, or neutral, sinks such as voids. To explain the observed void results, some mechanism must be invoked that allows a net flux of interstitials to the voids. It seems impossible to envisage any process taking place at a dislocation network to give this effect; instead such a situation is most likely to be achieved by some cumulative process inhibiting the jump of vacancies into a void. The presence of vacancy loops in the system supports this proposal. In particular it is significant

Table 1 The variation of void and loop parameters in Mo and TZM during neutron irradiation at 450°C

Material	Neutron dose (nm^{-2})	Average void size (nm) { $\pm 5\%$ }	Void density (m^{-3}) { $\pm 10\%$ }	Void swelling (%)	Loop density (m^{-3}) { $\pm 25\%$ }	Void/loop lattice parameter* (nm) { $\pm 5\%$ }
Mo	1.5×10^{26}	2.4	2.8×10^{23}	0.22	$\sim 6 \times 10^{20}$	22.2
Mo	3.5×10^{26}	4.4	1.3×10^{23}	0.57	$\sim 6 \times 10^{20}$	23.1
Mo	8.0×10^{26}	4.7	3.5×10^{22}	0.20	6×10^{22}	24.0
TZM	1.5×10^{26}	2.9	1.7×10^{23}	0.24	8×10^{22}	22.0
TZM	3.5×10^{26}	4.7	6×10^{22}	0.34	6×10^{22}	21.8
TZM	8.0×10^{26}	VNF	VNF	<0.01	1.3×10^{23}	—

VNF, voids not found.

* These values close to those suggested for 450°C by Moteff and Sikka³ who have studied the variation of void lattice parameter with irradiation temperature.

that the sharp rise in vacancy loop density in molybdenum takes place after void shrinkage processes have begun. If the voids acquire an effective preference for interstitials to add to the normal interstitial bias of the dislocation network, then it is not surprising that the vacancy concentration in the lattice could rise and allow the net absorption of vacancies in loops. However, the exact behaviour of the loops must clearly depend on their interstitial bias. It is interesting that the survival of the vacancy loops at a time when large voids are still growing (a situation certainly found in the medium-dose TZM specimens) supports the recent proposal¹⁰ that small vacancy loops must have a weaker interstitial bias than the network dislocations. The rather uniform loop size could also be significant in this context, as the vacancy loops might only grow to a size at which their neutrality with respect to the network is lost.

These results demonstrate that in both molybdenum and TZM, neutron irradiation can induce void growth and later, without any change in irradiation parameters, induce void shrinkage. A self-consistent explanation of the void and associated loop behaviour is that some mechanism first causes the smaller voids and later the larger to lower their probability of vacancy capture and hence allow a net influx of interstitials to their surfaces.

The void shrinkage in two materials of widely differing purity and the relatively slow increase in the effect during irradiation point to the mechanism being associated with the migration of transmutation products to the void surfaces. The segregation of alloying elements¹¹ and transmutation products¹² to voids by the flow of defects is already well known, but Marwick¹³ has recently derived expressions showing that solute atom segregation at voids can result in a void becoming biased against vacancies. Finally, if the effects reported here on molybdenum and TZM are found, or can be induced (perhaps by suitable alloy tailoring) in other materials, the technological interest could be considerable.

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Fixation of radioactive waste in high silica glasses

THE safe disposal of large quantities of high-level radioactive wastes from spent power reactor fuels, or reprocessing is a problem of considerable concern because of the danger which may arise from their release into the environment. We describe here a multi-barrier vitrification process which is conducted at moderate temperatures (below 900 °C) and which yields the fixation of radioactive wastes as oxides in a high-silica matrix. Some special characteristics of this process are discussed, including the excellent chemical durability, high thermal and structural stability of the glass, and the possibility of multibarrier protection.

The waste materials contain a wide variety of isotopes which

Table 1 Room temperature dissolution of candidate glass matrices

	High silica glass bar	Without bar	Borosilicate glass	Others
Matrix dissolution rate ($\text{g cm}^{-2} \text{d}^{-1}$)	5×10^{-10}	5×10^{-10}		
Concentration of radioactive waste	0.5%*	15%		
Rate of dissolution of waste material ($\text{g cm}^{-2} \text{d}^{-1}$)	2.5×10^{-12}	7.5×10^{-11}	3×10^{-4} to 3×10^{-3}	10^{-4} - 10^{-7}

* Concentration in the protective barrier; interior concentrations were at 15%.

emit all forms of radiation and therefore must be isolated from the environment. Most wastes are processed to salt form in aqueous, usually acidic solutions. The radioactivity levels of the waste materials in concentrated form are very high, with a high associated thermal power output¹. Waste forms may be kept at relatively cool temperatures (80 °C) with constant cooling. However, if cooling is not possible within the first century after removal from the reactor, the wastes may attain temperatures of several hundred degrees during this period. Although the actual thermal history of the solidified wastes is still a matter of debate it is becoming apparent that storage below 100 °C is desirable to maintain the structural and chemical integrity of the disposal medium. In addition, the fixation materials will receive high radiation doses and may undergo structural rearrangements. Finally, due to the constantly occurring radioactive decay processes, many isotopes will alter their chemical state and properties; most significantly, some species will give rise to secondary products in different physical states, such as the formation of He by α -emission. These transformations may stress the containers intolerably and lead to break-up or explosions which in turn expose a far larger surface area of the waste material to environmental attack. Therefore, the rate of chemical dissolution of the radioactive wastes in aqueous and saline solutions, and the structural stability, strength and He gas permeability of the various fixation materials determine the safety of the disposal systems for these wastes.

One promising approach to the problem of disposal is the process of fixation in oxide glasses². Glasses can withstand high ambient temperatures better than plastics or concrete, and have better durability than metals, especially in saline solutions. However, properties of glasses vary extensively with composition, and while glasses in general exhibit better chemical and physical durability than most other materials, a wide range of durabilities are possible depending on composition. For example, a glass such as B_2O_3 is immediately dissolved in water, yet other glasses show only small traces of corrosion even after millions of years³. In fact, with some glass compositions, the resistance to chemical attack achieved may be so high as to make possible beneficial uses of nuclear waste materials, such as for Cs and Sr radiation sources, without any danger of contamination of the environment.

The glass fixation schemes proposed in the past involve melting glass at relatively high temperatures⁴. Radioactive waste materials, however, must remain relatively cool to minimise the vaporisation of the volatile radioactive fission products including the alkali metals and the oxides of Ru. The most promising previous scheme using a zinc borosilicate glass was a compromise necessitated by the requirement to stay below 1,100 °C during melting². The compromise involved choosing a glass with very low silica content (<40%) to lower the melting temperature. The chemical durability exhibited by this glass, while better than that of many glasses, is considerably less than is achieved in good chemical labware, for example.

Good chemical durability in glass is generally achieved by

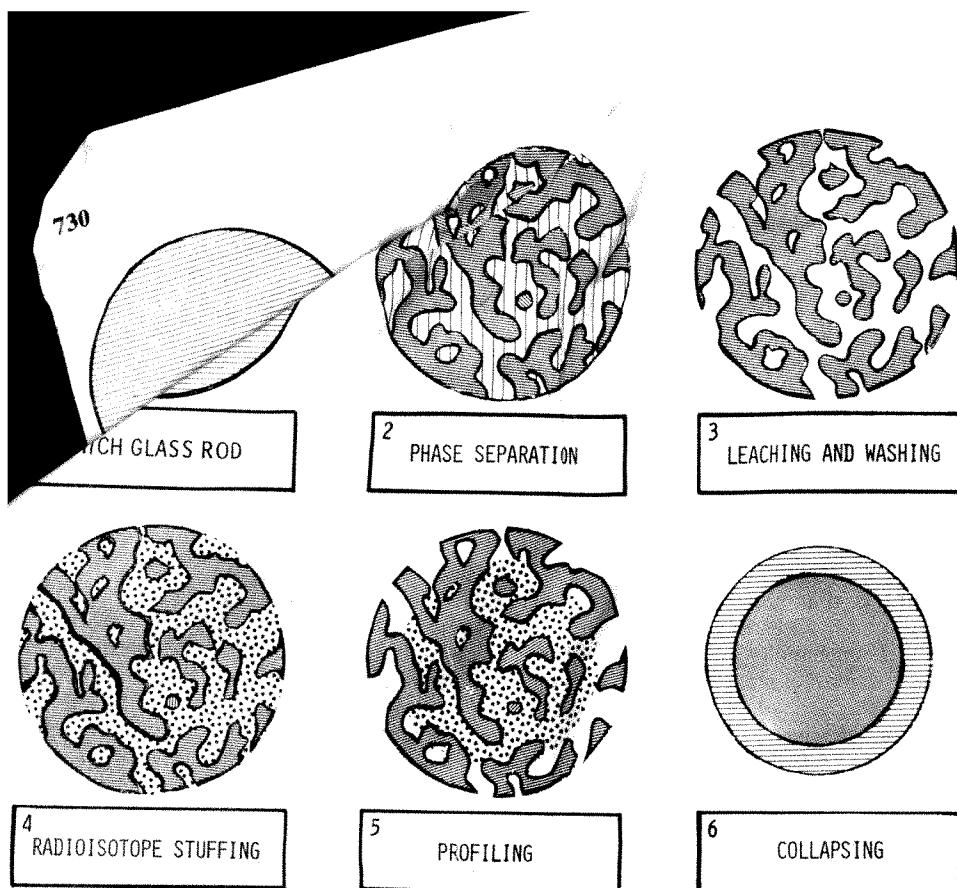


Fig. 1 Diagram of molecular stuffing process for the fixation of radioactive wastes.

increasing its silica content and decreasing alkali ion content as much as possible. This effect was observed with tektites retrieved from the ocean floor which show negligible chemical attack after 750,000 yr when the silica content is above 60% by weight³. These tektites, when measured in the laboratory, exhibit dissolution rates in water of approximately $0.5 \text{ \AA d}^{-1}$ (ref. 6).

Tektites have also provided information concerning the resistance of glass materials to devitrification over extended periods. Samples 35 Myr old have been found which are completely glassy and moon rocks have shown glassy phases over 4,000 Myr old without traces of crystallisation⁷.

The clue to high chemical durability and high structural stability seems to be high content of silica and other glassformers and low content of the alkali metals and other structure modifiers. Preliminary measurements of the compaction of glasses under high-level irradiation have shown that silica glass undergoes relatively little change⁸. Therefore, it is clear that high silica glasses will optimise the properties which characterise a desirable material for the fixation of radioactive wastes. However, preparation has been a problem since high temperatures (1,800 °C) are needed to melt glasses with a high silica content.

Recent research with a process for adding dopants to high silica porous glasses at relatively low temperatures (below 900 °C)⁹ has led us to develop a method suitable for the fixation of radioactive waste materials in a high silica glass.

The process uses a high-silica porous glass which can be prepared by melting an alkali-borosilicate glass, heat-treating it to induce phase separation into a high silica and a low silica phase and dissolving the latter by leaching in an acid solution which does not attack the high-silica phase. A porous glass with an interconnected porous structure and pore sizes from 100 to 500 Å is thus formed and consists of 96% SiO_2 and 4% B_2O_3 . The melting, phase separation and leaching operations are shown in steps 1, 2 and 3 of Fig. 1.) These steps are non-radioactive and may be conducted in a separate plant. Step 1 which requires a temperature of 1,400 °C, however, causes no problem of volatilisation of toxic materials since this is a simple non-radioactive glass melting operation.

The porous glass is then taken to a waste fixation plant and is subsequently soaked in a solution containing the prescribed

radioactive waste materials in salt form (see Fig. 1, step 4). These can be nitrates or other salts which decompose to oxides below 700 °C. After a soaking period of several hours, which allows the pores to fill completely with the solution, the laden porous glass is removed from the solution and the salts present in the pores are precipitated by undercooling. The porous glass is then soaked in a clean solvent which removes the waste material salts from an outer layer of porous glass at the surface of the glass object (see Fig. 1, step 5). The solvents are subsequently evaporated and the porous glass is heated slowly to between 625 and 700 °C to complete the drying and to decompose the waste material salts to an oxide form. Further heating to 900 °C sinters the structure by viscous flow, thus collapsing the pores and yields, on cooling, a solid glass object containing the radioactive waste materials interstitially in the interior (core region) and a high silica envelope on the outer surface (cladding region) (see Fig. 1, step 6). The core region consists of 75% SiO_2 and approximately 20% radioactive waste loading; the cladding region consists of 95% SiO_2 and is relatively free of waste materials (less than 0.5%). The cladding region can be made with any desired depth. An additional benefit of the difference in composition between the core and the cladding regions is the large surface strengthening obtained in these samples arising from a difference in the contraction of the two regions in cooling after fabrication. The clad region being of higher silica content has a lower coefficient of thermal expansion and is therefore fabricated with a large surface compressive stress which enhances the strength and stress corrosion resistance of these samples¹⁰.

We have made many samples with a number of dopants, most notably caesium oxide because it poses a significant problem as a radioactive waste material with its low vaporisation temperature, its high diffusion coefficient in glass and its high level of γ -radiation.

When caesium was used as a single dopant, the caesium oxide distribution in the finished rods was 15% by weight in the core and <0.5% in the clad, with the remainder of the glass being 4% B_2O_3 and the rest silica. (Note that this is a concentration of SiO_2 above 90 mol per cent.) We report, below, data on caesium-doped samples. We have also made samples containing mixtures of Fe, Sr, Y, Zr, Cs, Ba, La, Ce and Nd as nitrates, simulating a

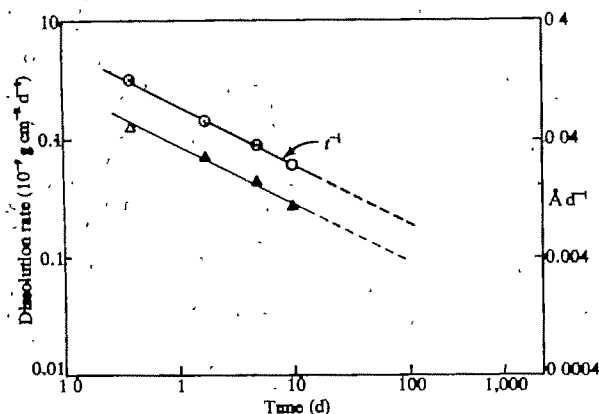


Fig. 2 Time dependence of room temperature dissolution rates for a high-silica glass matrix and for one with a 15% dopant concentration. O, Doped glass (15% loading); A, base glass.

Pw7—a waste stream² with equally successful results.

We have tested the chemical durability of rods containing 15% Ca_2O and rods without dopant by immersing them in distilled water at room temperature and at 100 °C for various times up to 10 d in a Teflon beaker. The chemical durability was related to the amount of silica released by the rods into the distilled water. The dissolution rate at room temperature is shown in Fig. 2 as a function of time for both types of glass. After 10 d in water, at room temperature the dissolution rate of the loaded rod is down to 0.02 Å d^{-1} ($5 \times 10^{-10} \text{ g cm}^{-2} \text{ d}^{-1}$) following a square root behaviour with time. The base glass dissolution rate is down to 0.01 Å d^{-1} after the same time period. At 100 °C the dissolution rate of the doped glass was measured to be $4 \times 10^{-6} \text{ g cm}^{-2} \text{ d}^{-1}$ after 2 d and $2 \times 10^{-6} \text{ g cm}^{-2} \text{ d}^{-1}$ after 4 d.

There are two mechanisms which may control the rate of release of caesium into the bath. The first corresponds to caesium ion diffusion through the glass to the surface, while the second corresponds to the dissolution of the surface itself. The diffusion coefficient for caesium in this glass at 20 °C has been estimated to be $1 \times 10^{-29} \text{ cm}^2 \text{ s}^{-1}$ from electrical conductivity measurements¹¹. This predicts a caesium release rate of $3 \times 10^{-18} \text{ g cm}^{-2} \text{ d}^{-1}$. This value is so small that despite the wide discrepancy usually seen between diffusion coefficients calculated from conductivity measurements and leach rate measurements, it is most likely that the rate of release of caesium from the cladding region of the glass into the bath will be controlled by the rate of surface dissolution and will have a value of $2 \times 10^{-12} \text{ g cm}^{-2} \text{ d}^{-1}$ at room temperature. This number is the product of the measured dissolution rate ($5 \times 10^{-10} \text{ g cm}^{-2} \text{ d}^{-1}$) and the caesium concentration in the clad (0.5%). Since the cladding region is expected to be 1 mm thick, it will take over 1 Myr to dissolve it.

The matrix dissolution rates measured for these samples are extremely low—lower than those measured for long-lived geological glass specimens. Compared to the other glass media being considered for radioactive waste fixation (see Table 1), these high silica glasses exhibit matrix dissolution rates 1,000-fold lower, in comparable conditions, without the additional benefit of the protection which the cladding region offers. As the cladding region developed by this process only contains 0.5% loading, then until the entire clad has been leached, the dissolved glass only contains a 0.5% concentration of waste materials while the glasses without the protective barrier contain concentrations of 15–30%. This provides an additional reduction by a factor of 30–60 in calculating the rate of release of waste materials from glasses made by the molecular stuffing process, giving a cumulative improvement of 10,000 to 1,000,000 times other glass encapsulation processes (see Table 1). Note that even without the barrier, improvements between 300 and 30,000 are still obtained.

These results show that the high-silica glass matrix produced

by the porous glass process could be of value for effective long-term chemical fixation of radioactive wastes. Several questions remain to be studied, such as the effect on durability of pressure, of high levels of radiation and of chemical transmutations.

In conclusion, we have demonstrated that the fixation of radioactive wastes is feasible in high-silica glasses of good chemical durability. An analysis of leaching rates measured on this high-silica glass shows a clear advantage over other fixation media.

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Is CS_2 a precursor for atmospheric COS?

THE stratospheric sulphate layer¹ may play a major part in the Earth's global radiation budget^{2,3}. Recent measurements^{4–6} indicated that carbonyl sulphide (COS) has an appreciable concentration, ranging from 0.3 to 0.5 p.p.b. in the Earth's troposphere. The gas is relatively inert^{7,8} in the troposphere and may therefore diffuse to the stratosphere where, through photolysis^{7–9}, it may provide a major source of sulphate for the formation of the stratospheric sulphate layer. Thus, the origin of atmospheric COS is of particular interest in the stratospheric sulphur cycle. Kurylo¹⁰ suggested that the reaction of carbon disulphide (CS_2) with OH might form COS as a reaction product. We investigate this possibility here through global COS budget analysis in the light of new atmospheric and laboratory measurements. Recent measurements showed that CS_2 has a tropospheric concentration of around 0.1 p.p.b.³. The gas has previously been detected in seawater samples by Lovelock¹¹. These measurements seem to suggest that the ocean might be a source for atmospheric CS_2 .

The atmospheric sinks for COS are,



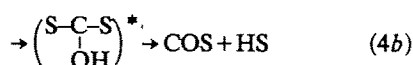
and



Reaction (1) dominates in the stratosphere and may account for a global removal rate of COS of about $2.4 \times 10^4 \text{ ton (S) yr}^{-1}$ based on our 1-dimensional model calculation¹² using measured photolysis cross-sections^{13,14}. Reaction (2) is relatively unimportant. The rate constant k_3 for reaction (3) is uncertain. Removal of COS in the troposphere, however, could be dominated⁸ by reaction (3) if k_3 exceeds $1 \times 10^{-15} \text{ cm}^3 \text{ s}^{-1}$. Kurylo¹⁰ recently reported a value of $5.7 \times 10^{-14} \text{ cm}^3 \text{ s}^{-1}$ for

k_3 at 296 K, in apparent conflict with an upper limit $k_3 \leq 7 \times 10^{-15} \text{ cm}^3 \text{ s}^{-1}$ reported by Atkinson *et al.*¹⁵. Assuming a tropospheric OH abundance¹⁶⁻¹⁸ of 10^6 cm^{-3} , one would estimate global removal rates of COS due to reactions (1-3) of $\sim 2.2 \times 10^6 \text{ ton (S) yr}^{-1}$, $2.2 \times 10^5 \text{ ton (S) yr}^{-1}$ and $2.4 \times 10^4 \text{ ton (S) yr}^{-1}$ corresponding to the cases $k_3 = 5.7 \times 10^{-14} \text{ cm}^3 \text{ s}^{-1}$, $k_3 = 5 \times 10^{-15} \text{ cm}^3 \text{ s}^{-1}$ and $k_3 = 0 \text{ cm}^3 \text{ s}^{-1}$ respectively. The obvious question is whether one can account for the global COS balance in each of the above three cases representing different atmospheric lifetimes of COS.

Atmospheric oxidation of certain sulphur-carbon containing species such as CS_2 and DMS (CH_3SCH_3) might be possible sources for atmospheric COS. For instance, the reaction of CS_2 with OH,



might produce COS¹⁰. The rate constant k_4 is $1.85 \times 10^{-13} \text{ cm}^3 \text{ s}^{-1}$ according to Kurylo¹⁰ and $\leq 7 \times 10^{-14} \text{ cm}^3 \text{ s}^{-1}$ according to Atkinson *et al.*¹⁵. The products in reaction (4) have not been identified. However, note that reaction (4a) is endothermic¹⁰ by 23 kcal mol⁻¹, while (4b) is exothermic¹⁰ by 35 kcal mol⁻¹. Assuming that reaction (4) proceeds primarily through branch (4b), the flux of COS obtained from integrating the continuity equation is

$$\phi_{\text{COS}}(z=0) = \int_0^\infty \{k_3[\text{OH}][\text{COS}] + J[\text{COS}] - k_{4a}[\text{OH}][\text{CS}_2]\} dz \quad (5)$$

Where J is the photolysis rate of COS and $[i]$ denotes the concentration (molecule cm^{-3}) of the species i . The quantity ϕ_{COS} defined here may be interpreted as a measure of the deficit in the COS budget and may be readily calculated using a standard one-dimensional model¹². If we adopt the observed mixing ratios of COS and CS_2 of 0.4 and 0.1 p.p.b. (parts per 10^9) (refs 4-6) respectively, a first order balance in COS budget may be achieved by adjusting the values k_3 and k_{4a} . For instance, in the above three cases where $k_3 = 5.7 \times 10^{-14} \text{ cm}^3 \text{ s}^{-1}$, $5 \times 10^{-15} \text{ cm}^3 \text{ s}^{-1}$ and $0 \text{ cm}^3 \text{ s}^{-1}$, the corresponding values for k_{4a} have to be 1.7×10^{-13} , 1.7×10^{-14} and $2 \times 10^{-15} \text{ cm}^3 \text{ s}^{-1}$ to balance the COS budget. Note that for the first case, the pair of values (k_3 , k_{4a}) is consistent with the values reported by Kurylo¹⁰. While the values for the two latter cases are consistent with the upper limits set by Atkinson *et al.*¹⁵.

Note that when reaction (3) is the dominant loss process for COS ($k_3 \gg 2 \times 10^{-15} \text{ cm}^3 \text{ s}^{-1}$), a simple relationship between the ratios k_3/k_{4a} and $[\text{CS}_2]_0/[\text{COS}]_0$, where the subscript '0' denotes surface concentration, can be derived from the consideration of COS budget balance. When $\phi_{\text{COS}} \approx 0$ (that is: approximate balance), equation (5) becomes

$$\int_0^\infty k_3[\text{OH}][\text{COS}] dz \approx \int_0^\infty k_{4a}[\text{OH}][\text{CS}_2] dz \quad (6)$$

from which we can derive a ratio $[\text{CS}_2]_0/[\text{COS}]_0$ which is independent of OH concentration by noting

$$k_3[\text{OH}]_0[\text{COS}]_0 H_3 \approx k_{4a}[\text{OH}]_0[\text{CS}_2]_0 H_{4a} \quad (7)$$

where H_3 and H_{4a} are the appropriate scale heights for reactions (3) and (4b) respectively. Since H_3 and H_{4a} are of similar order, the ratio $[\text{CS}_2]_0/[\text{COS}]_0$ is given by

$$R_A = \frac{[\text{CS}_2]_0}{[\text{COS}]_0} \approx \frac{k_3}{k_{4a}} = R_L \quad (8)$$

Equation (8) relates an atmospheric quantity R_A with a laboratory quantity R_L and confirmation of equation (8) will strongly suggest CS_2 as a precursor for COS. Clearly, measurement of k_{4a} requires the detection of COS as a product from reaction (4).

The only atmospheric CS_2 data reported to date are those by Sandalls and Penkett⁵, which seemed to be quite scattered with values ranging from 0.07 to 0.2 p.p.b. However, the lower values should be more appropriate for global conditions. Taking a typical COS concentration⁴⁻⁶ of ~ 0.4 p.p.b. the ratio R_A is ~ 0.18 – 0.5 in harmony with the quantity, $R_L = 0.3$ derived from laboratory measurements of k_3 and k_{4a} by Kurylo¹⁰. In view of the present uncertainties in both atmospheric and laboratory data, the agreement between R_A and R_L may be fortuitous. Nevertheless, relation (8) and similar analysis on other sulphur-carbon containing species (for example, DMS) would provide useful guidelines for identifying the appropriate atmospheric and laboratory measurements which may lead to a better understanding of the origin of COS, and might have interesting implications on the origin of the stratospheric sulphate layer.

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⁸⁷Rb–⁸⁷Sr age of Saint Mesmin chondrite and dating of gas-rich polymict breccia

CHONDRITES are generally regarded as being the oldest objects in the Solar System. However, most of them have suffered severe secondary effects, such as metamorphism, shock and irradiation. The gas-rich polymict breccias have had the most complex history of the chondrites. Some grains of polymict breccias have been exposed to solar irradiation and trapped solar-type rare gases before compaction of the meteorite. Evidence indicates that they were formed in a regolithic environment at the surface of their parent body¹. However, the timing of the successive events which affected the gas-rich chondrites is largely unknown. Saint Mesmin (chemical group LL), is probably the best studied example²⁻⁷ of this class of meteorites. It consists of a dark matrix, rich in solar-type noble gases, which contains light gas-poor xenoliths². The matrix is mineralogically unequilibrated and its material belongs to the chondritic petrological types 4 and 5 (ref. 3). In contrast the xenoliths have been strongly metamorphosed and re-equilibrated and belong to the petrological types 6 and 7 (ref. 3). Also veins of olivine microporphyry run across the meteorite³. Finally, a tectonically incorporated piece of chondrite, of the chemical group H (type H4), has been found in Saint Mesmin⁴. Here, we deal with ⁸⁷Rb–⁸⁷Sr dating of small 10 mg, hand-picked pieces, of the various components of Saint Mesmin. These pieces have been

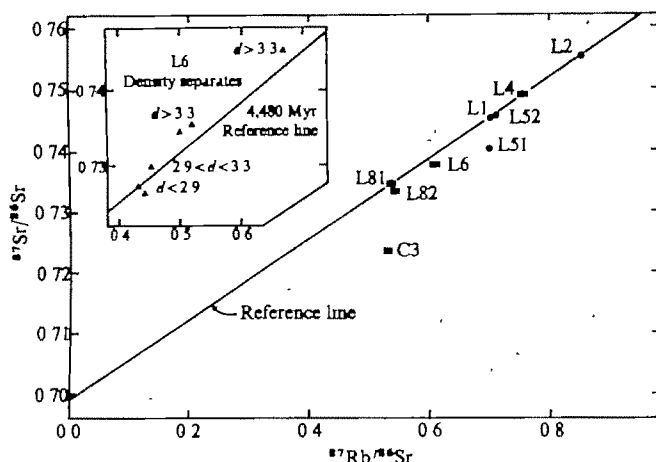


Fig. 1 $^{87}\text{Sr}/^{86}\text{Sr}$ versus $^{87}\text{Rb}/^{86}\text{Sr}$ plotted for the light xenoliths. Sample names are as given in other studies³⁻⁷. Two digit numbers indicate independent analyses for the same xenolith. Petrographic type is 7 for C3 and L2, 6 for the others³. The inset shows the data for the heavy liquid density fractions from the L6 xenolith. Reference line, 4,480 Myr; $\lambda = 1.42 \times 10^{-11} \text{ yr}^{-1}$; $^{87}\text{Sr}/^{86}\text{Sr} = 0.69877$.

studied by the other techniques³⁻⁷ and have been named by Pellas. Light xenoliths are labelled L1 to L8 and dark samples D1 to D8. One particular light xenolith of petrological type 7 is named C3. Two samples of the type H4 xenolith are named D1 and DD respectively. Where several pieces of the same sample had been analysed independently, we added a second digit to the sample name (for example L81 and L82). We have also analysed heavy liquid fractions of one light xenolith (L6) and of one sample of the H4 type xenolith (DD).

Experimental techniques follow those of Birck and Allègre⁸ and those reported elsewhere⁹. In this study, however, dissolution has only been made in a HF-HNO₃ mixture. New columns and labware meant that some blanks were high (up to 0.1 ng Rb and 0.4 ng Sr) compared with our usual ones (0.05 ng Rb and 0.1 ng Sr).

The results are given in Figs 1 and 2 for light xenoliths and dark samples respectively. (Raw data may be obtained from the authors.) Data for the light xenoliths scatter in the $^{87}\text{Rb}/^{86}\text{Sr}$, $^{87}\text{Sr}/^{86}\text{Sr}$ diagram (Fig. 1). However, most of them plot on a line of age 4,480 Myr ($\lambda^{87}\text{Rb} = 1.42 \times 10^{-11} \text{ yr}^{-1}$), not significantly different from our 'primitive isochron' for chondrites⁹. Our data for heavy liquid density fractions, from the L6 xenolith, are shown on both sides of the reference isochron (inset). If we assume that experimental perturbations by heavy liquids have been negligible, this scatter shows that the ^{87}Rb - ^{87}Sr clock has been partially reset. The repartition of the points can indicate that Rb and Sr redistribution has been closed at the scale of the xenolith. As our samples are rather small, they are probably unrepresentative of each light xenolith. As most of the data plot on the 4,480 Myr reference line this can indicate that all xenoliths are that old, but that the ^{87}Rb - ^{87}Sr system has been perturbed between the minerals of some or all of them. This is also supported by the 4,500 Myr old ^{87}Rb - ^{87}Sr age of Saint Mesmin whole rock¹⁰.

Similarly, samples of the dark components do not define a straight line in Fig. 2. These dark components are, however, of various types: D7 and D8 are samples of the gas-rich matrix; whereas D7 plots on the reference line at 4,480 Myr (samples D71 and D72), D8 plots significantly below (samples D81 and D82). This reveals that the ^{87}Rb - ^{87}Sr system in the matrix has been perturbed at a time younger than 4,480 Myr.

D6 is a gas-poor black xenolith⁷. Our two analyses (D61 and D62) plot on both sides of the reference isochron. As for black hypersthene chondrites¹¹, this result could indicate when this black xenolith was reheated. The slope of the two point-line defined by D61 and D62 yields an age of 1,500 Myr.

Samples of the two microporphyric inclusions D3 and D5 have been analysed⁶. All of them (D3, D51 and D52) plot on the reference isochron. This agrees with their old K-Ar as well as their ^{39}Ar - ^{40}Ar ages^{7,12}. This result is of great importance as these inclusions have been melted and have intruded the meteorite after some brecciation has already occurred⁶. It seems therefore, that the meteorite was brecciated very early in its history. It is interesting that the dark parts of Krähenberg, which are similar to these microporphyric inclusions⁶, also have a 4,600 Myr-old ^{87}Rb - ^{87}Sr age¹³, although this measurement lacks accuracy. Assuming that LL chondrites have had a single parent body, this could indicate that a melting event occurred quite early in its history, and that the melt intruded these two meteorites.

Finally, we have analysed the H4 xenolith (samples D1 and DD), as well as heavy liquid density fractions from sample DD (inset). Whereas the analyses for the whole rock and the heavy fractions of the comparatively large (70 mg weight) sample DD plot on the reference isochron, that for the smaller sample D1 plot well below this line. Figure 3 shows that Rb and Sr are mainly contained in two mineral phases of the xenolith. Whereas the heavier fraction (olivine?) forms an Rb and Sr - poor abundant reservoir, fraction $2.9 < d < 3.1$, consists of Rb- and Sr-rich sparse minerals. The abundances of Rb and Sr in sample D12 (Fig. 3), as well as the position of this sample in the ($^{87}\text{Rb}/^{86}\text{Sr}$ against $^{87}\text{Sr}/^{86}\text{Sr}$) diagram (Fig. 2, inset), indicate that the second phase is enriched in D12 compared with sample DD. Thus, D12 is probably unrepresentative of the average xenolith. Our data on sample DD indicates that this H4 xenolith of Saint Mesmin is about 4,500 Myr old. Its ^{87}Rb - ^{87}Sr system has been perturbed later, possibly due to post-shock reheating. Note that the age defined by DD and the Rb and Sr-rich fraction ' $2.9 < d < 3.1$ ', is 1,200 Myr which roughly agrees with the 1,400 Myr K-Ar age of this xenolith. The strong orientation of the metallic patches in DD indicates that it has been severely shocked. As there is no clear effect in the adjacent parts of the meteorite, DD was probably shocked before its incorporation in Saint Mesmin, and possibly inside the H-chondrite parent-body, 1,400 Myr ago.

Dodd and Jarosevitch⁶ suggest that at least two brecciation events affected the microporphyric inclusions of Saint Mesmin. As discussed above, a first brecciation occurred very early in the history of this meteorite. Our data are ambiguous with regard to younger episodes. First we consider the model ages of our samples relative to the $^{87}\text{Sr}/^{86}\text{Sr}$ initial ratio of chondrites, $^{87}\text{Sr}/^{86}\text{Sr} = 0.69877$ (refs 9, 14). Except for sample C3, they are all older than 4,000 Myr. This could indicate that the irradiation and brecciation history of Saint Mesmin is that old. Such a model

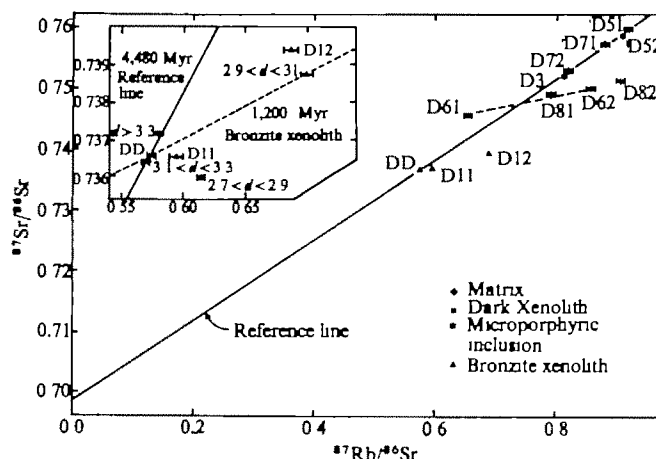


Fig. 2 $^{87}\text{Sr}/^{86}\text{Sr}$ versus $^{87}\text{Rb}/^{86}\text{Sr}$ plotted for the dark xenoliths. For general information see Fig. 1. The inset shows the data for the H-type xenolith.

has been supported by Pellas from U-Th-He and K-Ar data older than 4,000 Myr for most of these samples⁴. However, the two-point isochron of the D6 xenolith may be significant. With the data of the H4 xenolith (sample DD) this can indicate that Saint Mesmin was not yet compacted at that time, and was situated close to the surface of its parent body. This has been argued by Schultz and Signer⁷ on the basis of the K-Ar Age of the H4 xenolith.

Saint Mesmin is composed of 4,480-Myr-old components, many of which have been subsequently thermally disturbed. The foreign H type component can be understood as a residue of an impacting body at the surface of the Saint-Mesmin parent-body. Although their interpretation is not unique, the chronological

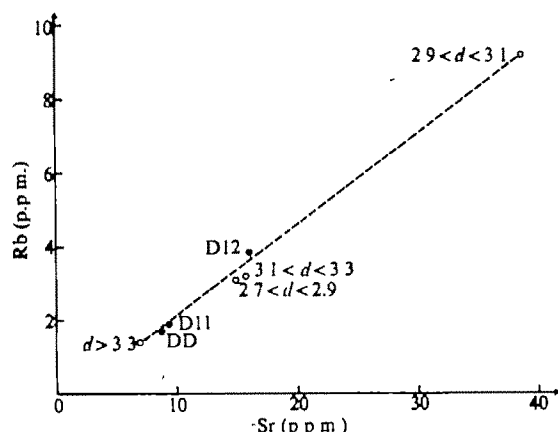


Fig. 3 Sr/Rb diagram for the samples and the density separates from the H4 type xenolith. Any fraction can be considered as a mixture of two main minerals respectively contained in fractions $d > 3.3$ and $2.9 < d < 3.1$. D12 is an unrepresentative sample of the mean xenolith.

data support a formation of Saint Mesmin from a regolith at the surface of its parent planet, as recently as 1,400 Myr ago. In particular, the chronological data indicate that the solar irradiation of some grains from the matrix does not correspond to an early irradiation, such as a T-Tauri phase of the Sun.

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A Morarian age for the 'younger Moines' of central and western Scotland

MUCH of our recent knowledge of the Moine metasediments^{1,2}, which extend from the Moine Thrust across the Great Glen Fault to the boundary of the Dalradian Supergroup^{3,4} (Fig. 1), stems from the western Moine region. Here, late-Precambrian, Morarian ages (740-780 Myr) have been established from pegmatites in the Morar Division⁵, and a Grenvillian age ($1,030 \pm 50$ Myr) from the Granitic Gneiss of Ardgour in the Glenfinnan Division⁶. The 'Central Highland Granulites', generally regarded as 'younger Moine'^{2,8}, are little known geologically. Here we briefly outline the results of field (by M.A.J.P.) and radiometric investigations (by O.v.B.) in the Central Highland Granulites and the western Moines, which have fundamental implications for the evolution of these extensive metamorphic rocks, and for the nature of the Grenvillian-Caledonian interval in this sector of the North Atlantic Crust.

Field relationships in the Central Highlands Granulites indicate that the region consists of two fundamentally different assemblages of Moine rocks: a basement, and an overlying cover (Fig. 1). The new name 'Central Highland Division' is used here to describe the basement rocks following Johnstone's use of the term division^{1,7}. The basement and cover are separated everywhere by a zone of tectonic break, called the Grampian Slide. Harris *et al.*⁴ have proposed using the lithostratigraphical term 'Grampian group' for rocks which clearly correspond with the cover assemblage, and their incorporation into the Dalradian

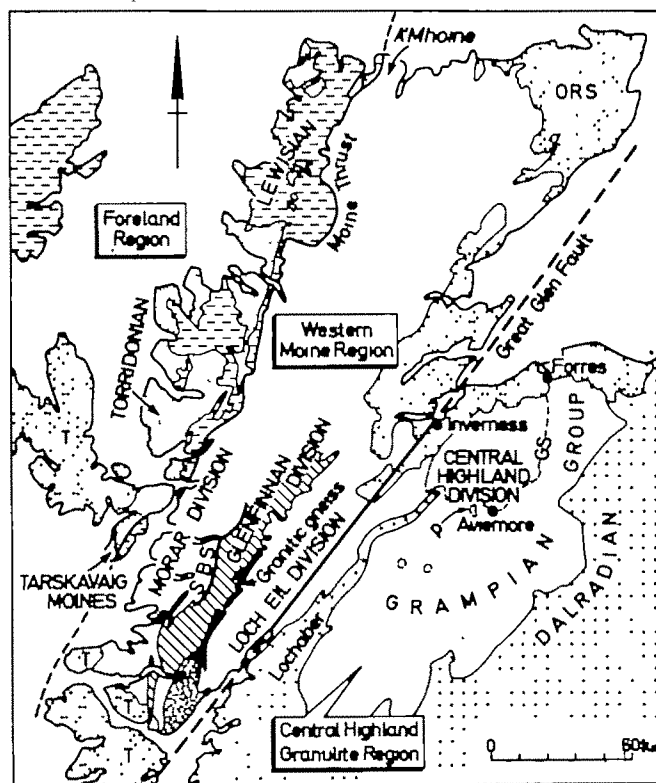


Fig. 1 Sketch map showing the geological setting of the Central Highland Division and the Grampian group: the Central Highland Granulite Region encompasses the outcrop areas of both. P, by Aviemore, centre of the Spey-Dulnain region; GS, approximate trace of the Grampian Slide; ORS, Old Red Sandstone; T, Tertiary and Mesozoic.

Table 1 Rb-Sr isotopic data for muscovite samples in pegmatites

Pegmatite no. and locality	Geological setting	Muscovite sample no.	Rb (p.p.m.)	Sr (total) (p.p.m.)	$^{87}\text{Rb}/^{86}\text{Sr}$	$^{87}\text{Sr}/^{86}\text{Sr}$ (Ri = 0.710)	Apparent age (Myr $\pm$ 0.007)
AB 4 (NH 79500920)	Basement	A	364	13.9	80.64	1.3683	573 $\pm$ 13
		B	367	20.0	62.18	1.2365	594 $\pm$ 13
		C	376	17.0	67.84	1.3303	640 $\pm$ 13
AB 90 (NH 79070937)	Basement	A	320	15.6	62.65	1.3071	668 $\pm$ 13
		B	329	14.1	72.30	1.4201	688 $\pm$ 13
DR 11 (NH 77291305)	Host uncertain	A	450	12.6	113.64	1.7005	611 $\pm$ 11
AF 20 (NH 77821126)	Cover-basement contact	A	317	17.7	54.35	1.1983	630 $\pm$ 14
		B	358	12.9	86.92	1.5695	693 $\pm$ 12
AF 20/8 (NH 77821126)	Cover	A	308	30.6	29.99	1.0116	705 $\pm$ 19
		B	305	29.2	31.13	1.0290	718 $\pm$ 19

All ages calculated with $\lambda^{87}\text{Rb}$ of $1.42 \times 10^{-11} \text{ yr}^{-1}$. Pegmatites AF20, AF20/8 have well preserved D2 fabrics; in AB4, AB90, D3 fabrics modify D2. DR11 is crenulated and retrogressed by a later, post D3 event.

Supergroup. We prefer to describe the cover rocks informally, as the 'Grampian group'.

The Central Highland Division forms most of the ground between Inverness, Forres and Aviemore (Fig. 1). Its dominant rocks are coarse to medium grained, variably gneissose siliceous and quartzofeldspathic metasediments which usually contain variably deformed concordant migmatite. These are interfolded with coarse, commonly migmatitic, quartzites, and with belts of coarse pelitic biotite-gneisses which frequently bear tabular kyanite and sillimanite.

In contrast with the basement, the rocks of the extensive Grampian group are finer grained, less metamorphosed, structurally simpler and essentially non-migmatitic. They form major formations of siliceous flags, semipelitic flags and pelitic schists. The presence of sedimentary structures in most lithologies should make it possible to elucidate regional tectonics and lithostratigraphical successions in this extensive region⁷. Mapping shows that some of the thick pelitic belts of the Central Highland Granulites generally thought to be Dalradian infolds in the Moine^{2,3,8}, such as the 'Grantown Series' and the Ord Ban-river Dulnain belt⁹, belong to a low stratigraphical level in the Grampian group and young upwards without a break into some of the Eilde Flags^{2,7,8}. Reconnaissance indicates that other pelitic belts, such as the Slochd-Streens of Findhorn^{7,9}, belong to the Central Highland Division.

Key evidence for the age relationships of these two rock assemblages stems from structural-metamorphic, stratigraphical and radiometric studies in the Spey-Dulnain watershed (centred on P in Fig. 1), where a late upfold brings up a core of Central Highland Division rocks within the Grampian group in the form of a tectonic window⁷. In this window, at least three very early generations of structures can be recognised, each associated with high-grade metamorphism. The earliest of these is a regional gneissosity. The second takes the form of penetrative isoclinal folding of this gneissosity, associated with migmatization and a high degree of mobilisation. The third forms a large regional recumbent fold which refolds the earlier structures.

The rocks of the Grampian group do not share these structures: they young consistently upwards for more than 1.5 km above their tectonic base, and lack intensive folding. Their earliest folds are associated with cover-basement sliding which truncates, shears out and superimposes new foliations on the early structures in the underlying basement. This contrast in the structural and metamorphic complexity between the Central Highland Division and the Grampian group is best interpreted in terms of an older basement separated by sliding from a younger cover.

In this Grampian group, the schistosity (S1) of the earliest deformation, D1, is highly overprinted by the effects of a major

event, D2. S1 can only be seen where it is folded around the hinges of F2 folds, and no F1 folds have been found. D2 resulted in a regional schistosity subparallel to the bedding, accompanied by kyanite-grade amphibolite facies metamorphism and by sliding in the cover-basement contact zone, where D2 fabrics are those of coarse, platy-tectonic schists.

A subsequent major event, D3, resulted in a new regional schistosity associated with lower grade amphibolite facies metamorphism and renewed sliding in the Grampian Slide zone. D3 sliding was associated with the growth of a penetrative fabric of replacement muscovite in the movement zones. In the tectonic schists, the D3 overprint of D2 produced platy muscovitic D3 fabrics, in which D2 fabrics are granulated and aluminosilicates are retrogressed. Subsequent deformations will be described elsewhere.

In this region, the D2-D3 Grampian Slide forms a zone 30-200 m thick, in which both basement and cover rocks are variably tectonised into tectonic schists, and in which tectonic slices of basement are present in the cover rocks.

In this slide zone a distinctive suite of concordant-sub-concordant foliated pegmatite veins occurs at localities as far as 60 km distant⁷. The pegmatite veins, 1 cm to 7 m thick, are present in both tectonised basement and cover rocks. They do not appear above or below the slide zone, and are interpreted as a syntectonic suite emplaced into movement zones. Rb-Sr analyses of 10 muscovite samples from five foliated pegmatites in the Dulnain watershed (Fig. 1) yield a grouping of ages within the range 718 $\pm$ 19 Myr to 573 $\pm$ 13 Myr (Table 1). The spectrum of ages in individual pegmatites corresponds with field distribution of D2 and D3 strains in the rocks of the slide zone. The higher ages are from pegmatites which share well preserved D2 slide-fabrics with surrounding tectonised rocks. Conversely, the lower ages come from pegmatites in zones more thoroughly modified by the later D3 fabrics.

This limited spread of ages indicates that the age of pegmatite emplacement into the movement zone, and the age of the D2 sliding, are unlikely to be much older than 718 $\pm$ 19 Myr. Thus, the pegmatites and the D2 event correspond with the Morarian ages of pegmatites in the western Moine region⁵. The same age pattern also implies that the D3 event which partly resets the radiometric clocks in retrogressive metamorphic conditions accompanied by liberal fluid activity, is younger than 573 $\pm$ 13 Myr, and is therefore clearly Caledonian.

It follows that the cover rocks of this region—rocks belonging to the lower part of the Grampian group—are older than the Morarian pegmatites. Correspondingly, as already indicated, the Grampian group cover is believed to be younger than the Central Highland Division basement. We tentatively correlate the latter with the strikingly similar Glenfinnan Division rocks from which the Grenvillian (1,030 $\pm$ 50 Myr) migmatitic

Granitic Gneiss of Ardour (Fig. 1) is thought to have developed *in situ*^{6,7,14}.

Viewed in this light, the D1–D2 events in the Grampian group fall within the Grenvillian–Caledonian interval, and are considered here to signify the reality of a limited orogenic event of Morarian age (broadly between 750 and 850 Myr) within the Moines (see ref. 5). Additional support for such a Morarian event comes from recent Rb–Sr whole-rock analyses of large samples of semipelites from the north-east Ox Mountains and Lough Derg inliers in western Ireland. These data yield an 860 ± 115 Myr isochron and indicate a post-Grenvillian maximum age (M. B. Max and C. D. Long, personal communication).

Field and radiometric evidence therefore indicate that at least the lower part of the Grampian group represents sedimentation during the Grenvillian–Morarian interval, and that its rocks were affected first by the Morarian event, and subsequently by the Caledonian orogeny. These rocks are referred to informally as being of Morarian age.

This model poses the problem of a Morarian–Caledonian interval, the nature of which remains unresolved. The metasediments of the uppermost part of the Grampian group are reported to pass into the overlying Dalradian by a sedimentary transition^{3,10,11}, whereas in some areas tectonic contacts intervene between the two^{2,7,12}. Apart from sections near its tectonic base⁷ and others adjacent to the Dalradian^{3,10,11}, much of the Grampian group still awaits detailed examination. It may be significant that in the Grampian group west of Loch Erich, Thomas¹³ reports the presence of an older and a younger series of metasediments separated by a major slide.

West of the Great Glen Fault, in the Loch Eil Division^{1,2}, the rocks along and to the south of Loch Eil appear to closely match certain formations of the Grampian group in their lithologies, sedimentary structures and in their apparent degree of structural–metamorphic complexity. In the region of Glen Tarbert, the rocks match those of the Central Highland Division^{7,14}.

Nearer to the Moine Thrust, a case has been made for a Grenvillian age for the early metamorphism and deformation in the Morar Division^{15,16}. However, there is now mounting evidence^{5,14} that the Precambrian event in these rocks may have been the Morarian event, and that they may be broadly equivalent in age to the Torridonian sediments deposited on the foreland between 1,000 and 800 Myr (ref. 17)—a long standing correlation¹⁸. This Morar Division is separated from the almost certainly Grenvillian (see ref. 6) rocks of the Glenfinnan Division by a major break, the Sgurr Beag Slide (Fig. 1). If the Morar Division is indeed of Morarian age, then the Sgurr Beag Slide assumes a similar structural position in separating Grenvillian basement from Morarian cover, as the Grampian Slide east of the Great Glen Fault.

The lower part (at least) of the Grampian group, probably parts of the Loch Eil Division, the Morar Division, the Tarakavaig Moines (Fig. 1)—already correlated with the Torridonian Sleat Group¹⁷—and the rocks of the north-east Ox Mountains and Lough Derg inliers of Ireland, may all form parts of one major assemblage, to which the arkosic Torridonian of the foreland would also belong. Such an assemblage is regarded as being analogous to a combination of groups which, as has yet to be demonstrated in the field, may make up a Moine Supergroup of Morarian age.

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A Caledonian blueschist from the Irish Dalradian

THE occurrence of the blue amphiboles glaucophane and crossite in metamorphosed basic rocks is characteristic of terranes metamorphosed at high pressures. Most examples are from relatively recent fold belts. We record here a Caledonian blueschist from the Dalradian Supergroup in South Achill Island, County Mayo (Fig. 1). It is the first 'old' blueschist from Britain to occur in a regularly bedded and folded sequence and may imply an early high pressure metamorphic event in this part of the Dalradian, evidence for which was generally destroyed by either erosion or later metamorphism.

The blueschist outcrops on Claggan Beach, 0.75 km south-west of Ashleam Bay (Fig. 1). The rocks exposed on the beach are mostly metasediments. Psammites predominate on the north side with interbedded pelitic schists, while metabasites occur on the south side. The metabasites are predominantly albite–epidote–chlorite schists but contain epidosite pods a few tens of cm in diameter and a more continuous boudinaged epidosite layer about 1 m thick that contains crossite and is therefore referred to as a blueschist.

The geology of the area has been described elsewhere^{1–3}. The basic rocks have been ascribed to the Upper Dalradian by Harris and Pitcher⁴ but a Middle Dalradian age has been recently proposed by Max and Long⁵.

The blueschist is fine grained (0.1–0.2 mm) and predominantly composed of epidote that is typically near $\text{Ca}_{2.08}\text{Fe}_{1.01}\text{Al}_{2.12}\text{Si}_{3.11}$ (calculated from microprobe analyses on an anhydrous basis) but with up to 2.30 Al atoms at some analysed points. Crossite occurs throughout the rock but is also concentrated into thin layers visible in hand specimens. Crossite compositions range from $\text{Na}_{1.88}\text{Ca}_{0.15}\text{Fe}_{2.02}^{\text{tot}}\text{Mg}_{2.28}\text{Al}_{0.80}\text{Si}_{8.20}$ to $\text{Na}_{1.92}\text{Ca}_{0.07}\text{Fe}_{1.51}^{\text{tot}}\text{Mg}_{2.12}\text{Al}_{1.36}\text{Si}_{8.15}$, tending to be richer in glaucophane at the margins. The rock also contains quartz, albite (>99% Ab) and hematite, with small granules of sphene sometimes associated with the hematite. Quartz and albite tend to occur together in felsic segregations, and crossite occurs both with these phases and in regions of epidote only.

Crossite grains often show marginal alteration to finer grained amphiboles, especially where quartz, albite and hematite are also present. Crossites enclosed in epidote are often unaltered. Two distinct alteration products are recognised, but there may be some gradation between them. They are a bright green barroisitic amphibole which usually replaces crossite directly, and a colourless to pale green actinolite that may mantle either the crossite or the barroisite. Microprobe analysis of these alteration products is difficult because of their fine grain size, but the barroisite in particular seems to be of rather variable composition. One point gave $\text{Na}_{1.54}\text{K}_{0.02}\text{Ca}_{0.65}\text{Fe}_{2.06}^{\text{tot}}\text{Mg}_{2.30}$

$Mn_{0.02} Al_{1.18} Ti_{0.01} Si_{7.69}$. The actinolite is rich in Na and Al, near $Na_{0.47} Ca_{1.55} Fe_{1.49} Mg_{3.29} Mn_{0.01} Al_{0.70} Ti_{0.02} Si_{7.67}$.

Crossite has not been found in separate smaller epidosite pods on the same beach, but the barroisitic and actinolitic amphiboles are both present and show similar mutual relationships. Stilpnomelane is also present in some of these smaller pods. We infer from these observations that crossite formed early in the metamorphic history of the rock and subsequently became unstable.

Crossite schists of this type are typical of many high pressure metamorphic belts throughout the world⁶ and, in the absence of jadeite and lawsonite, are probably best assigned to a high pressure greenschist sub-facies rather than the glaucophane schist facies. In such terrains the blue amphibole occurs in oxidised metabasites only and this may partly explain its scarcity in the Dalradian.

The Claggan blueschist we describe here is the first to be reported from the Dalradian and it is also the first from the British Caledonides to occur in a regularly bedded and folded sequence; the similar crossite schists at Ballantrae and Anglesey are isolated occurrences in areas of complex, highly faulted geology. It therefore seems that at least this part of the Dalradian underwent an early high pressure metamorphism and the observed breakdown of crossite may reflect a change in conditions towards a conventional Barrovian facies series. The Barrovian regional metamorphic grade in South Achill is very low¹ and it is therefore the most likely part of the Irish Dalradian for early assemblages to be preserved.

Ernst⁷ has documented the variations in blueschist abundances with age and suggests that the scarcity of pre-Mesozoic

blueschists results from changes in the thickness of lithospheric plates with time. Modern blueschist facies assemblages with jadeite, lawsonite or aragonite may not have been stable in the Palaeozoic Earth. However, blueschists may have formed in the past but have been particularly susceptible to destruction during orogeny. England and Richardson⁸ calculate that blueschists will tend to be rather rapidly destroyed by erosion after orogeny. Thus they interpret the scarcity of old blueschists as resulting from the destruction of blueschist belts which were once present. In addition, relaxation of the thermal gradient might destroy high pressure assemblages that remained at depth. The discovery of a crossite schist in the Dalradian may accord with both these theories, but it does provide evidence that traces of former high pressure metamorphism can be very largely destroyed.

Thus in South Achill the moderately high pressure crossite schists, formed early in the Grampian orogeny, were largely destroyed by subsequent metamorphism. It is possible that other blueschists formed from Dalradian and related rocks were uplifted and destroyed by erosion rather than further metamorphism. The evidence for this is the presence of detrital ferroglaucofan and crossite in probable mid-Ordovician rocks of the Longford-Down region recently reported by Sanders and Morris⁹.

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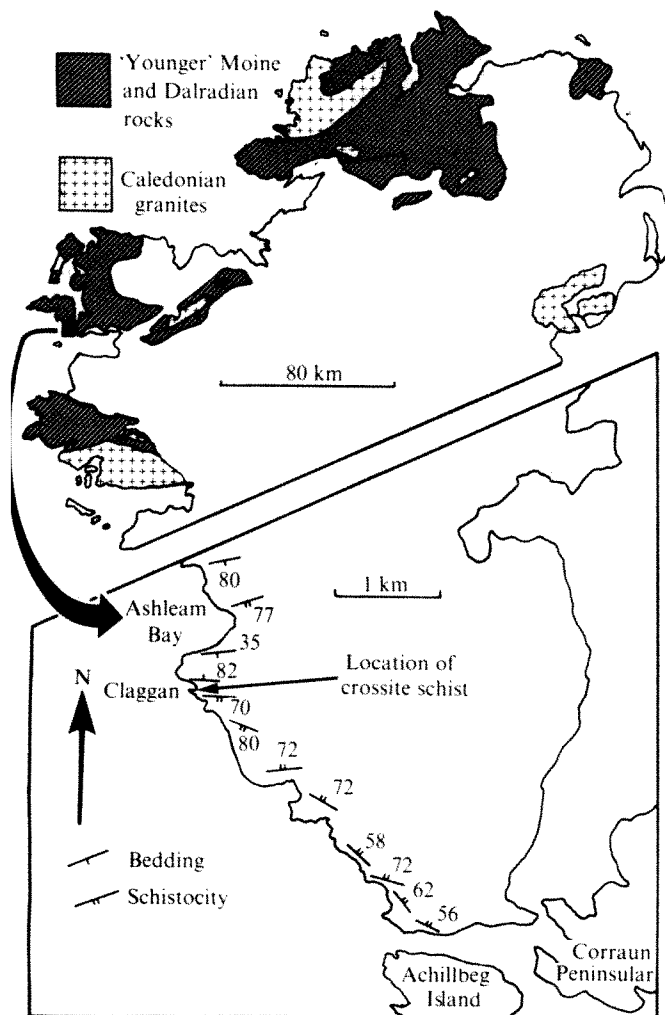


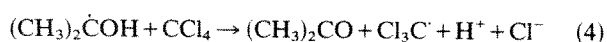
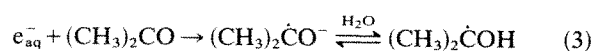
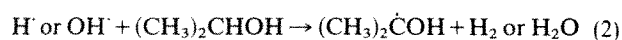
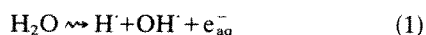
Fig. 1 Map showing the location of the blueschist occurrence in relation to the 'younger' Moine and Dalradian rocks of Ireland.

Direct observation of a free radical interaction between vitamin E and vitamin C

VITAMIN E (α -tocopherol) and vitamin C (ascorbic acid) react rapidly with organic free radicals, and it is widely accepted that the antioxidant properties of these compounds are responsible in part for their biological activity¹⁻⁵. Tissue vitamin C levels are often considerably greater than those of vitamin E, for example in liver the values are approximately 2 mM and 0.02 mM, respectively. Nevertheless, vitamin E is considerably more lipophilic than vitamin C, and in biomembranes has been found to be the more potent antioxidant, particularly with respect to lipid peroxidation; penetration to a precise site in the membrane may be an important feature of the protection against highly reactive radicals⁶. Tappel has suggested that the two vitamins act synergistically, vitamin E acting as the primary antioxidant and the resulting vitamin E radical then reacting with vitamin C to regenerate vitamin E⁷. We now report direct observation of this interaction, which we feel may be an important feature in the maintenance of vitamin E levels in tissues.

As vitamin E is poorly soluble in water, conventional pulse radiolysis techniques, involving aqueous solutions and oxidising radicals such as $OH^\cdot$, cannot be used to generate the related free radical (Vit E[•]). We have shown that the trichloromethylperoxy radical $Cl_3COO^\cdot$ can be generated in aerated water-isopropanol-acetone mixtures containing carbon tetrachloride,

reactions (1)–(5). The radical is a moderately reactive one-electron oxidising agent and has been shown to react with various nucleophilic compounds, with rate constants of the order of 10^7 – 10^8 $M^{-1} s^{-1}$ (ref. 8).



On pulse radiolysis of such a solution containing vitamin E we have observed the formation of a transient species with an absorption spectrum similar to those previously assigned to phenoxy radicals⁹. We attribute the absorption to the radical, Vit E[•]. Free radical scavenging actions by vitamin E have been ascribed to the initial reaction of the phenolic hydroxyl group with the production of a phenoxy radical¹¹. The rate of its formation was first order in vitamin E concentration corresponding to:



$$k = 5 \pm 1 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$$

The absorption decayed over a period of 3 ms. However, in the presence of vitamin C its spectrum changed rapidly, a decrease in absorption at 425 nm occurring simultaneously with an increase at 360 nm. These changes are attributed to the formation of the vitamin C radical (Vit C[•]) in view of previously published data concerning its absorption spectrum^{2–4}:

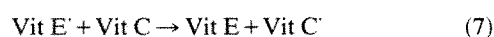


Figure 1 shows spectra of the transient species present 10 μ s, 400 μ s and 1,600 μ s after an aerated solution containing isopropanol (50%), acetone (10%), carbon tetrachloride (0.04 M), α -tocopherol (3.4×10^{-3} M) and ascorbic acid (1.60×10^{-3} M) neutralised with sodium hydroxide was irradiated with a 0.2- μ s pulse of 4 MeV electrons from the Brunel linear accelerator.

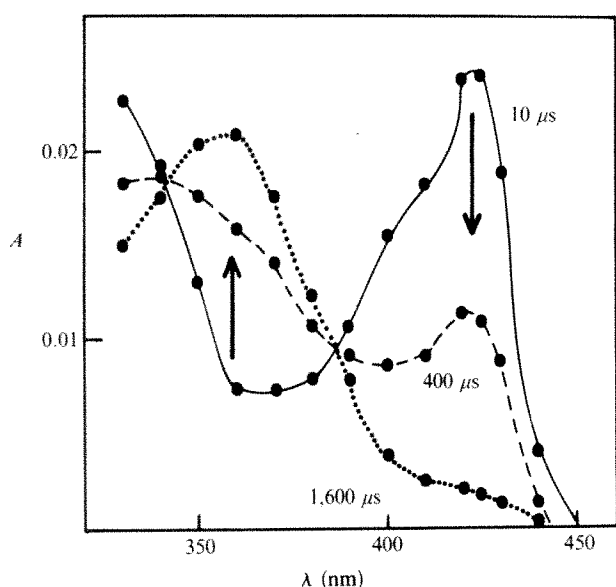


Fig. 1 Absorption spectra at various times after pulse radiolysis of an aerated solution of vitamin E and vitamin C. (—●—) vitamin E[•], (---●---) vitamin C[•], see text.

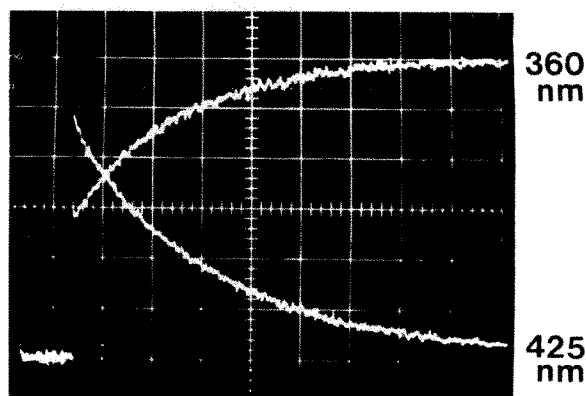
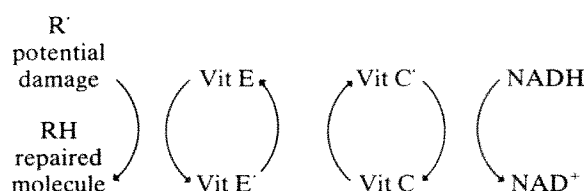


Fig. 2 Oscillograms obtained at 425 nm (decay of vitamin E[•]) and at 360 nm (grow-in of vitamin C[•]) for the reaction between the vitamin E radical and vitamin C (2.88×10^{-3} M ascorbate). Ordinate, 1% absorption per large division; abscissa, 100 μ s per large division.

After 10 μ s the spectrum is principally that of Vit E[•] with a small contribution (10%) from Vit C[•] whereas after 1,600 μ s the spectrum is that of Vit C[•] alone. Oscillogram traces of the decay of Vit E[•] at 425 nm and the concurrent grow-in of Vit C[•] at 360 nm are shown in Fig. 2 for a similar solution but containing 3.2×10^{-3} M ascorbic acid. Both the decay and the formation of the absorbing species were exponential, with first order rate constants proportional to vitamin C concentration. From measurements at four vitamin C concentrations the rate constant for reaction (7) was $1.55 \pm 0.2 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$.

Such a rapid interaction may well be pertinent to protection from free radical-mediated damage *in vivo*: the recycling of vitamin E at the expense of vitamin C may account in part for the fact that clinically overt vitamin E deficiency has not been demonstrated in man. Moreover in certain conditions the vitamin C radical can itself be enzymatically reduced back to vitamin C by a NADH-dependent system¹⁰. Thus this allows the repair of an otherwise potentially damaging organic free radical R[•] to be linked to NADH oxidation and a wide range of normal biochemical processes.



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Opposite cerebral hemispheric superiorities for visual associative processing of emotional facial expressions and objects

STUDIES of patients with unilateral cerebral lesions¹ or surgically disconnected hemispheres² suggest a functional asymmetry in processing visual information by the two hemispheres. The right hemisphere (RH) seems to favour matching of pictures of objects by similarity of shape (apperceptive matching), whereas the left hemisphere (LH) favours matching by similarity of meaning or conceptual category (associative matching). As, on the other hand, considerable recent experimental evidence supports the hypothesis that the RH might have a distinct role in processing emotional information³⁻⁸, it would be surprising if the RH lacked the capacity of associative processing which is important in emotional communication. Using tachistoscopic half-field presentation the present study produces evidence for a RH superiority for associative processing in response to adequate stimuli such as emotional facial expressions, whereas the LH is more efficient in detecting similarity of function between objects whose shapes are very different.

Twenty-four adult right-handed normal subjects (12 men, 12 women, 20-30 yr old) performed two visual half-field experiments. The method was identical in both experiments. Subjects had to fixate a central point in a two-channel tachistoscope. Two pictures were then simultaneously shown to them for 150 ms. One of the pictures, a stylised drawing, was always presented in central vision at the point of fixation. The other, a photograph, was presented to either the left or the right side of the drawing, either in the left or the right visual field (LVF/RVF). With exposure a timer was started. If the subject felt that the two pictures represented the same meaning, he pressed a button to stop the timer, otherwise no response had to be given. Equal numbers of positive and negative instances were presented randomly and equal runs of right and left hand responses were used in alternation. Each subject participated in four sessions, on four different days. A session consisted of 96 projections for each experiment. A total of 384 pairs of pictures per subject and experiment were shown.

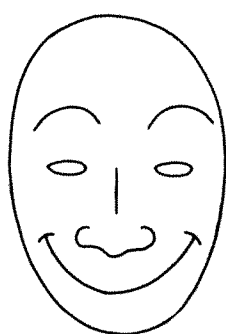


Fig. 1 'Happy', an example of a common emotional expression in two faces of different shape. The faces were photographed as close as possible according to the features outlined by Ekman and Friesen⁹. To enhance affectivity, the two persons whose faces were photographed were known to all subjects. The stylised drawing was always presented in central vision, subtended a maximum visual angle of 1.7° on each side of the point of fixation. The photograph of the same size was presented either on the left or the right side of the drawing, within 2.2-5.6° of visual angle from fixation point. The 24 subjects were subdivided into two groups of 12 (6 men, 6 women) to whom photographs were presented with faces or objects orientated to the right or the left respectively. The order of presentation was alternated between the experiments, balanced for sex of subjects and picture orientation.

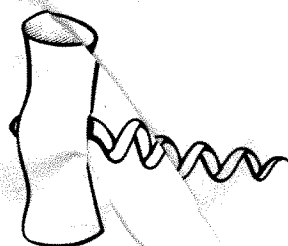


Fig. 2 'Corkscrew', an example of two different objects belonging to a common category to which

In experiment 1, subjects were presented with pairs of pictures, one representing a stylised drawing of a male or female face in profile, showing the face with an emotional facial expression, that is, 'happy' or 'astonished'. All possible combinations of the two faces and six photographs were used. Variance analysis of correct decisions revealed faster times for LVF than for RVF ($F(1.368) = 15.72$; $P < 0.001$), indicating RH superiority (Fig. 3) and a significant decrease in time in the course of the testing ($F(3.368) = 6.44$; $P < 0.001$), suggesting a 'learning effect'. Differences between hands and interactions did not differ significantly ($P > 0.05$). Neither the sex of the person being tested nor the orientation of faces on the photographs had any significant influence on the difference in times of decision between visual fields. There were 24% errors. Analysis of errors revealed a significant decrease in the course of the testing, independent of visual fields ($F(1.6) = 27.47$; $P < 0.005$). No other differences in errors between visual fields, hands, sex of subjects or orientation of pictures could be found.

In experiment 2, presentation and data analysis were identical to those in experiment 1. Drawings and photographs represented three different categories of objects, that is 'corkscrew' (Fig. 2), 'key' and 'brush'. Times for correct decisions were shorter for RVF than for LVF ($F(1.368) = 11.31$; $P < 0.001$), indicating LH superiority (Fig. 3) and the learning effect was again significant ($F(3.368) = 10.72$; $P < 0.001$). Again there was no significant difference in times of decision between hands and interactions. Neither the sex of subjects nor the orientation of objects on the photographs had an influence on the difference in time between the visual fields. There were 4% errors equally distributed between visual fields, hands, sex of subject and orientation of pictures. No decrease in errors in the course of testing could be found.

The above findings show that associative processing of non-verbal visual information is stimulus dependent and that there is a functional asymmetry of associative processing by the two hemispheres. A possible mechanism, which would very probably favour the LH, could be 'inner verbalisation'. To reduce possible differences in the capability of verbalisation, we used as a reference picture a highly stylised drawing, which allowed easy verbalisation of the emotional facial expression as well as of the object classes. Nevertheless the behaviour of the subjects indicated very different levels of awareness of the correctness of decisions for emotional facial expressions as opposed to object categories. Often, erroneous object judgments were followed by rapid recognition of the errors, whereas for emotional expressions subjects repeatedly expressed the sense of having made an error, when in fact the manual response had been correct. However this is an incidental observation which needs to be explored systematically.

To conclude, we found that visual associative processing is not exclusively a function of the left hemisphere, but that both

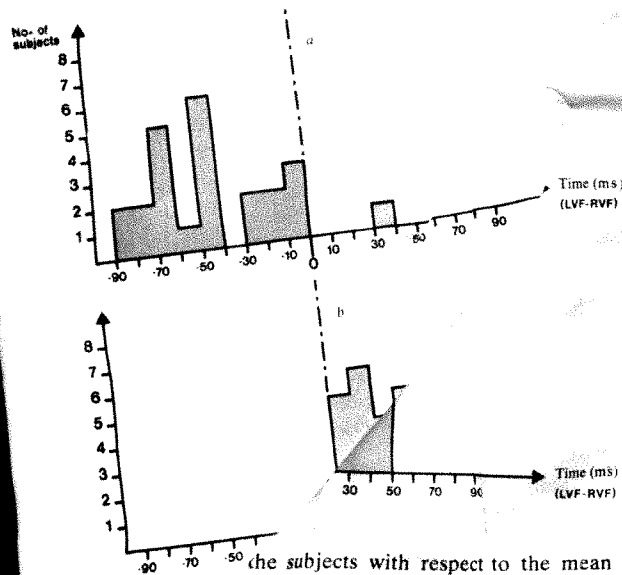


Fig. 3 Differences in LVF, thus indicating right hemisphere differences. *a*, Experiment 1 (emotional expression); *b*, Experiment 2 (object).

hemispheres are capable of visual associative performance. There are, however, two different associative procedures: The left hemisphere seems to be superior on an associative task requiring recognition of a common category to which different objects belong, whereas the right hemisphere seems to be superior on an associative visual task requiring recognition of a common emotional facial expression in different faces. Although it is not proven that the emotional feature in the facial expression was critical in producing the right hemisphere superiority, this would be consistent with the recently expressed view, that the RH might have a distinct role in processing emotional information.

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Naloxone dose dependently produces analgesia and hyperalgesia in postoperative pain

If endorphins modulate pain, then naloxone should affect pain behaviour even in subjects who have not received exogenous opiates. However, some naloxone studies claim a lack of effect^{1,2}, others a hyperalgesic effect³⁻⁵, and others both hyperalgesic and analgesic effects^{6,7}. Such discrepancies may arise from the different methods used to induce and assess pain⁸, and from variation in dose. We know of only one previous human study which used multiple doses of naloxone⁶; this indicated a dose-dependent bi-directional effect. In the present study, a clinical pain paradigm was used to generate a dose-response curve for naloxone. We report that naloxone produces analgesia at low doses and hyperalgesia at high doses.

The patients studied were 35 males and 42 females, aged 19-32 yr. Detailed methods were published previously⁸. Subjects underwent a standardised surgical procedure for the removal of impacted mandibular third molars after premedication with 10-20 mg of diazepam. During surgery, 15-40% nitrous oxide and local anaesthesia with 3% mepivacaine without vasoconstriction (effective duration 45-75 min, ensuring reversal of anaesthesia before experimental interventions) were used. Naloxone can have effects which are not specific for opiates. In fact, a naloxone interaction with both diazepam^{9,10} and nitrous oxide¹¹ has been reported. However, these interactions are unlikely to have a significant effect on the conclusions of this study for reasons discussed previously⁸.

The patients received placebo as their first medication 2 h after the onset of local anaesthesia. One hour later, naloxone was given double blind in doses of 0 (placebo), 0.4, 2, 7.5 or 10 mg. Patients rated their pain by a mark on a 10-cm horizontal line which had the words 'no pain' at the left (0 cm) end and 'worst pain ever' at the right (10 cm). The validity of this scale has been discussed elsewhere⁸. Pain was measured 5, 20 and 60 min after each injection. The pain at 60 min, the time of greatest placebo effect, was used in the present analysis. Pain, with this paradigm, increases over time. This was demonstrated by administration of placebo by a secret intravenous infusion line so that the patients did not know that they had received anything, and the surgeon did not know what he had administered (a blind placebo)¹². Placebo responders were those subjects in whom pain decreased or was unchanged 1 h after placebo administration. Placebo non-responders were those patients whose pain increased over that hour.

Figure 1 shows dose-response curves for the change in pain and mean pain levels, 1 h after naloxone. For placebo responders, the dose-response curve is bi-directional. Compared with placebo, naloxone at low doses (0.4 and 2 mg) produces analgesia, whereas at higher doses (7.5 and 10 mg) it produces hyperalgesia. The naloxone-induced reduction in pain level is significantly greater than that produced by placebo both at the 0.04 mg dose ($0.01 > P > 0.005$, by the Student *t* test) and at the 2 mg dose ($0.05 > P > 0.025$) of naloxone. At the 7.5 and 10 mg doses, the change in pain is significantly greater than that produced by placebo ($0.05 > P > 0.025$). Figure 1b also demonstrates a bi-directional dose-response curve when pain level, rather than change in pain, is plotted.

When the analysis is carried out for placebo non-responders, naloxone has relatively little effect (Fig. 1a). In fact, if all non-responders are compared with placebo (0 mg), there is a slight hyperalgesia ($0.1 > P > 0.05$). When responders and non-responders are pooled, the bi-directionality is still apparent (Fig. 1a).

The finding that naloxone has a bi-directional dose-response curve present in placebo responders, may resolve some of the apparent conflict among previous reports. Grevert and

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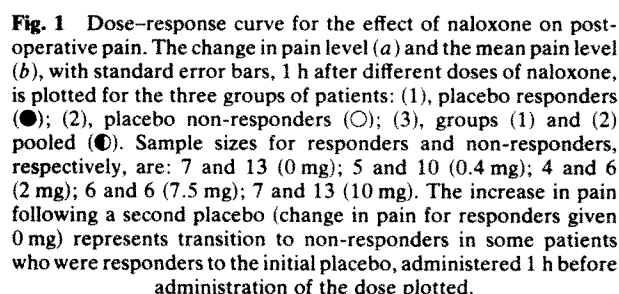


Fig. 1 Dose-response curve for the effect of naloxone on post-operative pain. The change in pain level (*a*) and the mean pain level (*b*), with standard error bars, 1 h after different doses of naloxone, is plotted for the three groups of patients: (1), placebo responders (●); (2), placebo non-responders (○); (3), groups (1) and (2) pooled (◐). Sample sizes for responders and non-responders, respectively, are: 7 and 13 (0 mg); 5 and 10 (0.4 mg); 4 and 6 (2 mg); 6 and 6 (7.5 mg); 7 and 13 (10 mg). The increase in pain following a second placebo (change in pain for responders given 0 mg) represents transition to non-responders in some patients who were responders to the initial placebo, administered 1 h before administration of the dose plotted.

Antiviral effect of interferon by the host in vivo may be mediated by the host

INTERFERON has been shown to act both *in vivo* and *in vitro*. In interferon-treated cells *in vitro* the cycle of the virus is inhibited at the transcriptional level, depending on the virus. Viral replication is also the direct inhibition of viral replication has been the mechanism by which interferon has been shown to be effective *in vivo*. We report here results that show the effect of interferon against viral infection *in vivo* and the inhibition of the viral replication of the same virus in

To test the antiviral effect of human leukocyte interferon (HLI) *in vivo*, six rhesus monkeys were infected intradermally with different doses of vaccinia virus (strain of the Rijks Instituut voor de Volksgezondheid, RIV)² of which three were treated daily with HLI administered intramuscularly. The HLI was prepared as described previously³. The preparation used in these experiments had a specific activity of 2.1×10^6 units per mg protein. In control monkeys the lesions reached their maximum at day 7. Daily intramuscular injections of 5×10^5 units HLI per kg body weight during 8 d completely inhibited the development of the typical vaccinia-induced skin lesions (Fig. 1). No skin lesions were detected during the observation period of 4 weeks following infection. However, we were unable to correlate this distinct *in vivo* effect of HLI with a corresponding inhibition of the cytopathogenic effect (CPE) of the same virus on diploid rhesus monkey skin fibroblasts or on Rous sarcoma virus transformed human fibroblasts (RSb cells). Up to 10^4 units of HLI failed to protect the cells against the CPE of vaccinia virus (RIV strain), although the CPE of vesicular stomatitis virus (VSV) was effectively inhibited (Table 1). Surprisingly, vaccinia virus was resistant to interferon in our *in vitro* system, but the same results were obtained *in vitro* with the vaccinia virus strain WR and with smaller challenge doses (10^{-3} TCID₅₀)⁴. Also no anti-vaccinia activity could be detected in the sera of the monkeys 4 h after the first injection with HLI. At this time, the sera showed the maximum titre of antiviral activity when tested against VSV. Similar experiments performed with human diploid skin fibro-



Fig. 1 *In vivo* effect of human leukocyte interferon (HLI) in rhesus monkeys infected intradermally with vaccinia virus. Monkeys were treated with HLI from day -1 to day 7 at 5×10^5 units kg⁻¹ i.m. They were vaccinated intradermally at day 0 with the following preparations: 10^7 TCID₅₀ ml⁻¹ vaccinia virus (a); 10^6 TCID₅₀ ml⁻¹ vaccinia virus (b); 10^5 TCID₅₀ ml⁻¹ vaccinia virus (c); 10^7 TCID₅₀ ml⁻¹ vaccinia virus, subjected to UV- and heat-inactivation (d); NaCl (e). For each dilution, 0.05 ml were injected at three sites. The vaccination sites 12 d after infection are shown in a rhesus monkey treated with HLI (left) and in a control monkey (right). Three monkeys were treated with interferon and three served as controls.

Table 1 *In vitro* inhibition of the cytopathogenic effect (CPE) of vaccinia virus by human leukocyte interferon (HLI) and serum from rhesus monkeys treated with HLI

Preparation	RSb cells		Skin fibroblasts from rhesus monkeys	
	VSV	Vaccinia virus	VSV	Vaccinia virus
HLI, 10^4 units ml ⁻¹	10,000	<1	10,000	<1
Pre-serum*	<10†	<10	ND	ND
Post-serum*	350†	<10	ND	ND

Rous sarcoma virus transformed human fibroblasts (RSb) or monkey skin fibroblasts were plated in microtitre plates, grown to confluency (4×10^4 cells per well) and treated with serial dilutions of HLI containing 10^4 units per ml of rhesus monkey sera (before and after injection of 5×10^5 units HLI kg⁻¹). After overnight incubation, the supernatant was removed and cells were infected with vesicular stomatitis virus (VSV) or vaccinia virus (multiplicity of infection 0.25 TCID₅₀ per cell). The incubation was terminated when untreated infected control cells showed more than 90% CPE. The cells were stained with crystal violet. Both viruses were always tested simultaneously in the same microtitre plate and an interferon standard preparation was included. The activity of interferon is expressed as the reciprocal of the highest dilution giving 50% protection. The titres are given as reference units (tested against standard preparation 69/19).

* Before and after 4 h after injection of rhesus monkeys with 5×10^5 units of HLI per kg.

† Mean titre of three monkeys. ND, not determined.

blasts and rhesus monkey kidney cells showed identical results. There was also no inhibition of the production of infectious vaccinia virus (unpublished results).

These findings indicate that interferon can be effective *in vivo* against a virus which is insensitive to its antiviral action in various cell types. Likewise, it has been shown by others⁵ that interferon can inhibit *in vivo* the growth of tumour cells which are resistant to its growth inhibitory action *in vitro*. Interferon may activate several defence systems of the host, for example, the cytotoxicity of the natural killer cells and the macrophages^{6,7}. Perhaps such 'aggressive' cells can selectively destroy virus-infected cells *in vivo*.

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Thrombin and epidermal growth factor become linked to cell surface receptors during mitogenic stimulation

THERE is much evidence that interaction with specific cell surface receptors is a key event in the action of polypeptide hormones and growth factors. Many analyses of hormone-receptor interactions have been carried out based on the assumption that hormone binding to the cell receptors is a reversible reaction¹⁻³. Indeed, many studies have demonstrated that bound hormones dissociate from their receptors. However, in most systems the possibility could not be excluded that a significant fraction of the hormone molecules that bind to cells become linked to their receptors. Our present studies with the polypeptide mitogens ¹²⁵I-thrombin and ¹²⁵I-epidermal growth factor (¹²⁵I-EGF) show that a significant percentage of the

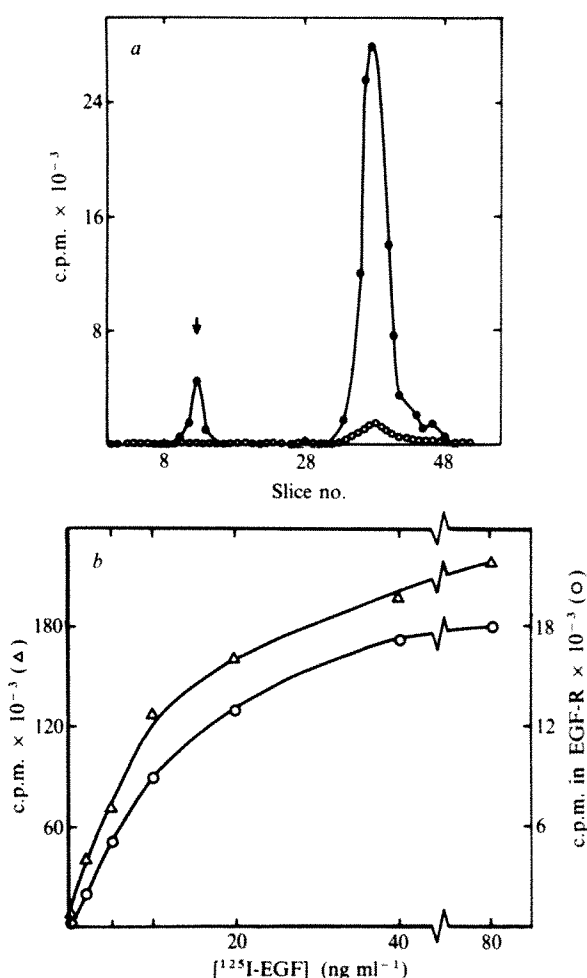


Fig. 1 Linkage of ¹²⁵I-EGF to cell receptors. *a*, Cultures were rinsed with DV medium containing 15 mM hydroxyethyl-*N,N'*-piperazine sulphonate, pH 7.3 (DV HEPES medium), and incubated for 1 h at 37 °C with 3 ml DV HEPES medium containing 0.1% bovine serum albumin (BSA) and either ¹²⁵I-EGF (10 ng ml⁻¹) (●) or ¹²⁵I-EGF plus 2 μg ml⁻¹ unlabelled EGF (○). The cultures were rinsed six times with 2 ml ice-cold phosphate-buffered saline (PBS) and the cells solubilised in 0.2 ml 0.1 M Tris buffer (pH 6.8) containing 3% SDS, 1% 2-mercaptoethanol, 2.0 mM phenylmethylsulphonyl fluoride and 0.6% *N*-ethylmaleimide. The samples were incubated in a boiling water bath for 5 min and applied to disc gels containing 5–20% linear polyacrylamide gradients and 0.1% SDS. The gels were cut into 2-mm slices, dried, and the radioactivity measured with a γ-counter. The arrow indicates the 170–190 K component. *b*, The experiment described in *a* was carried out using ¹²⁵I-EGF at the indicated concentrations. The radioactivity in the bands was corrected for nonspecific binding by subtracting the radioactivity bound in the presence of 2 μg ml⁻¹ unlabelled EGF. ○, c.p.m. in the 170–190 K component (EGF-R); △, total specifically bound radioactivity (c.p.m. in EGF-R plus c.p.m. in unlinked ¹²⁵I-EGF).

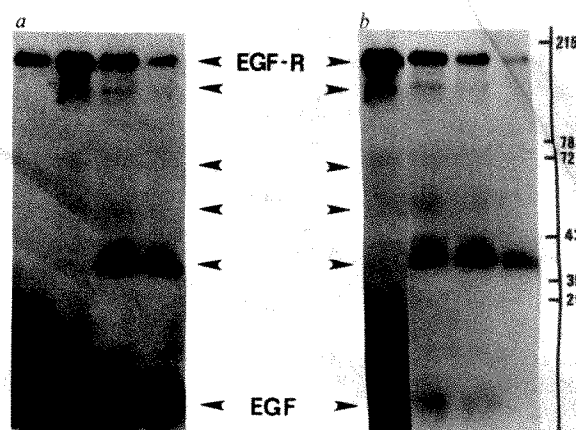


Fig. 2 Time courses of formation and degradation of EGF-R. *a*, As described in Fig. 1*a*, HF cells were incubated with ¹²⁵I-EGF (20 ng ml⁻¹) for various periods, rinsed, solubilised and heated. Samples (20 μl) and red blood cell marker proteins were applied to slab gels containing a 5–20% linear polyacrylamide gradient and 0.1% SDS. The gels were fixed, stained with Coomassie blue, and dried. Autoradiographs were produced using Kodak XOMAT R film. Lanes 1–4 show the results of incubating cells with ¹²⁵I-EGF for 10, 60, 180 and 420 min, respectively. *b*, HF cell cultures were incubated with ¹²⁵I-EGF (20 ng ml⁻¹) as described in Fig. 1*a*, and were quickly rinsed six times with ice-cold PBS. The cultures were reincubated at 37 °C with 3 ml DV HEPES medium for various periods. The cells were then rinsed twice with ice-cold PBS, solubilised and heated as described in Fig. 1*a*. SDS-PAGE and autoradiography were carried out as described in *a*. Lanes 1–4 show 0, 60, 120 and 180 min incubations, respectively, following removal of ¹²⁵I-EGF-containing medium.

molecules that are specifically bound on human fibroblasts become linked to cell surface receptors. This finding has broad implications for the way in which these molecules might stimulate cell division. It also raises the question of whether linkage of polypeptide hormones to their receptors is a general phenomenon.

Human foreskin (HF) cells (passage 7–11) were grown in Falcon tissue culture dishes containing Dulbecco–Vogt modified Eagle's medium (DV medium) and 5% calf serum as previously described⁴. Cells for experiments were seeded in 60-mm dishes at 3×10^4 cells per cm², and were incubated in serum-containing medium for 3 d, then in serum-free medium for 2 d. EGF was purified by the method of Savage and Cohen⁵. EGF⁶ and thrombin⁷ were iodinated by published procedures. Both proteins contained an average of 0.7 I-atoms per molecule. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using disc and slab gels was carried out by the method of Laemmli.⁸

We have recently used the procedures of Das and Fox to radiolabel the EGF receptors on HF cells with ¹²⁵I-EGF-NAPEDE, a photoactivable cross-linking derivative of ¹²⁵I-EGF⁹. We found that the EGF-receptor complex had a molecular weight (MW) similar to the 190,000 dalton (190 K) EGF-receptor complex reported in mouse cells¹⁰. During this work, we discovered that some of these receptors also became labelled by ¹²⁵I-EGF that lacked a photoactivable group. Figure 1*a* shows the distribution of radioactivity in gel slices after SDS-PAGE of solubilised HF cells that had been incubated with ¹²⁵I-EGF (10 ng ml⁻¹) for 60 min at 37 °C. In this and the following experiments, the cells were solubilised by boiling in 3% SDS and 1% 2-mercaptoethanol, conditions that disrupt noncovalent interactions between most proteins. As shown, about 90% of the radioactivity was found in a rapidly migrating band. We identified this material as ¹²⁵I-EGF on the basis of its MW (about 6,000). In addition, 6–9% of the radioactivity specifically bound to the cells was contained in a 170–190 K component. This component was absent when a vast excess of unlabelled EGF was present in the binding medium to reduce interaction of ¹²⁵I-EGF with its receptors (Fig. 1*a*), or when ¹²⁵I-EGF was incubated without cells. These results showed that a significant fraction of bound ¹²⁵I-EGF became directly linked to cellular receptors for EGF. This linkage was probably

covalent because the labelled 170–190 K component survived treatment with 6 M guanidine-HCl and 0.1 M 2-mercapto-ethanol¹¹.

The amount of ^{125}I -EGF that became linked to receptors (EGF-R) increased with increasing concentrations of ^{125}I -EGF and was maximal at 40 ng ml^{-1} ^{125}I -EGF (Fig. 1b). This was about the same concentration dependence displayed by total specific ^{125}I -EGF binding. Thus, at all ^{125}I -EGF concentrations examined, linked ^{125}I -EGF accounted for a relatively constant fraction (6–9%) of the total specifically bound ^{125}I -EGF. Also, the amount of EGF-R varied with duration of ^{125}I -EGF incubation roughly in parallel with total specific ^{125}I -EGF binding. Figure 2a (lane 1) shows that EGF-R and also ^{125}I -EGF specifically bound but not linked (EGF) were apparent after only 10 min incubation at 37°C . The amounts of both EGF-R and EGF continued to increase during the first hour of incubation with ^{125}I -EGF (lane 2), but decreased markedly during the next few hours (lanes 3 and 4). The decrease in ^{125}I -EGF binding that occurs during several hours of incubation with ^{125}I -EGF is caused by a decrease in the number of EGF receptors (down regulation)^{12,13}. Apparently, the receptors that were linked to ^{125}I -EGF were down-regulated with the other EGF receptors.

The rate at which ^{125}I -EGF became linked to receptors suggested that this reaction took place at the cell surface. Consistent with this view, EGF-R formed during a 10-min incubation with ^{125}I -EGF was completely removed by a 10-min treatment with trypsin ($10\text{ }\mu\text{g ml}^{-1}$) at 37°C . However, after a

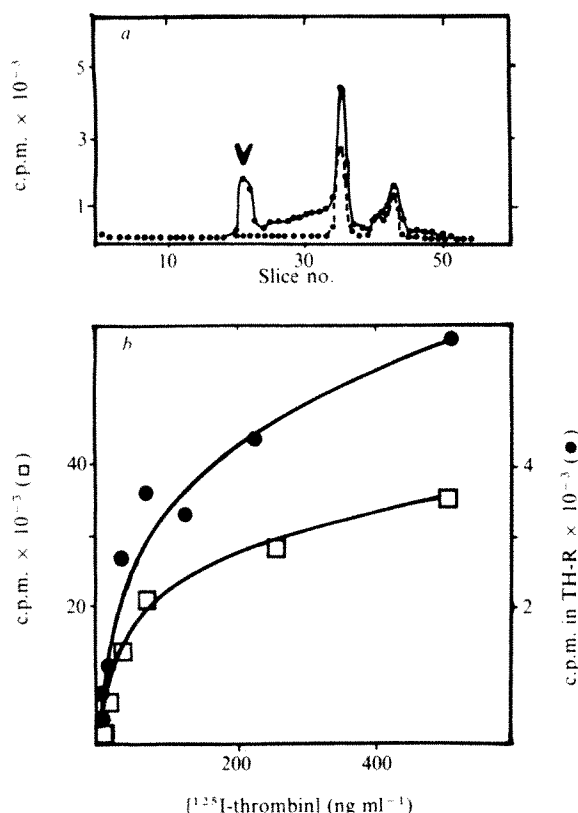


Fig. 3 Linkage of ^{125}I -thrombin to cell receptors. *a*, Cultures were incubated at 37°C for 20 min with DV HEPES medium, and the medium was replaced with DV HEPES medium containing 0.5% BSA and either ^{125}I -thrombin ($0.25\text{ }\mu\text{g ml}^{-1}$) (●) or ^{125}I -thrombin plus $5\text{ }\mu\text{g ml}^{-1}$ unlabelled thrombin (□). The cultures were then incubated for 90 min at 37°C and rinsed, solubilised and heated as described in Fig. 1a. The samples were applied to disc gels containing 7.5–15% polyacrylamide linear gradients and 0.1% SDS. The radioactivity in gel slices was determined as described in Fig. 1a. The arrow indicates the 65K component. Unlinked ^{125}I -thrombin migrated in gel slices 34–37. *b*, The experiment described in *a* was carried out with ^{125}I -thrombin at the indicated concentrations. The radioactivity in the 65K and unlinked ^{125}I -thrombin bands were corrected for nonspecific binding by subtracting the radioactivity that bound in the presence of $5\text{ }\mu\text{g ml}^{-1}$ unlabelled thrombin. □, Radioactivity in the 65K component and bound but unlinked ^{125}I -thrombin; ●, c.p.m. in the 65K component.

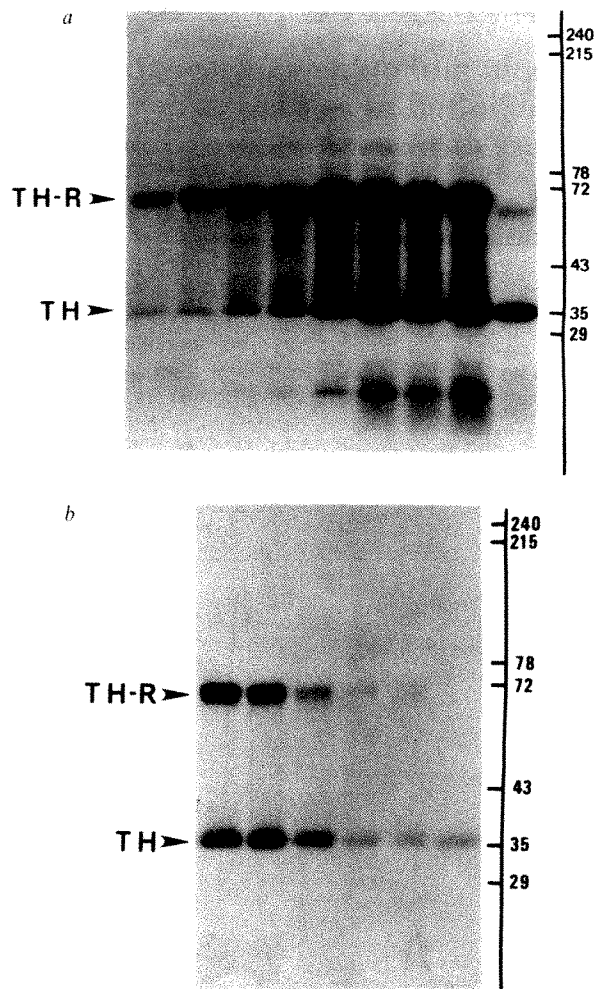


Fig. 4 Time courses of the formation and disappearance of Th-R. *a*, Using the procedures described in Fig. 3a, HF cells were incubated with ^{125}I -thrombin (50 ng ml^{-1}) for various periods at 37°C , and prepared for SDS-PAGE as described in Fig. 1a. Samples were applied to slab gels containing 5–20% linear polyacrylamide gradients and 0.1% SDS, and autoradiographs were made. Lanes 1–8 show the results of 20 s, 40 s, 80 s, 160 s, 9 min, 30 min, 60 min and 90 min incubations, respectively. Lane 9 shows the result of a 30-min incubation with ^{125}I -thrombin when $8\text{ }\mu\text{g ml}^{-1}$ unlabelled thrombin was present. *b*, Cultures incubated with 50 ng ml^{-1} ^{125}I -thrombin for 90 min were quickly rinsed six times with 2 ml ice-cold PBS, and incubated for various periods at 37°C with 3 ml DV HEPES medium containing $8\text{ }\mu\text{g ml}^{-1}$ unlabelled thrombin. The cells were then rinsed twice with cold PBS and processed for SDS-PAGE as described in Fig. 1a. The samples were applied to slab gels containing 7.5–15% linear polyacrylamide gradients and 0.1% SDS, and an autoradiograph was prepared. Lanes 1–6 show the results of 0, 10, 30, 270 and 360 min incubations, respectively, following removal of ^{125}I -thrombin-containing medium. In lane 6 cultures were treated as in lane 4, except that the ^{125}I -thrombin incubation was carried out in the presence of $8\text{ }\mu\text{g ml}^{-1}$ unlabelled thrombin.

1-h incubation with ^{125}I -EGF, most of the EGF-R was insensitive to trypsin, suggesting that it had entered the cells. During ^{125}I -EGF incubations longer than 1 h, iodinated components of approximate MWs 70,000, 50,000 and 36,000 appeared (Fig. 2a, lanes 3, 4). Figure 2b shows that these resulted from processing or degradation of EGF-R. When cultures incubated with ^{125}I -EGF were rinsed and incubated at 37°C with medium containing no EGF, the unlinked ^{125}I -EGF disappeared from the cells within 1–2 h, but much of the EGF-R remained (Fig. 2b, lanes 1–3). During the next 2 h, most of the EGF-R was degraded to a component of MW 36,000, and additional products of 70,000 and 50,000 MWs (Fig. 2b, lanes 3, 4). Degradation of EGF-R occurred more rapidly if EGF was present in the medium. Similar results have been obtained in studies with mouse cells in which the fate of the EGF receptor covalently linked to ^{125}I -EGF through a photoactivated cross-linking reagent was studied^{9,10}.

These results with EGF prompted us to determine whether thrombin, another highly purified growth factor for HF cells¹⁴, also became linked to its receptors during mitogenic stimulation. Fibroblasts possess a large number of specific high-affinity thrombin receptors (about 2×10^5 receptors per cell with a K_d of 1×10^{-9} M in mouse cells)¹⁵. Binding to these receptors is apparently necessary for thrombin-stimulated cell division¹⁵. When HF cells were exposed to ¹²⁵I-thrombin ($0.25 \mu\text{g ml}^{-1}$) for 90 min at 37 °C, most of the specifically bound radioactivity migrated with material larger than ¹²⁵I-thrombin, and about one-third of the specifically bound radioactivity was linked in a well defined complex of MW 65,000 (65 K component) (Fig. 3a). Because the MW of thrombin after reduction is about 30,000 (ref. 16), this indicated linkage to a receptor of about 35,000. (However, these MWs are only rough estimates, as thrombin is a glycoprotein.) The remainder of the specifically bound radioactivity migrated with free thrombin and heterogeneous material that was smaller than the 65K cross-linked complex but larger than thrombin. As with EGF-R, the ¹²⁵I-thrombin-receptor complex (Th-R) survived treatment with 3% SDS and 1% 2-mercaptoethanol in a boiling water bath for 5 min, suggesting that this linkage is also covalent. The Th-R probably resulted from interaction of ¹²⁵I-thrombin with cell receptors because it was absent either when ¹²⁵I-thrombin was incubated without cells (data not shown), or when a vast excess of unlabelled thrombin was added to inhibit interaction of ¹²⁵I-thrombin with its receptors (Fig. 3a).

Cellular formation of Th-R was apparent at a thrombin concentration of 2 ng ml^{-1} , and approached a maximum at $0.4 \mu\text{g ml}^{-1}$ ¹²⁵I-thrombin. The concentration dependence of Th-R formation and total specific ¹²⁵I-thrombin binding exhibited a similar concentration dependence (Fig. 3b). Interestingly, the dose dependence of thrombin for stimulation of HF cell DNA synthesis approximately corresponds to that for Th-R formation (unpublished results), consistent with the possibility that Th-R transmits the mitogenic signal.

Formation of Th-R by HF cells incubated with ¹²⁵I-thrombin at 37 °C could be detected within 20 s, and reached steady-state within 30 min (Fig. 4a). In contrast, the amount of bound but unlinked ¹²⁵I-thrombin (Th) increased more slowly. The rapidity of Th-R formation suggested that this linkage occurred at the cell surface. This was confirmed by the finding that a 10-min treatment with trypsin ($10 \mu\text{g ml}^{-1}$) at 37 °C removed Th-R formed after a 3-min incubation of cells with ¹²⁵I-thrombin. After a 90 min binding incubation most of the Th-R was insensitive to trypsin, suggesting that it had been internalised. We found no evidence of discrete degradation fragments from Th-R. When cultures incubated with ¹²⁵I-thrombin were rinsed and incubated at 37 °C with medium containing unlabelled thrombin, Th-R gradually disappeared over 4 h without the appearance of other bands (Fig. 4b).

We have reported here a novel interaction of human cells with two protein mitogens, EGF and thrombin. Both molecules became linked to their specific cell surface receptors, both linkages seemed to be covalent, and in both cases the coupled growth factor-receptor complexes apparently entered the cells. These linkages were not caused merely by transfer of ¹²⁵I from the growth factors to receptors, as chymotryptic digestion of EGF-R and Th-R released polypeptide fragments of ¹²⁵I-EGF and ¹²⁵I-thrombin, respectively.

The finding that polypeptide factors become linked to cell surface receptors is unexpected but not inconsistent with current knowledge. The direct linkage of EGF to cell surface receptors of normal and transformed murine cells and isolated membranes has been found simultaneously by Linsley *et al.* and is reported in the accompanying paper¹⁷. Insulin and trypsin have also been reported to become tightly associated with cell components^{18,19}, although it is unclear from these reports whether these associations survive treatment with SDS. In studies on the interaction of ¹²⁵I-insulin with H-4 hepatoma cells, a significant quantity of what seems to be an insulin-receptor complex (MW 120–130,000) was found, and was resistant to treatment with

SDS and 2-mercaptoethanol (C. Hoffman and D. F. Steiner, personal communication). Direct linkage of polypeptide hormones and growth factors to cell surface receptors might be a general phenomenon which will facilitate both the identification of these receptors and study of their metabolic fate. The role of polypeptide-cell surface receptor complexes in the action of these polypeptides remains to be studied. However, Shechter *et al.* recently concluded that the growth stimulatory effects of EGF "may be mediated by occupation of only a negligible fraction of very high-affinity binding sites" (ref. 20). The present data are consistent with the possibility that this "very high-affinity" binding represents EGF-R.

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Direct linkage of epidermal growth factor to its receptor

EPIDERMAL GROWTH FACTOR (EGF), a potent polypeptide mitogen, has been studied intensively by those concerned with the molecular events leading to cell proliferation^{1–4}. The initial, reversible interaction of EGF with a specific cell surface receptor and the subsequent internalisation and degradation of EGF, and by inference the EGF-receptor complex, are well documented, although the role of these events in mitogenesis is unknown^{4–6}. In an attempt to define this role, we used photoaffinity probes to specifically cross-link radiolabelled EGF to its receptor, a 185,000-dalton polypeptide on the surface of murine 3T3 cells⁷. These probes also enabled us to follow the metabolic fate of this receptor⁸. We have since noted that a small portion of the underivatized radiolabelled EGF that binds specifically to 3T3 cells or isolated 3T3 cell membranes becomes directly and irreversibly linked to a polypeptide of molecular weight (MW) 185,000. Here, we document this association and

show that the resulting complex has properties identical to those of the EGF-receptor complex originally identified by photo-affinity labelling.

When 3T3 cells were incubated with ^{125}I -EGF, and the proteins solubilised in ionic detergent solution and subjected to electrophoresis, 1–2% of the specifically bound label migrated as a band of apparent MW 190,000 (Fig. 1, lane A). The radioactivity in this band was reduced when unlabelled EGF was included with labelled EGF in the binding medium (Fig. 1, lanes C, E), an indication that the appearance of the 190,000 MW band requires specific binding of EGF.

EGF resembles certain other hormones in its capacity to reduce the number of available specific receptor sites when incubated with cultured cells^{3–6,8}. This ligand-induced reduction of binding activity is termed 'down regulation', and in the case of the EGF receptor, occurs by internalisation of the receptor protein⁸. When 3T3 cells were treated with unlabelled EGF to down-regulate the EGF receptor before addition of labelled EGF, formation of the direct linkage complex was greatly reduced (Fig. 1, lane G), a further indication that complex formation requires EGF specific binding. Also, when cells were incubated with labelled EGF for longer periods, radioactivity disappeared from the 190,000 MW direct linkage complex and appeared in the same three degradation products that are formed during down-regulation of the photoaffinity-labelled EGF-receptor complex (Fig. 1, lanes B, D, F)⁸. These data show that a small amount of the ^{125}I -radioactivity in specifically bound EGF becomes directly linked with its receptor.

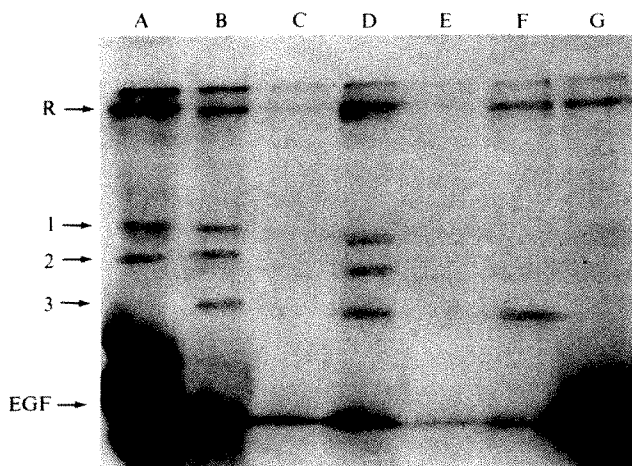


Fig. 1 Formation of the direct linkage complex and the derived metabolic products during down-regulation. Monolayer cultures of Swiss mouse 3T3 cells (clone 42) were grown on plastic dishes, as previously described⁷. Confluent cultures were washed three times with binding medium (DMEM, without NaHCO_3 but containing 10 mM HEPES and 1.0 mg ml⁻¹ bovine serum albumin). Some samples were incubated with 50 ng ml⁻¹ unlabelled EGF for 4 h at 37 °C to allow for down regulation of receptor. All samples were incubated for 30 min at 37 °C with iodinated EGF⁷, at 50 ng ml⁻¹ (1.5×10^3 c.p.m. per ng) in binding medium, and excess unlabelled EGF was added where noted. The ^{125}I -EGF preparations used contained 0.5–1.0 atoms of iodine per mol of EGF. The cells were washed to remove unbound labelled EGF, then incubated in binding medium at 37 °C for the indicated times, washed again, frozen, thawed and dissolved in electrophoresis sample buffer (ESB: 63 mM Tris, pH 6.8, 3% SDS and 10% glycerol). Aliquots of 100 μl of each sample were subjected to electrophoresis on a 10% acrylamide gel, using the discontinuous system of Laemmli¹¹. On completion of electrophoresis, the slabs were stained with Coomassie blue, destained, dried on filter paper, and autoradiographed on X-ray film (Kodak no screen NS-2T). The band of radioactively labelled material visible at the interface between the stacking and resolving gels in this experiment is not present when samples are boiled or subjected to shear forces before electrophoresis, conditions which do not quantitatively alter the amount of radioactivity present in the receptor band. (A) Formation of the direct linkage complex (R) by incubation of cells with ^{125}I -EGF for 30 min at 37 °C. (B) Formation of receptor degradation products (I, II and III) when samples from (A) were incubated in binding medium for an additional 30 min at 37 °C. (C) As (B), but with 500 ng ml⁻¹ of unlabelled EGF present during ^{125}I -EGF binding. (D) as (B), but with 60 rather than 30 min of additional incubation. (E) as (D), but with 500 ng ml⁻¹ unlabelled EGF present during ^{125}I -EGF binding. (F) As (B), but with 120 rather than 30 min of additional incubation. (G) Cells were incubated with 50 ng ml⁻¹ of unlabelled EGF for 4 h at 37 °C to down regulate the EGF receptor before ^{125}I -EGF binding.

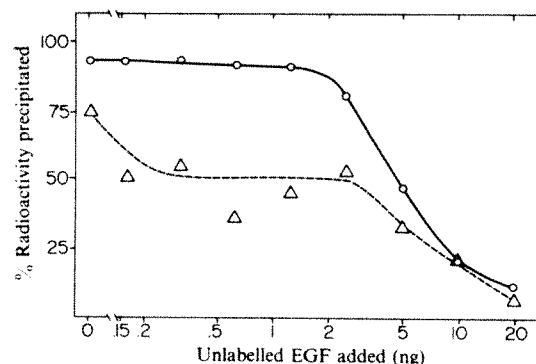


Fig. 2 Immune precipitation of specifically bound EGF and the EGF-receptor direct linkage complex. ^{125}I -EGF was bound to cells for 60 min at 23 °C as described in Fig. 1. After washing to remove unbound EGF, the cells from three 78-cm² dishes were scraped into binding medium and sedimented in a Brinkman microfuge. The cells were vigorously mixed for 2 min with 0.9 ml of 0.5% (v/v) Triton X-100 in binding medium and then incubated for an additional 10 min at 23 °C. Insoluble material was removed by sedimentation for 2 min in a microfuge; more than 90% of the cell-bound radioactivity and the bulk of the direct linkage complex remained in the supernatant fraction. Determination of immune precipitable total cell-bound EGF (O): 100 μl of the supernatant fraction were mixed with 10 μl of binding medium containing the indicated amounts of unlabelled EGF. To this, 10 μl of binding medium containing 1 μg of IgG fraction¹² of rabbit anti-EGF¹³ were added and the samples incubated for 30 min at 4 °C, after which, 10 μl of 10% (v/v) solution of formalin-fixed *Staphylococcus aureus*, an immune adsorbent¹⁴, were added. After a 15-min incubation at 4 °C, the immune adsorbent was sedimented for 2 min in a microfuge and the radioactivity present in the pellet and supernatant fractions measured to determine the per cent of total radioactivity which adhered to the immunoadsorbent. Determination of immune precipitable EGF-receptor direct linkage complex (Δ). Each pellet and supernatant fraction from the immunoadsorption experiment was treated to achieve the stated concentrations of the components in ESB, boiled and centrifuged to remove the immune adsorbent. Samples were subject to electrophoresis on a 10% acrylamide slab¹¹, and the region of the gel containing the direct linkage complex excised for measurement of radioactivity.

As direct linkage could result from transfer of labelled iodine from ^{125}I -EGF to its receptor, we used an antiserum specific for EGF to determine the immunoreactivity of the labelled 190,000 MW protein in non-ionic detergent extracts (Fig. 2). The bulk of the radioactivity initially present in both total cell-associated EGF and in the direct linkage complex was immune precipitated by antiserum to EGF. Also, unlabelled EGF similarly inhibited the precipitation of both the direct linkage complex and total cell-associated EGF. This last observation shows that much of the direct linkage complex consists of a single immunoreactive EGF molecule in stable association

Table 1 Time course of ^{125}I -EGF binding and direct linkage complex formation with a cell surface membrane preparation

	Incubation temperature (°C)	Incubation time (h)	
		1	2
Total binding (c.p.m.)	0	1,658	1,546
	23	3,547	2,777
	37	3,602	2,686
Direct linkage complex (c.p.m.)	0	10	49
	23	21	112
	37	43	120

3T3 cells were grown to confluence on 3,400 cm² of surface area in Dulbecco's modified Eagle's medium (DMEM) + 10% fetal calf serum (FCS) for preparation of cell surface membranes¹⁶. Particulate fractions banding at less than 40% (w:w) sucrose were pooled and collected by sedimentation. The membranes were suspended in binding medium and divided into three aliquots of 1.0 ml; 0.11 ml of ^{125}I -EGF (2.1×10^5 c.p.m. per ng, 500 ng ml⁻¹) in binding medium was added to each tube for incubation at 0, 23 or 37 °C. Incubations were terminated by layering a 0.1-ml aliquot from each tube above a 1 ml cushion of 10% (w:w) sucrose in binding medium and sedimenting for 2 min in a Brinkman microfuge. The pellets were washed with 1.5 ml binding medium. Total binding was corrected for background binding measured at zero time and was approximately 90% specific. The radioactivity in direct linkage complex was measured as described in Fig. 1 and was also corrected for background binding.

with its high MW receptor protein. However, this does not eliminate the possibility that the direct linkage complex forms through attachment of receptor to an artefactual and highly reactive EGF species produced during radioiodination. We therefore repeated the experiment described in Fig. 2 using unlabelled EGF and cells labelled metabolically with ^{35}S -methionine. Using these cells we identified a high MW ^{35}S -labelled protein band with electrophoretic mobility identical to that of the ^{125}I -EGF receptor direct linkage complex and which was only formed in cells incubated with EGF (data not shown).

Two independent experiments show that the photoaffinity-labelled EGF-receptor complex is degraded in the lysosomes⁸: the degradation products co-fractionate with lysosomal enzyme markers during organelle fractionation, and the degradation of the photoaffinity complex is inhibited by the lysosomotropic drug, chloroquine⁹. The degradation of the direct linkage complex is also markedly inhibited by chloroquine (Fig. 3a), and the rates of degradation (Fig. 3b) in the presence and absence of chloroquine are similar to those previously determined for the photoaffinity-labelled complex⁸.

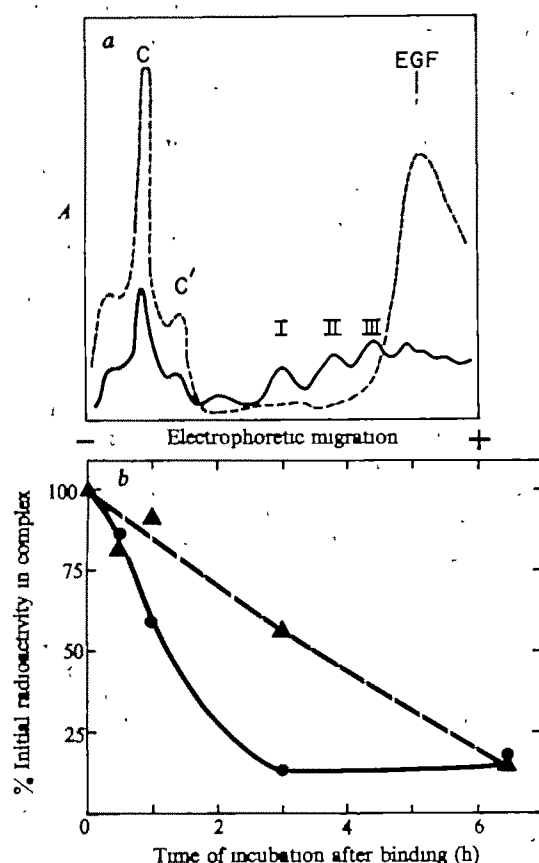


Fig. 3 a, Degradation of the direct linkage complex and inhibition of degradation by chloroquine. The conditions are essentially those described in Figs 1 and 2. Binding was for 1 h at 23 °C, following binding, and before preparation for electrophoresis, the cultures were incubated at 37 °C for an additional 75 min. The X-ray films were scanned with a densitometer, and the tracings are reproduced. —, Degradation of the direct linkage complex (C) into the proteolysis products characteristic of the EGF-receptor cross-linked complex. C' is a high MW product (142,000) that accumulates in the presence of chloroquine⁹. Bands I, II and III have apparent MWs of 59,000, 38,000 and 30,000 in this particular gel system. ———, Inhibition of direct linkage complex processing by chloroquine. Conditions were as described for Figs 1 and 2. Chloroquine (0.1 mM) was added 15 min before EGF and was present throughout the experiment. b, Effects of prior treatment with chloroquine on the rate of degradation of the direct linkage complex. Conditions were the same as in a, except that chloroquine was added 1 h before EGF. At the indicated times, samples were prepared for electrophoresis, which was carried out on a 10% acrylamide slab. The direct linkage complex was located on the dried gel by autoradiography. The amount of radioactivity present as direct linkage complex at an indicated time of incubation is expressed as a % of the amount of complex present before incubation. ●, No chloroquine added; ▲, plus 0.1 mM chloroquine.

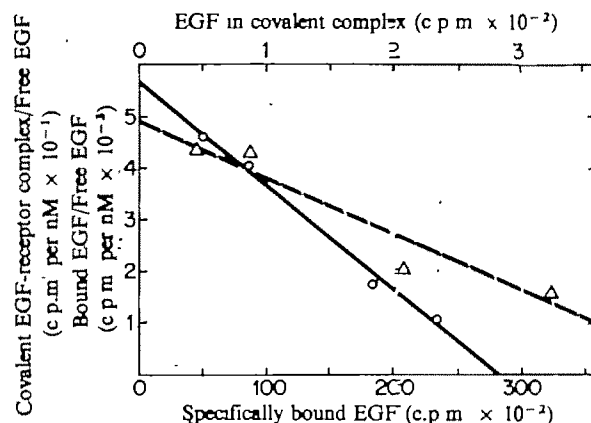


Fig. 4 Correlation between the concentration dependence of EGF binding and direct-addition complex formation. Cells grown as described in Fig. 1 were incubated for an additional day in DMEM plus 0.8% FCS. The cells were washed with binding medium, and total binding was determined at 23 °C after a 65-min incubation with iodinated EGF (2.3×10^4 c.p.m. per ng) at 3.75, 7.5, 37.5 and 75 ng ml⁻¹. Nonspecific binding was measured on duplicate cultures in the presence of 1 μg ml⁻¹ unlabelled EGF. Cultures were washed to remove unbound EGF and the samples were solubilised with 0.1 ml ESB and quantitatively transferred for determination of cell-bound radioactivity. Specifically bound EGF (○) was calculated by subtracting nonspecifically bound EGF from the total amount bound. The resulting binding data were plotted according to Scatchard²⁵, the slope of the curve is thus proportional to the K_A of binding. The samples were then processed for electrophoresis as in Fig. 1. The regions containing the direct linkage complex were excised from the dried gel and total radioactivity was corrected for the nonspecific radioactivity remaining when 1 μg ml⁻¹ unlabelled EGF was present. The specific radioactivity in the complex at a particular EGF concentration was calculated as the fraction of the total radioactivity applied at that concentration (Δ). Each point is the average of two determinations, except for the point at 75 ng ml⁻¹ EGF, which is the average of four determinations. For broken line, —slope = $K_{\text{formation}} = 0.112 \text{ nm}^{-1}$; for solid line, —slope = $K_A = 0.201 \text{ nm}^{-1}$.

If the direct linkage EGF-receptor complex is a complex of EGF and its physiological receptor, the saturability for reversible EGF binding and direct complex formation should be similar. The observed concentration dependence for complex formation, $K_{\text{formation}}$, closely approximated the equilibrium binding constant, K_A , for EGF and its physiological receptor in the intact cell (Fig. 4 and data not shown). Table 1, which shows that complex formation is a slower process than binding, may partly explain the slight discrepancy between $K_{\text{formation}}$ and K_A . This should be accentuated at lower EGF concentrations, where longer periods of time are required to achieve binding equilibrium. Accordingly, the apparent $K_{\text{formation}}$ is an underestimate of its true value. Table 1 also shows that complex formation is more sensitive to a temperature decrease than is binding, and indicates why direct linkage of EGF to its receptor was not observed in our earlier studies with ^{125}I -EGF photoaffinity probes^{7,8}. A comparison of the times and temperatures of exposure to EGF to receptor for direct labelling (Table 1) with those used in the photoaffinity labelling studies^{7,8} shows that direct complex formation would account for less than 10% of the cross-linked complex formed on photolysis^{7,8}.

Thus, we have demonstrated that a small fraction of the EGF specifically bound to its cell surface receptor becomes strongly and directly linked to a high MW protein. This complex and the photoaffinity-labelled EGF-receptor complex have the same biological fate. The biological and chemical properties of the complex, described above are those expected of a covalently linked EGF-receptor complex.

We are now investigating the chemical nature and mechanisms of formation of the complex. Its members are not joined by a disulphide bond—it is not dissociated by boiling in 5% 2-mercaptoethanol. Its formation does not seem to be catalysed by a transglutaminase reaction similar to that responsible for intermolecular protein cross-linking in the red blood cell¹⁰—complex formation is not increased by Ca^{2+} ion, or decreased by added amines such as histamine, glycine ethyl ester or cystamine. Alkylation of the amino terminus of EGF with

biotin¹⁷ did not affect EGF binding, but substantially inhibited direct complex formation, implicating a site on or near the amino terminus of EGF in direct complex formation.

We also have evidence for an EGF-receptor complex in SV40-transformed 3T3 cells, normal human fibroblasts and a human tumour cell line (A-431)^{5,18}; it is also formed when EGF is incubated with human placental membranes. Baker *et al.* (see accompanying paper) have independently detected a EGF-receptor complex in cultured human fibroblasts and have evidence of complex formation between thrombin and its receptor in human foreskin fibroblasts¹⁹. Recently, a complex of insulin and a protein of MW approximately 120,000–130,000 was detected in cultured H-4 cells incubated with ¹²⁵I-insulin; this complex resists dissociation in SDS and accounts for at least 10% of the insulin which binds specifically to these cells (C. Hoffman and D. Steiner, personal communication). A high MW form of ¹²⁵I-insulin, accounting for at least 10% of the radioactivity specifically bound to adipocytes, was detected by Kahn and Baird²⁰; this passes through Sephadex G-50 at the void volume in aqueous solution containing 0.1% Triton X-100, 4 M urea and 1 M acetic acid. We have incubated 1 nM ¹²⁵I-insulin with cultured L1-3T3 cells either before or after they differentiated to form adipocytes²¹ and find a substantial quantity of a component of MW 125,000 ± 5,000 which resists dissociation to form free insulin during 5 min of boiling in aqueous 3% SDS (Iwata, K. and C.F.F., unpublished observation). Taken together, these observations indicate that the direct linkage phenomenon may be a general one, a correlation between the high affinity specific binding of many polypeptide ligands and their cell surface receptors.

Although a biological role for direct linkage of hormones to their specific receptors has not been shown, Schechter *et al.* have concluded that a small fraction of "very high affinity binding sites"—possibly identical to the direct complex—may play a role disproportionate to their fraction of the whole in mediating EGF action²².

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Is stimulation of benzodiazepine receptor binding mediated by a novel GABA receptor?

A STEREOSPECIFIC, saturable, high affinity binding site for ³H-diazepam has recently been characterised in membrane fractions isolated from the brains of mammals¹, including man², which has been suggested to represent the pharmacological benzodiazepine (BZ) receptor^{2–4}. Electrophysiological⁵ and biochemical⁶ results indicate that BZs exert their main actions in the central nervous system by facilitation of the synaptic action of γ -aminobutyric acid (GABA). Evidence for an interaction between GABA and BZs at the receptor level has recently been obtained^{7–11}. Thus, GABA has been shown to stimulate BZ binding^{8–11} and there is preliminary evidence that this process is mediated by a specific GABA recognition site, as the GABA agonists muscimol⁸, imidazoleacetic acid¹¹, and 3-guanidino-propionic acid¹¹ also stimulate ³H-diazepam binding. We now report different structural requirements for stimulation of ³H-diazepam binding to rat brain synaptic membranes compared with those for inhibition of Na⁺-independent ³H-GABA binding¹². We suggest that stimulation by GABA of BZ receptor binding involves a novel type of a GABA receptor.

Specific BZ receptor binding was determined with previously frozen and extensively washed membrane fractions from rat brain cerebral cortex with 1 nM ³H-diazepam as labelled ligand¹¹. Specific binding of 5.8 nM ³H-GABA to Triton X-100-treated membranes⁷ was measured using a centrifugation assay¹³.

The structural analogues of GABA in Fig. 1 were examined as stimulators of ³H-diazepam binding. In general, the concentrations of GABA analogues required for 50% activation of ³H-diazepam binding (EC₅₀) were higher than those required for 50% inhibition of Na⁺-independent ³H-GABA binding (IC₅₀). A comparison of the ratios EC₅₀:IC₅₀ revealed remarkable differences. Dihydromuscimol, GABA, and 4-amino-3-hydroxybutyric acid, relatively flexible molecules which can adopt a variety of conformations, exhibited the lowest ratios. Muscimol, thiomuscimol, and *trans*-4-aminocrotonic acid, although still flexible molecules but compounds with partially flattened structures, displayed relatively higher affinities for Na⁺-independent ³H-GABA binding sites. Compounds with even more rigid and flattened structures such as isoguvacine and 4,5,6,7-tetrahydroisoxazolo(4,5-c)pyridin-3-ol (THIP) had very little effect on ³H-diazepam binding but were potent inhibitors of Na⁺-independent ³H-GABA binding. On the other hand, isoguvacine and THIP did not display properties of (partial) antagonists as they failed to modify the submaximal stimulation of ³H-diazepam binding induced by 3 μ M GABA or by 1 μ M muscimol (data not shown). These results point to the existence of a distinct GABA recognition site associated with the BZ receptor, as the structural requirements for the activation of BZ receptor binding are different from those for inhibition of Na⁺-independent ³H-GABA binding.

On extending some previous work^{8,9,11} we found that the effects of all GABA analogues which stimulated ³H-diazepam binding could be antagonised by low concentrations of ($\pm$)-bicuculline-methiodide (10 μ M). In contrast, picrotoxin (30 μ M) had no significant effects on basal or GABA-stimulated ³H-diazepam binding.

The proposed new GABA recognition site is different from the GABA high affinity uptake site as well as the Na⁺-independent ³H-GABA binding site, as potent GABA uptake inhibitors such as guvacine, β -alanine and nipecotic acid^{14,15} had no effect on ³H-diazepam binding. In contrast to Na⁺-dependent ³H-GABA binding, stimulation of ³H-diazepam binding was antagonised by bicuculline-methiodide, enhanced by freezing and thawing¹¹ and insensitive to pretreatment of the membranes with phospholipase A⁹.

Compound	Formula	Activation of ^3H -Diazepam binding EC ₅₀ (μM)	Inhibition of ^3H -GABA binding IC ₅₀ (μM)	EC ₅₀ IC ₅₀
(RS)-4,5-Dihydro-muscimol		0.22	0.041	5.4
Muscimol		0.35	0.015	23
GABA		1.15	0.14	8.2
trans-4-Amino-crotonic acid		1.15	0.060	19
Thiomuscimol		1.50	0.15	10
(RS)-4-Amino-3-hydroxy-butyric acid		17.8	2.60	6.9
3-Guanidino-propionic acid		71.0	3.50	20
Imidazola-acetic acid		89.0	1.20	74
Homomuscimol		280	8.0	36
Isoguvacine		>300	0.45	>500
THIP		>300	0.80	>500
Guvacine		>300	>100	—
(RS)-Nipeccotic acid		>300	>100	—
Gabaculine		>300	>100	—

In conclusion, stimulation of ^3H -diazepam binding seems to be the result of a specific interaction of GABA-like drugs with a novel type or different functional state of a GABA receptor. The distinct structural requirements for stimulation of BZ receptor binding might allow the development of new GABA agonists with selective therapeutic actions.

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Fig. 1 Stimulation of ^3H -diazepam binding and inhibition of Na^+ -independent ^3H -GABA binding by GABA analogues. Crude synaptic membranes isolated from rat cerebral cortex were frozen at -20°C for at least 18 h and then washed 5 times by rehomogenisation in 40 vol 50 mM Tris-HCl buffer (pH 7.4)¹¹. Specific ^3H -diazepam binding was determined in triplicates as previously described¹¹. The incubation medium contained membranes (about 250 μg of protein), 1.0 nM ^3H -diazepam in 100 mM Tris-HCl buffer pH 7.4 plus compounds to be tested. At the end of the incubation period (0 $^\circ\text{C}$, 90 min) 5 ml ice-cold buffer (50 mM Tris-HCl, pH 7.4) was added immediately and membranes with bound ^3H -diazepam were trapped on Whatman GF/B glassfibre filters. The filters were instantly washed with additional 5 ml ice cold buffer and specific binding was determined after subtraction of unspecific binding in the presence of excess (3 μM) unlabelled diazepam. In the presence of a saturating concentration of GABA (30 μM) ^3H -diazepam binding was stimulated between 81 and 128% over basal binding. The test compounds were used in at least four concentrations. The following compounds structurally related to GABA (not listed in Table 1) were examined at 100 μM concentration and found to be inactive (less than 10% stimulation) in stimulating specific ^3H -diazepam binding: glycine, taurine, glutamic acid, homocarnosine, baclofen, aminooxyacetic acid, γ -vinyl GABA, γ -acetylenic GABA, δ -aminovaleric acid; 5-amino-valerianic acid and β -alanine were weak activators with EC₅₀ values $>100 \mu\text{M}$. For the determination of specific ^3H -GABA binding crude synaptic membranes from rat cerebral cortex were frozen at -20°C for at least 24 h and treated for 30 min at 37°C with 0.01% Triton X-100⁷. After centrifugation at 40,000g for 15 min the pellet was dispersed with an Ultra-Turrax in 50 vol 50 mM Tris-citrate buffer (pH 7.1) without Triton X-100. This centrifugation was repeated twice. For specific ^3H -GABA binding¹³ aliquots of membranes were incubated in quadruplicate at 4°C for 5 min in 0.5 ml 50 mM Tris citrate buffer (pH 7.1) containing 5.8 nM ^3H -GABA, alone, or in the presence of 4 mM GABA, or in the presence of at least four concentrations of compounds to be tested. After incubation, the reaction was terminated by centrifugation for 10 min at 40,000g. The supernatant was discarded, the pellet rapidly rinsed with cold H_2O and dissolved in NCS (Amersham) solubiliser before counting. The a.c.i.m.s of ^3H -GABA or ^3H -diazepam binding measurements at a single drug concentration were less than 4%. IC₅₀ and EC₅₀ values were determined from log-probit concentration curves and were reproducible within $\pm 15\%$ of the values given (Means of 2-4 separate determinations).

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Regulation of muscle acetylcholine receptor synthesis *in vitro* by cyclic nucleotide derivatives

STRONG evidence indicates that muscle activity represses the synthesis of extra-synaptic acetylcholine receptor (AChR) in vertebrate skeletal muscle—(1) electrical stimulation of adult denervated muscle prevents the development of acetylcholine supersensitivity^{1,2} and blocks the incorporation of radiolabelled amino acids into the AChR protein³; (2) *in vitro*, electrical stimulation of embryonic myotubes also causes a decrease in the rate of AChR synthesis; moreover, the abolition of the spontaneous contraction of the myotubes by tetrodotoxin (TTX)

leads to an increase of newly synthesised receptor molecules⁴⁻⁶; (3) chronic paralysis of the neuromuscular junction by cholinergic antagonists increases the synthesis of extrasynaptic AChR in adult and embryonic myofibre⁷⁻⁹. In addition, it has been claimed that 'trophic' factors other than the neurotransmitter acetylcholine¹⁰⁻¹⁴ regulate the level of extrasynaptic AChR. We show here that the synthesis of AChR in cultured embryonic myotubes is modified by cyclic nucleotide derivatives, and we suggest that cyclic nucleotides may serve as intracellular signals¹⁵ in both activity-mediated and neurotrophic control of extrasynaptic AChR synthesis.

Myoblasts were obtained from the hind limbs of 11-d-old chick embryos by mechanical dissociation¹⁶, seeded in gelatin-coated plastic dishes and cultivated in a 3:1 mixture of Dulbecco's modified Eagle's medium and medium 199 (Gibco) containing 10% horse serum (Gibco) and 1% chick embryo extract¹⁷. From 48 to 96 h of culture, the cells were treated with 10^{-5} M cytosine arabinoside to minimise fibroblast proliferation¹⁸. After the second day, the media were changed daily. For the quantitation of cell surface AChR sites, the myotubes were labelled with 10 nM ^{125} I- α -bungarotoxin for 1 h at 37°C, washed three times with phosphate-buffered saline, solubilised in 1 M NaOH containing 1% Triton X-100, and the ^{125} I-radioactivity bound per culture dish was determined in a γ -counter. All toxin binding data were corrected for nonspecific binding determined in the presence of 10^{-5} M decamethonium (always below 3% of total binding).

Addition of the membrane depolarising agent veratridine¹⁹ to myotubes from 7-d-old cultures significantly decreased the

number of α -bungarotoxin binding sites per culture dish to below control levels (Fig. 1). In the same conditions, and in agreement with other authors⁴⁻⁶, blocking of the myotube electrical activity by TTX increased α -toxin binding. Up to the third or fourth day in culture, veratridine and TTX (data not shown) had little effect on AChR accumulation; presumably, at that stage the cells have not developed action potential sodium channels sensitive to these compounds.

In 2- and 7-d-old cultures, dibutyryl cyclic GMP decreased, whereas dibutyryl cyclic AMP and prostaglandin E₁ (PGE₁) increased the number of α -bungarotoxin binding sites per culture dish (Fig. 1). On the other hand, 4×10^{-4} M butyrate had little if any effect on AChR expression (Table 1). Thus, the effect of dibutyryl cyclic nucleotides is nucleotide specific.

Figure 2 shows the concentration dependence of the inhibiting action of dibutyryl cyclic GMP on AChR accumulation; half-maximal inhibition occurred at $\sim 7 \times 10^{-5}$ M dibutyryl cyclic GMP. On the other hand, the half-maximal increase in α -toxin binding sites caused by dibutyryl cyclic AMP required concentrations of $1.3\text{--}1.5 \times 10^{-4}$ M (data not shown).

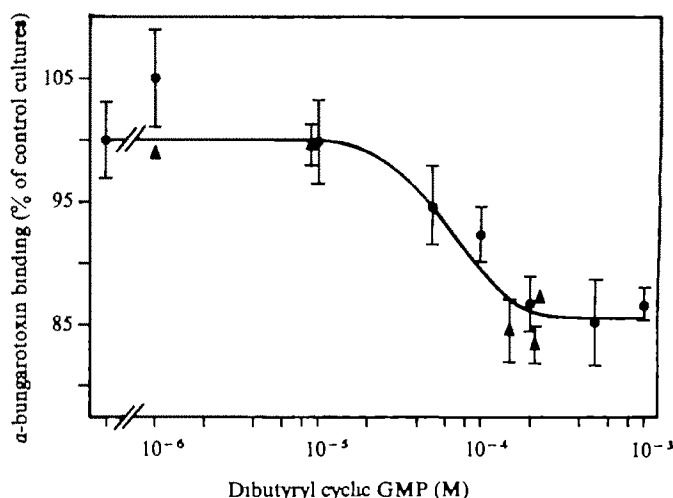


Fig. 2 Effect of increasing concentrations of dibutyryl cyclic GMP on the number of α -bungarotoxin binding sites in 4-d-old muscle cell cultures. Muscle cultures prepared as in Fig. 1 were treated with the indicated concentrations of dibutyryl cyclic GMP from 48 to 96 h of culture. ● and ▲, data from different independent experiments. The bars correspond to ± 1 s.e.m. ($n = 3\text{--}4$).

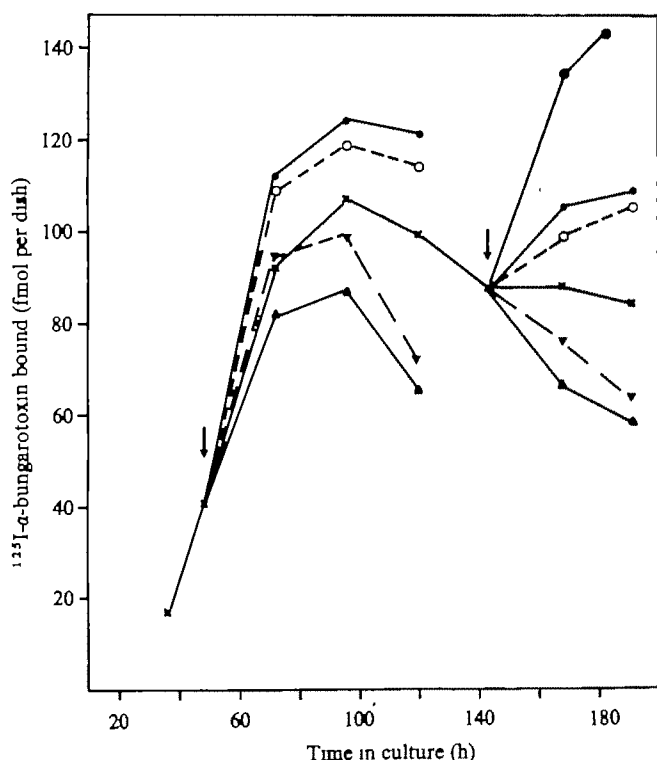


Fig. 1 Effects of veratridine, TTX, dibutyryl cyclic nucleotides and PGE₁ on the evolution of ^{125} I- α -bungarotoxin binding sites in muscle cultures. Myotubes were seeded at a density of 6×10^5 cells in a large number of 35-mm culture dishes. After 2 and 7 d in culture, the different reagents were added to several independent dishes. At the concentrations used, none of the additions had significant effects on the morphological differentiation of the cells. Culture condition: x, control; ▼, 1.5×10^{-6} M veratridine; ⊗, 10^{-6} M TTX; Δ, 2×10^{-4} M dibutyryl cyclic GMP; ●, 2×10^{-4} M dibutyryl cyclic AMP; ○, 10^{-5} M PGE₁. Each point represents the mean of at least two to three culture dishes.

Two alternative mechanisms may explain the effect of dibutyryl cyclic nucleotides on AChR levels: a change in the rate of AChR synthesis, or a regulation of its rate of degradation. Because of the great stability of the α -bungarotoxin-AChR complex, the degradation of AChR can be followed after pulse-labelling of the myotubes with ^{125} I- α -bungarotoxin, the loss of bound ^{125} I-radioactivity being a measure of intracellular degradation of the AChR protein^{20,21}. Figure 3 shows that dibutyryl cyclic AMP or dibutyryl cyclic GMP do not significantly alter the rate of AChR degradation in cultured muscle cells. Therefore, these cyclic nucleotide derivatives regulate the synthesis of AChR or its insertion into the cytoplasmic membrane.

In another series of experiments, pre-existing AChR sites were blocked with unlabelled α -bungarotoxin and the appearance of new AChR monitored by ^{125} I- α -bungarotoxin binding. When these experiments were carried out using young myotubes (between 2 and 3 d *in vitro*) cultures treated with dibutyryl cyclic GMP bound less and those treated by dibutyryl cyclic AMP or PGE₁ bound more ^{125} I- α -bungarotoxin than the untreated controls (Table 1a). As expected, veratridine had no significant effect. Seven-day-old myotubes produced similar

Table 1 Accumulation of ^{125}I - α -bungarotoxin binding sites after saturation of pre-existing AChR sites with unlabelled α -bungarotoxin

Culture condition	Concentration (M)	^{125}I - α -bungarotoxin bound (c.p.m. $\pm$ s.e.m. per dish)	n
a, 2-d-old muscle cultures			
Control	—	44,778 $\pm$ 3,420	8
Veratridine	1.5×10^{-6}	41,062 $\pm$ 1,396	5
Dibutyryl cyclic GMP	2×10^{-4}	32,413 $\pm$ 2,486	5
Dibutyryl cyclic AMP	2×10^{-4}	56,676 $\pm$ 4,848	5
PGE ₁	10^{-5}	59,333 $\pm$ 591	3
Butyrate	4×10^{-4}	46,536 $\pm$ 4,100	3
b, 7-d-old muscle cultures			
Control	—	15,023 $\pm$ 1,412	7
Veratridine	1.5×10^{-6}	8,688 $\pm$ 1,318	4
TTX	10^{-6}	25,799 $\pm$ 1,624	6
Dibutyryl cyclic GMP	2.5×10^{-4}	7,772 $\pm$ 339	4
Dibutyryl cyclic AMP	2.5×10^{-4}	18,868 $\pm$ 568	4
TTX + veratridine	$10^{-6} + 1.5 \times 10^{-6}$	21,469 $\pm$ 2,466	5
TTX + dibutyryl cyclic GMP	$10^{-6} + 2.5 \times 10^{-4}$	8,473 $\pm$ 393	5
TTX + dibutyryl cyclic AMP	$10^{-6} + 2.5 \times 10^{-4}$	33,260 $\pm$ 1,676	4
Veratridine + dibutyryl cyclic GMP	$1.5 \times 10^{-6} + 2.5 \times 10^{-4}$	8,001 $\pm$ 588	5
Veratridine + dibutyryl cyclic AMP	$1.5 \times 10^{-6} + 2.5 \times 10^{-4}$	11,155 $\pm$ 847	4
Butyrate	4×10^{-4}	15,978 $\pm$ 1,810	3

A large set of identical embryonic chick muscle cultures was prepared by seeding 6.8×10^5 cells per 35-mm culture dish. After 40 h in culture, α -bungarotoxin was added to one-third of the dishes at a final concentration of $0.2 \mu\text{g ml}^{-1}$, and the cells were incubated at 37°C for 8 h. The medium was then removed, and the cells washed free of unbound α -bungarotoxin. After the addition of fresh medium and the indicated reagents, the cells were cultivated for a further 24 h before the determination of ^{125}I - α -bungarotoxin binding. The remaining cultures were grown for 7 d and then subjected to the same experimental protocol as described above for the 2 d-old cells. n, Number of culture dishes.

results, except that veratridine became almost as potent as dibutyryl cyclic GMP in reducing the appearance of new surface α -bungarotoxin sites (Table 1b). Furthermore, in these spontaneously contracting cells the TTX-induced increase in the appearance of α -bungarotoxin sites was completely abolished by dibutyryl cyclic GMP and partially antagonised by veratridine; in contrast, the combination of veratridine and dibutyryl

cyclic GMP did not lower the number of α -bungarotoxin binding sites below the value observed with dibutyryl cyclic GMP alone. These observations suggest that membrane depolarisation by veratridine and dibutyryl cyclic GMP regulates AChR synthesis through a common intracellular mechanism. On the other hand, the effect of dibutyryl cyclic AMP superimposes on those obtained with both TTX and veratridine, and so probably involves a different regulatory pathway.

Further evidence for different mechanisms of action for dibutyryl cyclic GMP and dibutyryl cyclic AMP comes from measurements of total protein synthesis (Table 2). Veratridine, TTX and dibutyryl cyclic GMP did not significantly modify the incorporation of ^{35}S -methionine into total myotube protein, but dibutyryl cyclic AMP increased the latter by about 30% over control. Thus, the increase of AChR synthesis by this cyclic AMP analogue might be due to a general enhancement of protein synthesis, whereas its blocking by dibutyryl cyclic GMP seems restricted to this particular (or to a few) protein(s).

Our experiments therefore demonstrate that in cultured myotubes, dibutyryl cyclic GMP (1) represses AChR synthesis in a similar fashion to the depolarising drug veratridine, and (2) abolishes the development of 'acetylcholine supersensitivity' induced by TTX. Cyclic GMP therefore seems to be a suitable candidate for the intracellular messenger of the activity-mediated control of extrasynaptic AChR synthesis. Recent experiments by Beam *et al.*²² with invertebrate and vertebrate nerve-muscle preparations have shown that both nerve and direct electrical stimulation raise the content of cyclic GMP in the adult muscle. In invertebrates, the latter process is Ca^{2+} -dependent²². Interestingly, in chick myotubes D-600, a blocker of Ca^{2+} -channels, stimulates AChR synthesis²³. Moreover, experiments carried out with myotubes from a dystrophic strain of mouse indicate that mechanical contraction is not required for the regulation of AChR synthesis²⁴. It is therefore tempting to speculate that the regulation of AChR synthesis by muscle electrical activity involves successively: (1) the opening of TTX- or veratridine-sensitive Na^+ channels causing an entry of Ca^{2+} and therefore an increase of the cytoplasmic Ca^{2+} concentration; (2) the activation of guanylate cyclase by Ca^{2+} (or the inhibition of a phosphodiesterase); (3) the repression of AChR synthesis by cyclic GMP. Thus, both Ca^{2+} and cyclic GMP would correspond to the previously postulated 'shut off' factors¹⁴.

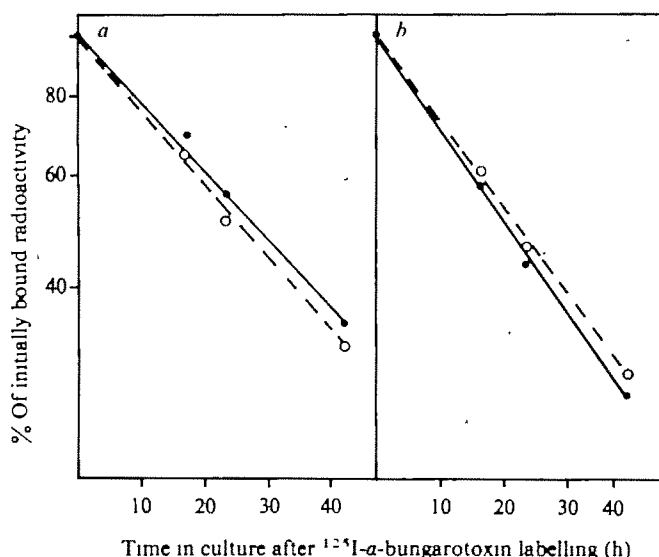


Fig. 3 Loss of ^{125}I - α -bungarotoxin binding sites in muscle cultures treated with dibutyryl cyclic nucleotides. 2-d-old muscle cultures prepared as in Fig. 1 were pretreated for 42–50 h with $2 \times 10^{-4}\text{M}$ dibutyryl cyclic GMP or dibutyryl cyclic AMP, respectively. The treated and parallel control cultures were then labelled by the addition of ^{125}I - α -bungarotoxin to a final concentration of 10 nM . After 1 h at 37°C , the cells were washed free of unbound toxin and cultivated in new medium containing the indicated additions. At different time intervals, duplicate dishes were removed, and the amount of bound ^{125}I - α -bungarotoxin radioactivity was determined. a: ●, Control; ○, $2 \times 10^{-4}\text{M}$ dibutyryl cyclic AMP; b: ●, control; ○, $2 \times 10^{-4}\text{M}$ dibutyryl cyclic GMP.

Table 2 Effect of TTX, veratridine and dibutyryl cyclic nucleotides on protein synthesis of chick embryonic myotubes in culture

Culture condition	Trichloroacetic acid-insoluble radioactivity (c.p.m. per dish $\pm$ s.e.m.)
Control	3,438 $\pm$ 240
Veratridine	3,452 $\pm$ 249
TTX	3,352 $\pm$ 60*
Dibutyryl cyclic GMP	3,428 $\pm$ 98
Dibutyryl cyclic AMP	4,442 $\pm$ 478

7-d-old muscle cultures prepared as described in Table 1 were treated with the above listed reagents for 20 h (concentrations identical to those listed in Table 1b); after 15 h, medium and additions were renewed. Then ^{35} S-methionine was added to the medium to a final concentration of $3 \mu\text{Ci ml}^{-1}$, and incorporation was allowed to proceed for 2 h. The cells were washed twice with phosphate-buffered saline, extracted in 1 M NaOH containing 5 mM L-methionine, and the trichloroacetic acid-insoluble radioactivity in aliquots of the extracts was determined by a glass fibre filter assay procedure. Number of dishes $n = 4$ or 5 except * $n = 2$.

As far as the effect of cyclic AMP and its derivatives is concerned, the following physiological implications might be considered. First, in cultures of myoblasts the level of intracellular cyclic AMP reaches a peak level shortly before fusion²⁵. The activation of adenylate cyclase may thus be essential for the coordinated induction of differentiated functions, including AChR synthesis in post-fusion myotubes²³. Second, our finding that AChR synthesis is regulated by compounds such as PGE₁ which change the intracellular levels of cyclic AMP offers a new approach to the old problem of 'neurotrophic' control of extra-synaptic acetylcholine sensitivity. Many extracellular factors may modulate the intracellular pool of cyclic AMP and thus superimpose on the cyclic GMP-mediated regulation of AChR by myofibre activity. The suppression of ACh supersensitivity by neural factors other than the neurotransmitter may, for example, result from the secretion of substances which inhibit adenylate cyclase (for example, insulin-like molecules) by the respective motor nerve terminals. In any case, the modulation of AChR levels by changes in the intracellular concentration of cyclic AMP probably represents a non-selective regulatory mechanism which may be connected with the general differentiation and maintenance of myofibre properties.

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Activation of steroid hormone-receptor complexes in intact target cells in physiological conditions

AT physiological temperatures glucocorticoids^{1–5}, in common with other steroid hormones⁶, initially interact with normal target cells such as the rat thymocyte by forming hormone-receptor complexes that seem first to be located in the cytoplasm and then rapidly become bound to the nucleus. At low temperatures (0–4 °C) the hormones, when incubated either with target cells or with cytosols from such cells, form 'non-activated' complexes that do not bind to nuclei; subsequent warming of the cells^{1–5}, or of the cytosols together with nuclei^{2,7–11}, leads to the formation of nuclear-bound complexes. As shown originally with oestrogens¹² and later with glucocorticoids^{2,7–18,13}, if cytosols with non-activated complexes formed at 0–4 °C are warmed to 20–37 °C in the absence of nuclei, the complexes become 'activated', and will then bind to nuclei even at low temperatures. Activation, which can also be brought about by increased ionic strength^{7–9,13}, gel filtration, dilution and other treatments¹⁴, has been studied extensively and is known to be accompanied by several changes other than enhanced affinity for nuclei. For glucocorticoid-receptor complexes the changes include enhanced affinity for DNA^{7,10,13–15} and altered mobility on phosphocellulose¹⁶, DEAE-Sephadex¹⁷ and DEAE-cellulose¹⁸ columns. This last property is the one we have used in our experiments. Despite the many published studies on activation, formation of non-activated complexes and subsequent activation have apparently never been demonstrated in cells or cytosols exposed to steroids at normal body temperatures only. Consequently, it is not known whether non-activated complexes and activation have any significant role in physiological conditions, and at least one well known scheme of steroid hormone action¹⁹ does not include the activation step. It is therefore possible that when steroid hormones bind to cytoplasmic receptors at physiological temperatures they immediately form activated complexes. The results described here show that for glucocorticoids this alternative can be excluded: when these steroids interact with receptors in rat thymus cells at 37 °C they initially form non-activated complexes, and subsequently give rise to activated complexes.

Thymus cells from adrenalectomized rats were prepared and incubated in Krebs-Ringer bicarbonate buffer (0.3–0.4 ml packed cells per ml cell suspension) with radioactive steroids using procedures described elsewhere²⁰. Cytosols containing the cytoplasmic steroid-receptor complexes from these cells were

obtained as supernatants following centrifugation (10 min, 2,000g, 3 °C) of broken-cell preparation obtained by diluting the cell suspensions sixfold in 1.5 mM MgCl₂ at 0–3 °C (refs 4, 5, 21). The MgCl₂ solution contained dextran-coated charcoal to adsorb free steroid^{5,21}. DEAE cellulose columns (0.7 cm diameter, 6 ml bed volume) were prepared with Whatman DE-52 cellulose that had been degassed under vacuum while suspended in 300 mM KH₂PO₄ and then washed with starting buffer (5 mM potassium phosphate, pH 7.4, with 0.5 mM dithiothreitol). All column procedures were carried out at 3 °C. Cytosols were applied as 0.7-ml aliquots. Each column was first eluted with 15 ml starting buffer to remove free steroid. This was followed by a 120-ml linear gradient from 5 mM to 300 mM potassium phosphate (pH 7.4) with 0.5 mM dithiothreitol¹⁸. The eluates were collected in 3-ml fractions, from which aliquots were taken for assay of radioactivity. Recovery in the eluates was 85–100% of applied radioactivity.

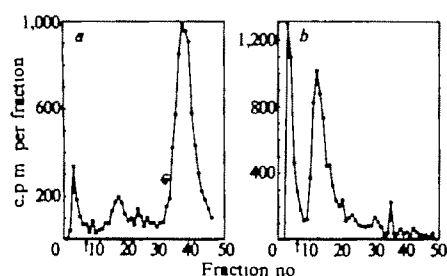


Fig. 1 DEAE-cellulose chromatography of cytosols containing predominantly non-activated (a) and activated (b) dexamethasone-receptor complexes. For the non-activated complex a cytosol was prepared from rat thymus cells incubated for 120 min with 7 nM [6,7-³H(N)]dexamethasone (specific activity, 33–40 Ci mmol⁻¹, NEN) at 0 °C, and chromatographed as described in the text. For the activated complex a similar cytosol was chromatographed after warming for 15 min at 25 °C. Numbers on the ordinate give the c.p.m. in the 3-ml fractions. The arrows on the abscissae indicate the start of the gradient elution. The peak to the left of the arrow, which probably consists of free steroid, was eluted with starting buffer.

The results in Fig. 1 on non-activated and activated ³H-dexamethasone-receptor complexes from rat thymus cells confirm and extend the results of Sakaue and Thompson¹⁸ on triamcinolone acetonide-receptor complexes from cytosols of rat liver, thymus and other tissues. As in their experiments the unwarmed cytosol, containing predominantly non-activated complex, gives a major peak on DEAE-cellulose chromatography that is eluted late in the gradient (fractions 31–45; following Sakaue and Thompson, we refer to this peak as Peak II). After warming to 25 °C for 15 min the cytosol gives a major peak eluted early (fractions 8–20; Peak I). The peak eluted with starting buffer (from fraction 0 to the arrow, Fig. 1) probably consists of unbound steroid, as the material in these fractions is adsorbed by dextran-coated charcoal, and in contrast to receptor-bound steroid does not bind to hydroxyapatite²². If the initial incubation with ³H-dexamethasone includes 10⁻⁶ M unlabelled dexamethasone, Peaks I and II disappear and the peak eluted with starting buffer is diminished. Sakaue and Thompson¹⁸ found that the steroid-receptor complex in Peak I binds much more efficiently to nuclei, chromatin and DNA-cellulose than that in Peak II; consistent with this, we have previously found that the complexes in warmed cytosols bind more efficiently to nuclei and DNA-cellulose than those in unwarmed cytosols^{2,15}. Our observations thus support Sakaue and Thompson's conclusion that Peaks I and II, respectively, represent activated and non-activated complexes.

The question of whether non-activated complexes are formed in physiological conditions can therefore be reduced to whether the complex initially formed when thymus cells at 37 °C are exposed to dexamethasone is eluted on DEAE-cellulose columns as the non-activated Peak II or as the activated Peak I.

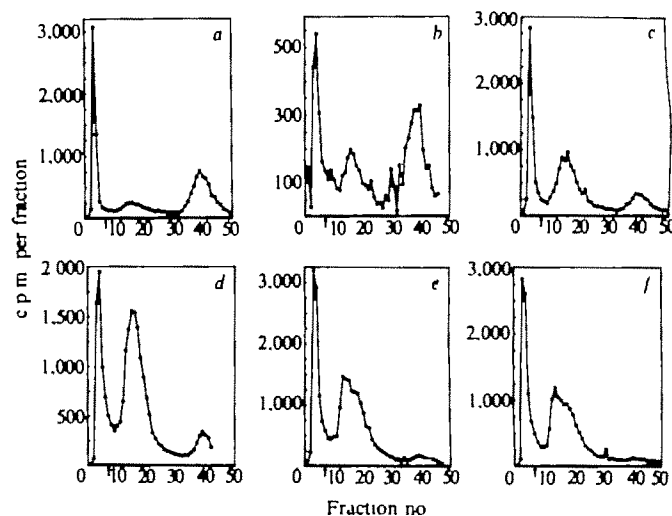


Fig. 2 Time course of formation of non-activated and activated dexamethasone-receptor complexes in rat thymus cells at 37 °C after addition of ³H-dexamethasone. Rat thymus cells incubated at 37 °C were exposed to ³H-dexamethasone for the times indicated. Incubations were terminated by a sixfold dilution of the cell suspension in 1.5 mM MgCl₂ at 0 °C, after which cytosols were prepared and chromatographed on DEAE-cellulose columns as described in the text. Each time of exposure represents a separate experiment, with free ³H-dexamethasone at concentrations of 50–100 nM. Ordinates and abscissae have the same meaning as in Fig. 1. Activated and non-activated complexes are associated, respectively, with the peaks at fractions 10–20 (Peak I) and 30–50 (Peak II). a, 0.25 min; b, 1 min; c, 2.5 min; d, 5 min; e, 15 min, f, 30 min.

The results in Fig. 2, which we have obtained consistently in several such experiments and have also confirmed using ³H-cortisol, show clearly that the non-activated complex is the first to appear. It is almost the only form present after 0.25 min, but is rapidly replaced over the next few minutes by activated complex and after 30 min is nearly undetectable. Figure 2 gives only the relative amounts of Peak I and Peak II at each time, but we have previously shown that in these conditions total cytoplasmic complex, that is, the sum of Peaks I and II, is formed with a half-time of about 1 min and reaches a fairly steady level by 5 min (ref. 5).

Thus, we conclude that the non-activated complex does have an important role in physiological conditions, apparently serving even at 37 °C as an obligatory intermediate in the initial formation of activated complex. In addition, the experimental approach shown in Fig. 2 provides kinetic and steady-state parameters on receptor transformations in intact cells that must be satisfied by any realistic model of receptor activation. Most models to date have been based on results obtained with cell-free systems. For example, it may be difficult to reconcile our results with a recent model of reversible activation, based on experiments with rat liver cytosols. In these, glucocorticoid-receptor complexes reach equilibrium with approximately equal amounts in the non-activated and activated forms, the latter form assayed by ability to bind to nuclei^{23,24}. In this connection, we have preliminary results suggesting that Peak I contains a minor component with little affinity for DNA-cellulose; we do not yet know whether this component is an experimental artefact or a third normal form of the cytoplasmic complex.

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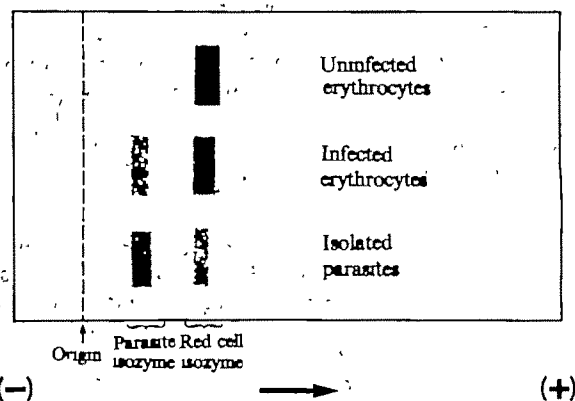


Fig. 1 Schematic representation of starch gel electrophoresis of lysates derived from normal and *P. berghei*-infected mouse erythrocytes and from isolated *P. berghei* parasites. Gels were stained for glutathione reductase (GR) activity with 2,6-dichlorophenol indophenol (DCIP) and 3-(4,5-dimethyl-thiazolyl-2)-2,5-diphenyl tetrazolium (MTT) in the presence of NADPH and GSSG¹³. Simultaneous staining of the other half of the gel with DCIP and MTT in the presence of NADPH but in the absence of GSSG resulted in no detectable staining, confirming that these isozymes represent GR. Note the appearance of a novel isozyme of GR in lysates from isolated parasites. The small amount of residual host cell GR in the latter sample is probably due to contamination of the isolated parasites with trapped host cell cytoplasm.

P. berghei (NYU-2 strain) was maintained in male Swiss White mice as previously described⁷. Blood from heavily infected mice (>70% of red cells infected) was collected after ether anaesthesia by axillary incision and aspiration into heparin. White cells and platelets were removed by filtration through powdered cellulose columns¹⁰. GSH¹¹ and glutathione reductase¹² were assayed as before.

P. berghei infected erythrocytes had more than twice the GSH content of uninfected red cells (Table 1). This difference was not an artefact due to changed size or haemoglobin content of

Dependence of plasmodial glutathione metabolism on the host cell

ONE inherited alteration of human red cell metabolism—quantitative deficiency of the X-linked enzyme glucose-6-phosphate dehydrogenase (G-6-PD)—has attained high frequency in many areas of endemic malaria and may protect against fulminant *Plasmodium falciparum* in some unknown fashion¹⁻⁵. G-6-PD catalyses the first step of the pentose phosphate pathway which provides reduced NADPH necessary for conversion of oxidised to reduced glutathione (GSSG → GSH)⁶. Erythrocytes deficient in G-6-PD are inefficient in generating NADPH and, when exposed to oxidants, lose GSH, accumulate oxidised haemoglobin and are destroyed by the reticuloendothelial system⁶. We hypothesised that, if the malaria parasite were to use red-cell NADPH for parasitic functions, the G-6-PD-deficient erythrocyte might be incapable of maintaining adequate GSH content. This would predispose the cell to premature destruction before the parasite matured, thereby limiting the severity of infection⁷⁻⁹. In exploring this hypothesis, we have investigated factors affecting GSH metabolism in malaria-infected mouse erythrocytes. We find that *P. berghei* malaria may utilise host-cell NADPH for the maintenance of parasite GSH. These observations may help elucidate both the parasite-induced red cell oxidant damage⁷ and the mechanism whereby G-6-PD deficiency protects against fulminant malaria infection.

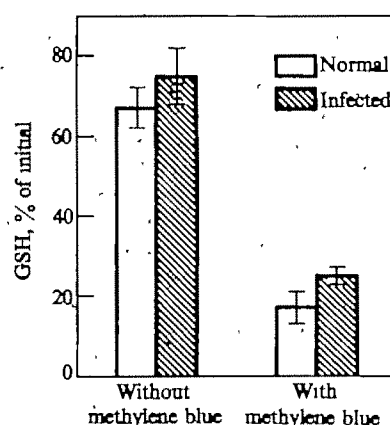


Fig. 2 Regeneration of GSH in whole erythrocytes previously treated with diamide. Suspension of uninfected and infected erythrocytes, haematocrit approximately 2 volumes per cent, were incubated with 0.25 mM diamide in 0.154 M NaCl for 30 s at 4 °C and washed in 0.154 M NaCl. Diamide-treated cells were resuspended to a haematocrit of approximately 2 volumes per cent in Hank's balance salt solution with 20 mg ml⁻¹ glucose, pH 7.4, with and without 500 μM methylene blue. Results are expressed as per cent of initial GSH concentration and represent the mean ± s.e.m. for 11 separate determinations.

infected cells and was apparent whether GSH concentrations were expressed per g haemoglobin, per ml packed red cells (Table 1) or even per 10^{10} red cells (data not shown). Nor was the difference explicable on the basis of decreased red cell age; our *P. berghei* infections last only 5–6 days from inoculation to death with no significant elevations in reticulocyte count.

To explore the possibility that the incremental GSH within infected cells might be parasite rather than erythrocyte GSH, we separated host cell cytoplasm from intact parasites by ammonium chloride lysis¹³. GSH analyses revealed that infected red-cell cytoplasm had normal GSH content, whereas isolated parasites contained most of the GSH found in whole infected cells (Table 1). We then investigated how the glutathione within the parasite might be maintained in the reduced state. Glutathione is constantly being oxidised and the reduction of GSSG to GSH is catalysed by glutathione reductase (GR), for which NADPH is the most important and, perhaps, sole substrate⁶.

Table 1 Concentrations of GSH and activity of glutathione reductase in uninfected and *P. berghei*-infected mouse erythrocytes, and in isolated parasites and host-cell cytoplasm separated by ammonium chloride lysis¹⁴

Group	N	GSH	
		$\mu\text{M per g Hb}$	$\mu\text{M per ml RBC}$
Uninfected red cells	20	5.6 (± 0.2)	1.9 (± 0.1)
Infected red cells	16	16.3 (± 1.5)*	3.9 (± 0.3)*
Group	N	Glutathione reductase (GR)	
		IU per g Hb	IU RBC
Uninfected red cells	15	14.7 (± 0.6)	4.6 (± 0.2)
Infected red cells	11	35.4 (± 4.0)*	7.8 (± 0.5)*
		GSH	GR
		$\mu\text{M per g protein}$	IU per g protein
Isolated parasites	10	12.0 (± 0.4)	15.5 (± 0.7)
Infected red cell cytoplasm	10	5.6 (± 0.3)	20.9 (± 2.3)

Figures in parentheses represent 1 s.e.m. Because the assay which we routinely use for GSH analysis utilizes 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), which may detect other reducing substances, we confirmed the identity of the GSH in infected cells and isolated parasites by chromatography on Biogel P-2 which showed that >95% of the DTNB reacting substance had a molecular weight similar to GSH.

* $P < 0.001$ when compared to values from uninfected red cells (Student's *t* test, two tailed).

Comparison of NADPH-dependent GR activities within uninfected and infected erythrocytes revealed marked increases within infected cells (Table 1). Although some mammalian forms of GR may use NADH as cofactor¹⁴, even at >1 mM NADH we were unable to detect GSSG reduction by either parasite or host cell GR. Starch gel electrophoresis of lysates derived from normal and infected erythrocytes, and from isolated parasites, indicated the presence of a unique parasite GR cathodal to the single host-cell isozyme (Fig. 1). This isozyme of GR in infected red cells is unlikely to be a modified form of the host-cell enzyme because of the large increase in enzyme activity within infected erythrocytes. This increased activity could not be explained by diminished red cell age because, as indicated earlier,

our *P. berghei* infections do not induce reticulocytosis. Furthermore, others have reported similar elevations of both GSH and GR activity in mouse erythrocytes infected with an entirely different species of malaria—*P. vinckei*¹⁶.

However, the source of the NADPH required for the activity of parasite GR was not clear. *P. berghei* (and other forms of malaria), lacks detectable G-6-PD activity^{17–19} and is, therefore, unable to utilise an intact pentose phosphate pathway for the generation of NADPH. Despite this, pentose shunt metabolism is accelerated in parasitised erythrocytes ($5\text{--}10\times$ normal)^{20,21}, although the activity of this pathway is low or absent in the parasite itself¹⁸. These observations suggested that the parasite might be oxidising red cell NADPH, perhaps through NADPH-linked reduction of parasite GSSG. The excess NADP so generated would, predictably, accelerate the host-cell pentose shunt⁶. To test the possibility that parasite GSSG might be reduced by external NADPH, we isolated parasites¹³ and reversibly oxidised parasite GSH to GSSG with diazene-dicarboxylic acid-bis-(*N,N*-dimethylamide) (diamide)²². As shown in Table 2, following brief treatment of parasites with diamide, incubation with exogenous NADPH supports the regeneration of GSH. This GSH regenerated during incubation must occur within the parasite because extensive washing of parasites does not result in a significant decrement in GSH concentration. Note

Table 2 Regeneration of GSH in diamide-treated isolated *P. berghei*

Substrate	Number	GSH, % of initial
None	20	17 (± 6)
NADPH (500 μM)	30	67 (± 14)*
NADPH (50 μM)	6	58 (± 11)*
NADP (500 μM)	8	16 (± 8)
D-Glucose (11 mM)	8	17 (± 4)
G-6-P (500 μM)	6	18 (± 8)
6-PG (500 μM)	6	19 (± 7)
G-6-P (500 μM) + NADP (500 μM)	6	38 (± 12)
6-PG (500 μM) + NADP (500 μM)	6	43 (± 14)

Suspensions of isolated parasites, approximately 10 mg protein per ml, were incubated with 0.7 mM diamide in 0.154 M KCl containing 20 mM Tris buffer, pH 7.4, at 4 °C for 30 min. The parasites were then washed in the KCl-Tris buffer to remove the unreacted diamide and resuspended to a concentration of approximately 25 mg protein per ml in KCl-Tris buffer. GSH determinations were done before and immediately after diamide treatment, and after 60 min incubation with the various additives at 37 °C. In all experiments, the mean of the initial GSH concentrations was $11.2 \pm 0.3 \mu\text{M}$ per g protein and GSH was undetectable immediately following diamide treatment. Results are expressed as per cent of initial GSH and represent the mean ($\pm$ s.e.m.).

* Significantly different from control (no substrate) at $P < 0.001$ by Student's *t* test, two tailed.

that even NADPH concentrations of 5×10^{-5} M (somewhat below the levels found in normal human erythrocytes⁶) will support reduction of intraparasitic GSSG. The failure of the GSH concentration to return to 100% of the initial values is probably due to diamide-induced damage to the parasite as well as possible loss of GSSG during diamide treatment. The glutathione reduction is specific for NADPH; incubation of isolated parasites with glucose, NADP, glucose-6-phosphate (G-6-P), or 6-phosphogluconate (6-PG) results in no reduction of GSSG (Table 2). Samples incubated with both NADP and G-6-P or 6-PG do regenerate some GSH (Table 2), possibly mediated by host-cell G-6-PD and 6-phosphogluconate dehydrogenase which, like other components of host cell cytoplasm, unavoidably contaminate preparations of isolated parasites.

Other observations indicate that the parasite is directly dependent on erythrocyte metabolism for reduction of GSSG.

In diamide-treated intact infected erythrocytes, glucose supports the regeneration of GSH (Fig. 2) within both host-cell cytoplasm and parasite (data not shown). In contrast, similar incubation of diamide-treated isolated parasites with glucose results in negligible reappearance of GSH (Table 2). Finally, parasite GSSG reduction within whole infected erythrocytes also appears to require NADPH. As shown in Fig. 2, methylene blue (which oxidises intracellular NADPH to NADP^{23,24}) blocks GSSG reduction in intact normal and infected erythrocytes incubated with glucose.

The utilisation of exogenous NADPH for the reduction of parasite GSSG to GSH, as well as the inability of isolated parasites to use glucose for this purpose, indicates an intimate metabolic dependence of the parasite upon the host red cell. The attendant oxidation of erythrocyte NADPH during reduction of parasite GSSG may explain both the increased oxidant sensitivity of infected erythrocytes, and the acceleration of pentose shunt activity within infected cells^{21,22}. Our results also suggest a mechanism whereby G-6-PD deficiency may protect against fulminant malaria infection. The utilisation of NADPH by both the host erythrocyte and malaria parasite would overwhelm the limited ability of G-6-PD deficient red cells to regenerate NADPH. The resultant decrement in cellular GSH may predispose the infected erythrocyte to premature, oxidant-induced destruction. Alternatively, the accumulation of GSSG may inhibit parasite protein synthesis²⁵. In either case, the reliance of the malaria parasite on host-cell NADPH may represent a site of action for new antimalarial drugs.

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Homogeneous sensitivity of human peripheral blood lymphocytes to radiation-induced chromosome damage

THE routine method for detecting genetic damage induced by environmental agents in man *in vivo* is to examine mitotic cells in short-term peripheral blood lymphocyte cultures for evidence of structural chromosome changes^{1,2}. Chromosome radiosensitivity in lymphocytes irradiated *in vivo* and *in vitro* is similar³, and so dose-response relationships have been established for different qualities and quantities of ionising radiations after *in vitro* exposure of blood samples⁴. These curves are used to estimate doses received in cases of accidental overexposure to radiation^{1,2,5} and in prediction of the sensitivity of human germ cells to heritable radiation damage⁶. Their shape is used to predict the effect of dose rate on chromosome aberration frequencies^{7,8} and to elucidate the mechanisms of induction of



Fig. 1 Metaphase cells at first (a) and second (b) mitosis after exposure of whole blood to 200 rad of X rays, incubation in medium containing BUdR, and harlequin staining¹³. d = dicentric, f = fragment. The harlequin-stained cell in second metaphase carries a dicentric and two identical fragments derived from duplication of a single fragment at first mitosis.

Table 1 Dicentric frequencies and proportions of metaphase cells which have undergone more than one mitosis at different times after irradiation of blood cultures from four donors

Donor	Treatment	Fixation time(h)	Metaphase cells at >1st division (%)	Dicentric per 100 cells			Poisson distribution of dicentrics in 1st divisions ^a χ^2 test, P =
				1st Mitoses	2nd Mitoses	All mitoses (not harlequin stained)	
A	200 rad + BUdR	40	3	48			0.70-0.80
		44	2	50			0.50-0.70
		48	2	54			0.10-0.20
		56	22	55			0.80-0.90
		60	35	55			0.50-0.70
	200 rad	40			26	52	
		44				53	
		48				53	
	BUdR	52	17			0	
		52				0	
A	200 rad + BUdR	48	2	43			0.02-0.05*
		56	30	43			0.20-0.30
		64	55	43	28		0.50-0.70
		72	73	43	20		0.50-0.70
		48				38	
	200 rad	72	85			0	
		72				0	
		64				0	
	BUdR	44	0	36			0.50-0.70
		48	0	40			0.20-0.30
B	200 rad + BUdR	52	20	47			0.70-0.80
		56	19	43			0.90-0.95
		60	34	41	14		0.30-0.50
		68	57	35	21		0.90-0.95
		44				38	
	200 rad	48				44	
		48				1	
		52	11			0	
	BUdR	52				0	
		52				0	
C	200 rad + BUdR	48	2	44			0.10-0.20
		56	23	44			0.98-0.99
		64	59	45	29		0.20-0.30
		48				35	
		72	90			0	
	200 rad	64				0	
		64				0	
		48	2	43			0.98-0.99
	BUdR	56	20	43			0.90-0.95
		64	57	42	22		0.90-0.95
D	200 rad + BUdR	72	70	41	27		0.80-0.90
		48				44	
		72	79			0	
		72				0	
		64				0	
	200 rad	48				0	
		72				0	
		64				0	
	BUdR	48				0	
		64				0	

Each value is derived from an analysis of 100 cells.

* A significant departure from a Poisson is expected by chance in the total of 22 samples analysed in these experiments

such aberrations⁸. However, accurate dose-response curves can be obtained only if cells are examined for chromosome damage at the first post-irradiation mitosis, because aberrations decline with successive divisions⁹. Crossen and Morgan¹⁰ used the harlequin-staining technique^{10,12} on phytohaemagglutinin (PHA)-stimulated lymphocytes exposed to bromodeoxyuridine (BUdR) to distinguish cells at first and later mitoses *in vitro* (Fig. 1). They have shown that at the conventional sampling time, about 48 h after stimulation, up to 53% of cells may be in second mitosis. Another problem is that the cells examined in first division at 48 h may not be representative of the entire cell population. Several workers¹³⁻¹⁵ have claimed that lymphocytes with different rates of response to PHA stimulation, undergoing first mitosis at different times after stimulation, carry different frequencies of aberrations. They have interpreted this as a sign that lymphocytes in the peripheral blood constitute a heterogeneous population in terms of chromosome radiosensitivity. If this were true, little significance could be attached to the shape of the dose-response curves. We report here that we have used the harlequin technique to show (1) that in spite of radiation-induced mitotic delay¹⁶ high frequencies of cells in second mitosis may still be found at 48 h, and (2) that there is no difference in aberration frequency in first-division cells sampled at various intervals after irradiation. Correct dose-response curves can therefore now be obtained with this method and any sampling time can be used provided that observations are confined to non-harlequin-stained (that is, first division) cells.

In preliminary experiments, X-ray-irradiated or non-irradiated blood samples were cultured in medium containing BUdR

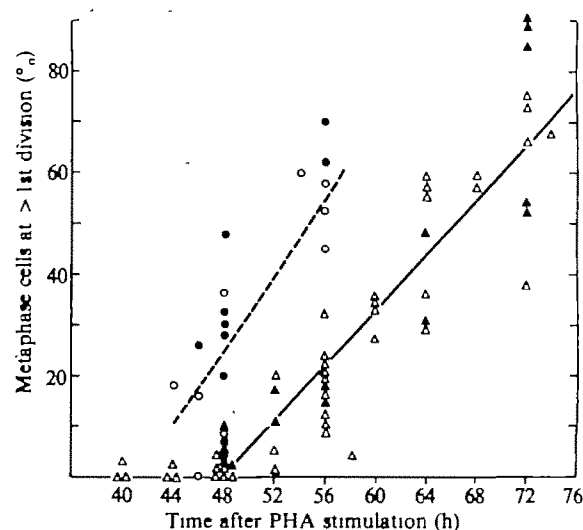


Fig. 2 Proportions of metaphase cells which have undergone more than one mitosis at different times after initiation of cultures of irradiated (150-300 rad of X-rays) or non-irradiated blood. Distinction between cells at first or later mitoses was made after harlequin staining¹² of cells grown in medium (80% Hams F10 + 19% fetal calf serum + 1.0% PHA) containing BUdR at 5 or 10 $\mu\text{g ml}^{-1}$. Cells were exposed to colcemid for 1 h before fixation. Data are from several experiments in which blood from six male donors (aged 21-33 yr) was used. Each point represents the analysis at 100 metaphase cells. Solid and dashed lines have been fitted by eye to the data for BUdR at 10 $\mu\text{g ml}^{-1}$ and 5 $\mu\text{g ml}^{-1}$, respectively. $\circ$, non-irradiated, BUdR 5 $\mu\text{g ml}^{-1}$; ∇ , non-irradiated, BUdR 10 $\mu\text{g ml}^{-1}$; $\triangle$, irradiated, BUdR 5 $\mu\text{g ml}^{-1}$; Δ , irradiated, BUdR 10 $\mu\text{g ml}^{-1}$.

at either 5 or 10 $\mu\text{g ml}^{-1}$, fixed at various times after culture initiation and harlequin stained. After 5 $\mu\text{g ml}^{-1}$, up to 48% of cells were in second mitosis by 48 h (Fig. 2) and 37% even after 300 rad of X rays. BUdR evidently induced some mitotic delay because the frequencies of cells beyond first division (Fig. 2) were, in general, considerably lower after 10 $\mu\text{g ml}^{-1}$ than after 5 $\mu\text{g ml}^{-1}$ (ref. 17). This suggests that in normal culture conditions, without BUdR, there may be even more than 50% of cells at second division by 48 h. Because the magnitude of mitotic delay is dose dependent¹⁶ there will be, at 48 h, a decreasing proportion of cells in second division with increasing dose, which will lead to an exaggeration of the curvature of the dose-response curve when the BUdR method is not used.

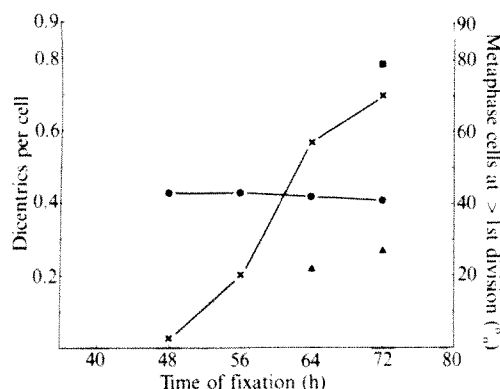


Fig. 3 Dicentric frequencies in cells from donor D (see Table 1) at first (●) and second (▲) mitosis after exposure to 200 rad of X rays. Also shown is the proportion of cells at >1st mitosis (×, irradiated; ■, control) at different sampling times.

To investigate aberration frequencies in first-division cells at different sampling times, whole blood from four healthy male donors (aged 21–33 yr) was exposed to 200 rad of X rays (290 kV, 8 mA, 100 rad min⁻¹) at 37 °C, cultured in medium with 10 $\mu\text{g ml}^{-1}$ BUdR, sampled between 40 and 72 h and harlequin stained. Non-irradiated cells were exposed to 10 $\mu\text{g ml}^{-1}$ BUdR or no BUdR, and some irradiated cells were cultured without BUdR. The results are shown in Table 1 and Fig. 3. There was no significant difference between sampling times in aberration (dicentric) frequencies in first-division cells (donor A, first experiment, $\chi^2_4 = 0.79$, $P = 0.90$ – 0.95 ; donor A, second experiment, $\chi^2_3 = 0$, $P = 1.0$; donor B, $\chi^2_3 = 2.46$, $P = 0.70$ – 0.80 ; donor C, $\chi^2_2 = 0.08$, $P = 0.95$ – 0.98 ; donor D, $\chi^2_3 = 0.063$, $P > 0.99$). BUdR did not induce aberrations (compare BUdR treatment with no treatment in Table 1). As in our preliminary studies (Fig. 2), with 10 $\mu\text{g ml}^{-1}$ BUdR the frequency of second divisions at 48 h was low. BUdR did not influence the frequency of aberrations induced by radiation (compare 200 rad only with 200 rad + BUdR at early fixation times before onset of second mitosis). Dicentric frequencies in second-division (harlequin-stained) cells were approximately half those in first-division cells, as expected if (1) 50% of dicentrics form bridges at first anaphase and 50% “fall-free” (ref. 18), and (2) cells with or without dicentrics at first mitosis are equally capable of undergoing second division. The means of the dicentric frequencies at all sampling times in first division cells from donors A (second experiment) B, C and D were similar, being 43.0, 40.3, 44.3 and 42.3 per 100 cells, respectively, but the frequency from donor A in the first experiment (52.4 per 100 cells) was significantly higher than these at the 5% significance level. The reason for this is not clear and repeat samples from the other donors will be required to assess the importance of this observation.

The distribution of dicentrics between first division cells at each fixation time conforms to a Poisson distribution (Table 1).

This provides further evidence of homogeneity of lymphocytes in chromosome radiosensitivity other than the equality of aberration yields in first-division cells at different sampling times.

We thank Miss Ann R. Currie and Mr J. R. Robinson for technical assistance. This research was supported by grants from the Cancer Research Campaign, the MRC and the International Atomic Energy Authority (Contract 1747).

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Corrigendum

In the letter ‘Nanosecond transient Raman spectra of photolysed carboxyhaemoglobin’ by K. B. Lyons *et al.*, *Nature* **275**, 565–566 (1978), paragraph 6 line 15 and paragraph 8 line 6 reads 0.002 Å not 0.02 Å. On Fig. 1c 3 cm⁻¹ should read 2 cm⁻¹.

Erratum

The following note in proof should have appeared in the Matters Arising item ‘Synergistic chelation therapy or mixed ligand complexes for plutonium and cadmium poisoning?’ by P. M. May and D. R. Williams, *Nature* **278**, 581; We would like to correct an error in our *Nature* paper⁴. In paragraph 4 and in Fig. 3 legend we reported that tablets of EDTA with salicylate or DNPS (Dimaval) protected mice from acute cadmium poisoning given 1 h post-cadmium. This should have read 5 min rather than 1 h as far as complete survival is concerned. Naturally, complete survival is observed 15 min but not 1 h post-cadmium for a single injection of the combination.

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reviews

Physics as natural philosophy

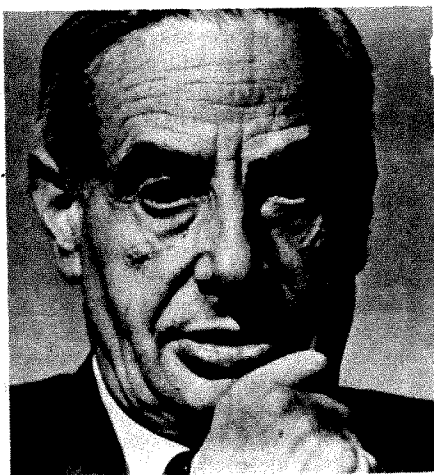
Victor F. Weisskopf

Memoirs of a Physicist in the Atomic Age. By W. M. Elsasser. (Adam Hilger: Bristol, 1978.) \$15.

I HAVE rarely read a memoir of a scientist which is as readable and captivating as this one. Scientists are not usually very good writers, but Walter Elsasser is. My judgement may be biased, as I belong to the same generation of scientists of Jewish origin brought up in the pre-Nazi German environment. The book gives a fascinating and tragic description of his slow and painful recognition as a young man that he did not belong to the society in which he grew up, although its cultural values were deeply ingrained in his thinking and feeling. In some ways he remained German all his life in the sense of Bach, Goethe, Schiller, Beethoven, Helmholtz and Freud. I think this is mainly why Elsasser keeps returning throughout the book to his belief that science should be natural philosophy and not a pragmatic study of nature. He has never felt comfortable in the Anglo-Saxon (or perhaps more typical, American) style of scientific research, although it is not as far from his ideal as he makes out.

In spite of its readability the book has a few shortcomings which will disappoint historians of science. Elsasser was in Göttingen at the time when quantum mechanics was beginning to take shape, but very little can be found in the book of the overwhelming excitement that must have been felt there, or of the discussions between Max Born, Pascual Jordan, James Franck and many others about the foundations of quantum mechanics. Also, too little is said about the enormous influence of Niels Bohr on the development of atomic physics at that time.

Elsasser did not have a happy life. In contrast to many other scientist-refugees, he did not become a full professor at a great university until the very end of his life. He had to work in industrial and governmental laboratories on subjects that were not his own choice, despite the fact that he was the person who initiated important insights into three of the most fundamental problems of physics: the interpretation of the results of the



Elsasser in 1974

Davisson-Kunsman (later Davisson-Germer) experiments as an effect of electron waves; the recognition of a shell-structure in atomic nuclei; the explanation of the Earth's magnetism as a self-generating dynamo in a liquid iron core. All three contributions were published as short notes: they were great flashes of ideas; and it was not Elsasser's style to elaborate details. Indeed, his note on the shell-structure of nuclei was all but forgotten when M. Mayer and H. Jensen rediscovered it 15 years later. They worked it out in detail and received the Nobel prize for their work.

These three contributions of Elsasser should have sufficed to secure him a high position in the scientific community. But he had bad luck all through his life as regards the recognition of his ideas and to his professional career—caused not only by external circumstances but also by his own personality. Elsasser was a difficult and complex character; he describes vividly some of the severe psychological troubles which he encountered in his youth. In spite of all these hardships he never lost his idealism and his devotion to what he calls "conceptual thought"—his interest in the essential and fundamental questions of science rather than in problem solving or empirical data collection.

Perhaps his lack of social success in the scientific community made him supercritical of some personalities and of the character of modern science. He describes the personality of P. Ehrenfest in such dark colours that he is not

recognisable to anyone who had personal contact with this warm-hearted, engaging and stimulating man. It is true that Ehrenfest was sometimes subject to moody periods; obviously a collision happened here between two difficult characters, that prevented Elsasser from recognising the unique humanity and charm of Ehrenfest. Also Hermann Weyl is censured because of his alleged lack of contact with reality in his books on relativity; an accusation which is in no way justified. Homi Bhabha too gets a rather heavy censure when he is accused of "modelling the properties of elementary particles in mathematical terms" and of trying to solve fundamental problems with inadequate means, as many of "those who come from cultures far from the circle of modern Western rationalism". Actually, Bhabha's contributions to particle physics were at least as fundamental as those of any Western physicist.

Elsasser was very much interested in the scientific explanation of the phenomena of life and was very sceptical as to the possibility of a physico-chemical explanation. He wrote three books about the subject, and had to modify his point of view to some extent after the discovery of the DNA double helix. But it is not easy to understand why he found it necessary to denigrate the importance and impact of Schrödinger's pioneering little book *What is Life?*, which has influenced a whole generation of biologists. Elsasser had a very high opinion of Schrödinger in every other respect. But he completely overlooked the fact that Schrödinger introduced for the first time the concept of an "aperiodic crystal", which was later found to exist in the form of DNA. Also, Elsasser seems to assume that the book was written after the discovery of the genetic code.

Altogether the disappointments which Elsasser suffered within the scientific community must have accumulated many prejudices in his thinking. He goes as far as to accuse the life sciences as a whole of being a "denial of science as a creative activity". He says that "scientific research was a process in which observation was related by a reciprocal interaction with thought . . . but now it seemed that in

the life sciences the prevailing approach... has assumed a quite different form"

In physics, he could not approve of the attitude of the younger generation of scientists towards their work: "The modern physics that had emerged... was world apart from the old physics that had for so long been called natural philosophy". Although there may be some truth in what he says about the pragmatic attitude of modern science, his outright condemnation is fundamentally mistaken. The spirit that drives the best of the younger generation—always only the best ones are counted—remains the urge for deeper knowledge and insight into the workings of nature. Maxwell and Darwin would have appreciated modern science if they were still alive.

These exaggerated condemnations do not detract from the intrinsic value of the book. On the contrary, they appear as part of the author's personality and help to bring out the profound predicament of a person brought up in the highly literate and idealistic atmosphere of the well educated central European middle class, when confronted with the spirit (or non-spirit) of the last quarter of our century.

Many episodes during the War are related with great skill. His description of the tensions and problems in F. Joliot-Curie's laboratory during the

German occupation is thoroughly fascinating, as the laboratory served partly as a scientific institution and partly as a centre of the resistance. Unfortunately, he does not mention the heroic role of Professor W. Gentner, who was sent to the laboratory as a German caretaker but, at the risk of his life, kept a helping and protecting hand over Joliot-Curie and his group.

Many deep thoughts and ideas are presented in this book. Elsasser has never shied away from posing the hardest questions and was never discouraged by the difficulties of finding answers. The question of body and mind is often brought up in this book, always illuminating some aspects and indicating the vastness of the problem. Here is one revealing quote: "We tend to look with condescension and pity on that period of history, called the Middle Ages, when men were so concerned with their souls that they often ignored the external world. It seems today that most men are so embroiled in the external world that they have lost sight of the vast dimensions of the world of psyche."

It is a deeply moving book, with some weaknesses, but also with much of the glory that goes with the quest for scientific knowledge. □

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Life from extraterrestrial sources

Colin Pillinger

Lifecloud: The Origin of Life in the Universe. By Fred Hoyle and N. C. Wickramasinghe. Pp. 189 (Dent: London, Toronto and Melbourne, 1978; Harper and Row: New York, 1979) £5.95; \$9.95

OVER the past couple of years a number of articles have appeared in the scientific literature laying the ground work for the Hoyle and Wickramasinghe hypothesis that chemical evolution occurred deep in space in interstellar gas clouds rather than in a 'primeval soup' on the Earth. In the present book they suggest that even very complex biological molecules are produced in this way and contributed to the primitive Earth by cometary impact. They contend that all the comparatively volatile materials in the atmosphere and oceans and even living cells are derived from an extraterrestrial source.

Lifecloud may be summarised as follows. The first three chapters deal with aspects of Darwinian theory. Chapters 4, 5 and 6 provide basic introductions to the functions of cells, the structure of biochemicals and atomic theory, respectively. Chapter 7 discusses how stars are formed, whereas 8 includes a catalogue of the wide range of molecular species in interstellar space now identified by radioastronomy and 9 and 10 are devoted to speculation on the nature of the dust associated with the molecular clouds (mostly the authors' own proposal concerning cellulose). The next two chapters consider the structure of comets and the evidence for biological precursors in meteorites; then come chapters on the birth of the Solar System and the early history of the Earth. Chapters 15 and 16 examine other bodies in our Solar System or associated with the Sun's neighbours as possible abodes for life; Chapter 17 develops the philosophy of the predator in evolution; Chapter 18, entitled "Invasion from the galaxy", is not an appraisal of the much publicised suggestions by the authors that certain epidemic illnesses were brought to Earth by extraterrestrial viruses, but an evaluation of the feasibility of colonising the Universe by space travellers. From the last chapter it is clear that Hoyle and Wickramasinghe favour the armchair

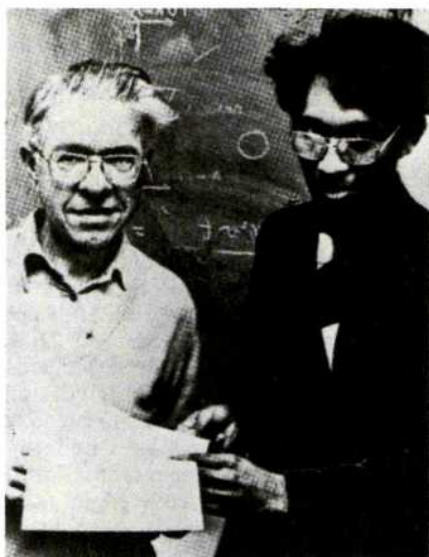
approach of attempting radio communication as an essential first step in exploration.

From the format of the book, it is quite clear that the authors are not writing for specialist readers. The amount of introductory material included suggests that the intended audience may have only a general scientific background. This being the case, in my opinion, the authors should have felt a duty to distinguish clearly what is well founded evidence and what is speculation. Many of their readers will not be equipped to make such a judgement. Bearing in mind that the actual text of the book begins on p 15, and ends on p 174 and the large number of chapters (19) leads to numerous blank or semiblack pages, an attempt has been made to cover an amazing amount of material in probably less than 150 pages. This inevitably means that certain very controversial ideas are presented as fact.

Personally I would have preferred to see a much longer book, more conservatively written and critically reviewing alternatives where they exist. For example, a major theme of the book requires that interstellar dust be identified as cellulose or polysaccharides and spectral evidence is presented to this effect. Most radio astronomers would agree that it is not possible, at present, to decide unambiguously between cellulose and the numerous other materials which have been suggested. Similarly, alternative substances could be responsible for the 2200Å extinction attributed by the authors to $C_6H_5N_3$, but none are discussed. An interesting point, in this respect, is the strength of the evidence required for proof of chemical structure by different scientific disciplines. The organic chemist would consider infrared and ultraviolet spectra, insist on mass spectrometry, like to have nuclear magnetic resonance and attempt coinjection using two gas chromatographic phases. If a natural product was involved its stereochemistry would need to be examined. For some astronomers a near match between comparatively featureless spectra seems to be adequate. Whereas the chemist would concede the difficulties of obtaining data and material for study, perhaps the astronomer could afford to be a little more sceptical. There is a long way to go before the identification of $C_6H_5N_3$ confirms the existence of porphyrins such as $MgC_{48}H_{48}N_4$.

Several instances occur in the book when the evidence presented by the authors needs a much more careful scrutiny than seems to have been the case. On p31, while discussing the possibility of Precambrian microfossils, it is suggested that trace quantities of certain biochemicals have been found associated with the observed structures. No evidence exists to demonstrate that the chemicals in question are due to the microfossils rather than the host rock, whereas strong

Hoyle and Wickramasinghe



arguments favour an interpretation that the samples have been contaminated, not during collection or in the laboratory when great care is taken, but by geological processes. Precambrian rocks contain both classes of compounds and stereoisomers which thermodynamically could not have survived the $1-3 \times 10^9$ yr since rock consolidation. Several groups (for example, Nagy; *Geochim. Cosmochim.* **34**, 525; 1970) were able to show that the porosities of cherts were far in excess of what might be expected, and percolating ground water could easily have transported recently deposited chemical fossils into the Precambrian record.

In examining the clues from meteorites the authors are again guilty of using only selected evidence. The investigation of organic molecules in these extraterrestrial samples are so fraught with controversy that many investigators have declined to study them further. Of the extractable compounds, only the amino acids by virtue of stereochemical arguments may be considered to be indigenous to the samples. Since amino acids are racemic mixtures, it is impossible to decide whether they are biological, that is, associated with meteorite 'microfossils' (assuming you believe these are not organised elements) or the remnants of an abiogenic synthesis. Many of the other classes of compounds which may be extracted by organic solvents from meteorites could be terrestrial contamination. Han *et al.* (*Nature*, **222**, 364; 1969) have shown clearly that easily recognisable amounts of terrestrial organics were accumulated by samples of the carbonaceous chondrite Allende during only a few hours in the terrestrial environment. Like other naturally occurring polymers, the insoluble organic material associated with carbonaceous chondrites is not particularly well characterised despite many heroic efforts. It is generally agreed to be a highly aromatic substance but few would accept an identification as cellulose

on the basis of similarities in the infrared spectrum alone. Perhaps some of the efforts now underway to identify the host phases of isotopically anomalous rare gases in certain meteorites might be more successful in recognising pre-Solar System material. Although a number of possibilities are currently being entertained, cellulose is not among them.

The book contains a number of trivial but real errors. The authors are obviously confused by meteorite terminology. A "fall" is a meteorite observed to fall and later collected; a "find" is a meteorite discovered accidentally and identified as such by its mineralogy, petrology, chemistry etc. Thus (p108) 90% of all "falls" (not finds) are stone meteorites; because of their vulnerability to weathering all known carbonaceous chondrites are "falls" not finds. There are well in excess of 1500 (about 2300) well catalogued meteorites (again not falls) (p 18

and p 108); since the Antarctic bonanza of recent years a great many more samples have been discovered. Many physicists would not agree that the solar wind imprinted the lunar magnetic field (p137). Finally it is to be hoped that the authors' knowledge of chemistry is not accurately reflected by the alarming number of mistakes in the structure of chlorophyll as depicted on p 51 or their choice of glycine (an amino acid without an asymmetric carbon atom) as an example of an optically active molecule (Appendix 2).

Although from my comments it does not seem that I was over impressed with this book, I recommend reading it. I look forward to seeing a revised edition, as I am in agreement with some of the authors' overstated sentiments. □

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Acknowledging global links

Peter J. Smith

Cosmos, Earth, and Man: A Short History of the Universe. By P. Cloud. Pp. 372. (Yale University Press: New Haven, Connecticut, and London, 1978.) £10.75.

In his Preface, Preston Cloud thanks "those anonymous critics of the several publishers who rejected an earlier draft of this book for their often cutting but nevertheless helpful comments". It is a gesture of characteristically Cloudian impishness, a humorous rounding-off to a piece of history that lesser people would rather have concealed. But joking apart, things have come to a pretty pass when one of America's best known Earth scientists can build up a collection of rejection slips and then only get published with the aid of a grant. In a market that publishers believe can support more than fifty practically identical general introductions to geology, it is nothing short of disgraceful to find a better book than most of them almost falling by the wayside.

For not only is Cloud a better stylist than most, he has a more original approach to content borne of a more original mind. His line is biogeology, which the American Geological Institute's *Glossary of Geology* defines as the "application of biological data to geology" but which in a lifetime of research Cloud has turned into something altogether more exciting than that dry definition would suggest. The advantage of having a foot in each

of two scientific camps is the acquisition of tendencies to spot connections and perceive correlations between developments parallel in time; and in *Cosmos, Earth, and Man* both are given rein. Where a pure anthropologist might see the ascent of man as a largely isolated phenomenon, Cloud relates man's evolution to the movement of continents, changes in climate and the encroachment of oceans:

"The breakup of the consolidated late Paleozoic continent of Pangaea, followed by the drifting apart of the pieces to form the present continents, set in motion a complex of processes that led to a general moderation of Mesozoic continental climates. Late Mesozoic changes and climatic deterioration that contributed to the extinction of the dinosaurs prepared the way for mammals to inherit the earth. Plate tectonics . . . may be related to the emergence of *Homo sapiens* . . ."

To be sure, our knowledge of global links is sparse and, where it exists, usually rather general. Nevertheless, it stands Cloud in good stead throughout the first three-quarters of his book where he works up from the nature of matter to space and planets, to the origin and evolution of the Earth and atmosphere, and to the origin of life and its evolution through plants and animals. It also helps in his discussion of the development of man; and up to this point the story that Cloud tells is as fascinating as some of the best fiction and surely has something new to impart not only to the layman, for whom the book is primarily intended, but also to all but the most erudite of Earth scientists. Up to this point, too, it has a convincing air of authority about it.

Unfortunately, perhaps, Cloud's predilections and the logic of the global linkage concept then lead him on inexorably to consider the future of

Homo sapiens in the context of the impending ecological crisis which, as hundreds of books and articles have told us already, is serious precisely because of global interactions. But however sincerely meant, this is the least satisfactory part of the book, partly because it is difficult to find anything genuinely new to say and partly because Cloud ignores or underplays an essential part of the argument.

His general solution, shorn of all detail, is "strategic research and planning involving all sectors of society". He may be right; but even though he acknowledges that the growth of technology since World War II has had unforeseen side-effects that must now be mitigated, he fails to observe that mitigation through planning itself has deleterious consequences. For as experience in Britain,

the USSR and, indeed, America has plainly shown, the quality of decision-making almost invariably deteriorates when placed in the hands of an anonymous and inflated state bureaucracy (which is what always develops from 'planning'); and the institution of planning, whether effective or not, is only ever achieved at the expense of freedoms. The cure may or may not be worse than the disease, but it needs more discussion than Cloud seems to suppose.

Even so, *Cosmos, Earth, and Man* is precisely the sort of book intelligent students and laymen should be reading. If everyone agreed with everything in it, it would probably be dull. Preston Cloud is never that. □

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Evocative of Dirac

R. H. Dalitz

Directions in Physics. By P. A. M. Dirac. Edited by H. Hora and J. R. Shepanski, with foreword by Sir Mark Oliphant. Pp. 95. (Wiley: New York and London, 1978.) \$16.45; £9.20.

THE editors of this book are to be congratulated on their initiative in persuading Professor Dirac, now retired from the Lucasian Chair at Cambridge and resident at Florida State University, Tallahassee, to make a lecture tour of Australia and New Zealand in 1975, and on their decision to record and publish these lectures.

The printed lectures are very evocative of Dirac. As one reads, one soon hears in one's mind the well-measured tones of Dirac's lecturing style. They capture precisely his simplicity of statement and clarity of logic. The lectures range quite widely in topic. The first lecture, "The Development of Quantum Mechanics", is primarily historical, and of particular interest concerning the precise steps of thought in the early development of non-relativistic quantum mechanics, as Dirac joined in following the initial ideas of matrix mechanics as put forward by Heisenberg.

The second lecture, on "Quantum Electrodynamics", is concerned with second quantisation, the particle representation corresponding to a wave field, and the field-theoretic formulation of the Hamiltonian for a system of charged particles. With relativistic quantum mechanics this leads Dirac to the prescriptions of renormalisation for



Dirac in 1978

dealing with the problem of divergent integrals in the perturbation calculation of quantum electrodynamic processes. Dirac is unwilling to accept the conventional renormalisation philosophy and feels that there is a change needed in the theory, "just about as drastic as the passage from the Bohr orbit theory to the quantum mechanics".

The other three lectures are more speculative. "Magnetic Monopoles" is a topic which Professor Dirac has found attractive from quite early times. Its discussion led him to his well-known result that magnetic monopoles, if they exist, should have a quantised strength, with magnitude given by the formula $n(hc/2e)$, where e denotes the unit of charge and n is an integer. In this lecture, Dirac gave attention to the question of how these magnetic monopoles might appear in experiment; and since the lecture took place soon after the publication of a cosmic ray investigation at Berkeley which claimed

to show evidence for the existence of a magnetic monopole, it was natural for him to discuss this evidence. The lecture almost coincided with the cogent criticisms made by Professor Alvarez of this interpretation of the Berkeley evidence. Dirac has, somewhat unwisely, added an appendix to this lecture, stating that the alternative interpretation by Alvarez is now disproved; in fact, Alvarez's interpretation still stands as a possibility, as does the interpretation given here by Dirac. In this situation few physicists today believe that this event gives any appreciable support for the existence of magnetic monopoles. As Dirac says, "one very much hopes that further examples will be discovered in future experiments", for that is the only way the matter can be settled.

The fourth lecture, on "A Positive-Energy Relativistic Wave Equation", is relatively technical, being concerned with a generalisation of the equation Majorana proposed in 1937 for neutral, massless fermions. This new equation finds serious difficulties when any attempt is made to extend it to the case of a charged particle; Dirac's conclusion is that the particles must be neutral or else the equation modified. The fifth and final lecture, "Cosmology and the Gravitational Constant", is based on Dirac's *Large Numbers Hypothesis*, that the very large dimensionless numbers occurring in our description of the Universe should be related with one another. Dirac focuses attention on three such numbers, (e^2/Gm_em_p) , $(m_e c^3/te^2)$ and $\sqrt{(M/m_p)}$, where G is the gravitational constant, M the mass of the Universe and t its age. Each of these numbers is at present of order 10^{39} . Dirac concludes that, in atomic units, G and M must vary with the age of the Universe, in particular that there must be a continuous creation of matter going on, a process which may be either evenly distributed over all of space or concentrated where there is already mass. The various possibilities can be distinguished by experiment, as Dirac points out, and he surveys the status of such experiments to date, leaving us with the hope that the question may become settled within a few years. This lecture is particularly interesting in the way it illustrates the simple and logical steps which Dirac's mind follows in his development of questions in physics.

These lectures have permanent value partly for historical reasons and partly because of the picture they give of the man. The lectures impinge on a number of interesting questions, for example, the relationship of Dirac's development of quantum mechanics with the ideas of Heisenberg. They give the reason (p16) for his proposal that the negative energy solution for the

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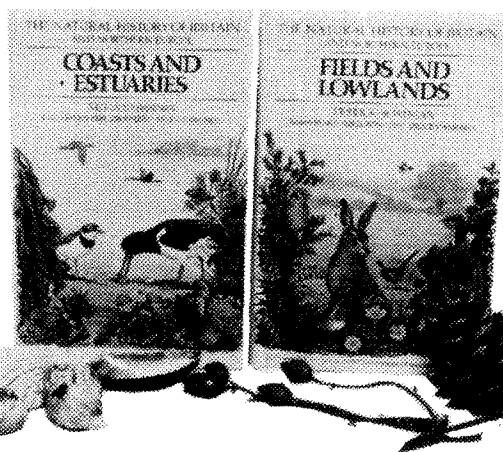
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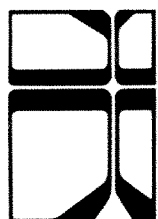
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electron might have been a representation of the proton. His firm belief that physics should follow "standard mathematics" is expressed very strongly in these lectures, and brings him to the point (p36) where he is willing to give up special relativity rather than accept renormalisation. One finds this a somewhat strange opinion from the man who, after all, introduced the δ -function into physics at a time when it was mathematically disreputable. The picture which he gives of the early days of quantum mechanics is most interesting, and there is one especially quotable remark, namely that "it was very easy in those

days for any second-rate physicist to do first-rate work . . . It is very difficult now for a first-rate physicist to do second-rate work". While accepting the latter proposition, we may remain somewhat sceptical about the former.

This book of lectures goes beyond history, although already interesting for that reason, and gives us also an interesting picture of Professor Dirac's ideas of recent years. Altogether, the book is very stimulating to read and deserves a place in every science library. □

R. H. Dalitz is Royal Society Research Professor in the Department of Theoretical Physics at the University of Oxford, UK.

From quarks via tokamaks, to quasars

P. T. Matthews

A Perspective of Physics. Vol. 2. Selections from 1977 Comments on Modern Physics. Introduced by Rudolf Peierls. (Gordon and Breach: London, New York and Paris, 1978.) £16.70.

UP to the outbreak of World War II the physicists who had discovered quantum mechanics and were engaged in developing its exciting implications in nuclear, atomic, molecular and solid-state physics and astrophysics, formed a close-knit international group, small enough for each individual to follow in some detail the activities of the whole. When work on basic physics started up again after the War, it was in much the same spirit; and it was not unreasonable to attempt to keep pace with the published literature as it appeared. This did not last long. The search for fundamentals led to a whole new international community of high energy, or elementary particle physicists, working in the newly discovered sub-nuclear domain, while each of the separate areas mentioned above developed into a major field of operation with its private army of specialists communicating within its own boundaries through an ever increasing flood of specialised literature.

The pace got hotter and by the mid-1950s the published literature became largely irrelevant because it took too long for an important advance to appear in print. Research groups working on related topics all over the world, but particularly in Western Europe and America, started communicating with each other by exchange of pre-prints and conference

reports. This scientific samisdat, even within a discipline such as high energy physics, tended to produce a fragmentation into subgroups very well informed within their own narrow speciality but increasingly isolated from each other. In 1967 *Comments on Nuclear and Particle Physics* was founded in a deliberate effort to counteract this trend. In the foreword to the opening volume it is explained that a group of distinguished physicists will each be asked "to comment on work that seems to him interesting and important" in the hope that new ideas would be made available "not only to specialists in the field, but also to colleagues working in related and unrelated areas of physics". Other issues in other fields followed and although the appearance of *Comments* did not change the face of physics, there is no doubt that over the years it has helped to break down the barriers of overspecialisation.

The volume under review is the second in an annual series which takes this process one stage further. It consists of a selection of reprints of some 25 articles which have appeared in

Comments on Modern Physics during 1977. The selection has been made by Sir Rudolph Peierls. The articles are split fairly evenly under the five main headings of nuclear and particle physics, solid-state, astrophysics, atomic and molecular and finally plasma physics. The selection is preceded by an introduction written by Sir Rudolph which straddles the whole field and so puts the individual articles into some coherent context. As the blurb on the dust cover rather grandly says, the whole is an attempt "to keep both the specialist reader and interested observer abreast of the most significant developments in all modern physics".

This is a very ambitious project which probably succeeds as well as could be expected, almost exclusively as a result of Peierls' masterly introduction. Without it the collected reprints would be a jumble of fragments which would add up to very little indeed. In 25 pages, which are commendably free from specialist jargon which so often obscures such articles, Peierls gives a broad brush account of the whole background which places each of the separate papers in an intelligible relation to the rest. The fact that this is done by someone already some years past official retirement, demonstrates by example that physics is still a coherent whole, and that one man prepared to make the effort can still see it as such and can follow its major developments all the way from quarks, via tokamaks, to quasars. □

Professor P. T. Matthews was Head of the Department of Physics, Blackett Laboratory, Imperial College, from 1971-76, and is now Vice-Chancellor of the University of Bath, UK.

Mechanism of organic evolution

Evolution. By C. Patterson. Pp. 197. (Routledge and Kegan Paul, in association with the British Museum (Natural History): London, 1978.) Hardback £5.95; paperback £2.95.

At the time of the Fifteenth International Congress of Zoology in 1958, the Natural History Museum in South Kensington, London, under Sir Gavin de Beer mounted a "permanent" evolution exhibit in the Main Hall of the Museum to commemorate in part the paper by Darwin and Wallace "On the Tendency of Species to form Varieties; and on the Perpetuation of Varieties

and Species by Natural Means of Selection", read at a meeting of the Linnean Society in London on 1st July 1858. To accompany the Exhibition, the Museum published *A Handbook on Evolution*, consisting of a general essay on natural selection and a guide to the exhibits. This *Handbook* was used by generations of students.

Twenty years later, the evolution exhibit has gone and the 1958 *Handbook* now seems very superficial. We are promised a new exhibit at the Museum in due course but in the meantime the Museum's Trustees (in association with Routledge and Kegan Paul) have produced a really excellent small book on *Evolution* by Colin Patterson, who works on fossil fish at the Museum. This is a completely new book which describes and summarises

the process and mechanism of organic evolution independently of any Museum exhibits. Dr Patterson is splendidly eclectic and suitably acerbic in his reviews of the concepts and controversies that contribute to our present understanding of the diversity of life.

As is proper for a taxonomist, Dr Patterson begins with a discussion of the idea of species; and as is proper for a palaeontologist, his concept of a species is three-dimensional (based on "community of descent" rather than "community of resemblance"), and includes the reality of intraspecific variation and differentiation. This leads him on to three chapters on basic genetics, and a further three on ecological and population genetics, culminating in a description of the origin of species—the subject which Darwin did not cover in his book on the *Origin of Species*; and then to a description of speciation in the Galapagos Islands. The last third of the book contains chapters on evolution beyond the species level, proof and disproof (in which Patterson largely discounts the Popperian view that evolution is metaphysics rather than science), the origin and early evolution of life, evolution and man (sadly playing down de Beer's arguments about neoteny in favour of regulatory genes, which amount to the same thing but are much more difficult to visualise), and a Who's Who of significant evolutionists (largely illustrated with portraits from the Linnean Society's collection).

The writing is concise but clear. I can think of no other book more suitable as a general introduction to the field of evolution as a whole, or for pre-university examinations in particular. There is a brief list of "further reading" which will be a useful guide to librarians.

Patterson steers a careful course through the minefield of evolutionary controversy; one of his reasons for claiming evolution is scientific is that the debates and progress pertinent to evolution have been about a series of specific questions—the relationship between ontogeny and phylogeny, the nature of particulate inheritance, the population genetics revolution in the 1930s, DNA in the 1950s and most recently the neutralist/selectionist argument. He skirts round the clade and grade dissension which is currently raising the temperature of systematists (*Nature*, 276, 759-60; 1978). Perhaps the only area in which Patterson is not entirely fair is in the relationship between evolution and Christianity; and he is hardly to be blamed because of the ability with which Christians from Soapy Sammy Wilberforce onwards have obfuscated their position.

The problem about evolution for Christians is that it seems on the surface to dispose of the need for a creator. The naive solutions are in Patterson's words:

"at one extreme the fundamentalist view that evidence of evolution, such as fossils, was built into the newly-created rocks to tempt us or test our faith. At the other extreme is the person to whom evidence of evolution only pushes the activity of the Creator further and further into the past. Both these modifications of the original creation myths are typical evasive moves, avoiding refutation or confrontation by modifying the original theory or erecting subsidiary defensive theories around it".

True, and Patterson comes close to

a positive approach to the old dispute when he points out that it is equally "depressing to be . . . a pointless experiment in protein chemistry or an experiment in ethics, which is the Christian message read from the same nihilist or 'nothing but' viewpoint".

The logical fallacy of 'nothing but-tery' has been recognised at least since the time of Aristotle; the fact that the same event may have more than one cause ought to have been assimilated by now—by both scientific reductionists and religious simplacists.

R. J. Berry

R. J. Berry is Professor of Genetics at University College, London, UK.

Appealing astronomy

Beyond the Moon? Written and presented by Simon Mitton. (ATV: London, 1979.) First programme transmitted in the UK: 09.10, 21 April, on ATV (Midlands). Other channels: morning, 22 April, 1979.

In view of its position as the most 'popular' of the physical sciences, astronomy has all too little television time allocated to it. ATV is therefore to be congratulated on running a seven-part introductory series, "both for those who are interested in the subject—and those who think they might be." In each programme Simon Mitton, as both writer and presenter, takes up a different strand and develops it from the historical point of view within a loose framework, which begins traditionally enough with the planets, and extends to encompass the stars, life in the Universe, interstellar travel, and the "far future".

The historical aspects are enlivened with biographical details which paint astronomers as real people; one appealing sequence was the summary of Newton's achievements in terms of the illustration on the current £1 notes. Despite Mitton's breathless enthusiasm, however, the production is too often constrained by a rigid studio format: presenter at desk with TV monitor. The programmes impress most through their studio demonstrations and film sequences. Demonstrations in the planetary programme range from antique orreries to a water bath for floating models of the low-density giant planets. Some of the demonstrations are unfortunately impressive at the expense of basic physics: the buoyancy of a helium balloon in air is adduced as evidence that the Earth's gravity is incapable of retaining helium atoms, and in a comparison of the weights of model gas molecules helium is represented as He₂. One surprisingly successful de-

monstration was a simple scale model of the Sun, Earth and Moon, that conveyed powerfully their relative sizes and distances, and the general emptiness of interplanetary space.

Film sequences from the Royal Greenwich Observatory and the Cambridge Institute of Astronomy provide strong links with the realities of observational astronomy. For example, the opening programme, though perhaps biased to the possibilities open to amateurs at the expense of the excitement of current research, is imaginatively set within the dome of the Isaac Newton Telescope; and in a later sequence, Mitton recounts the story of the discovery of Neptune from the dome of the Northumberland telescope, the instrument with which Challis almost forestalled Galle's identification of this new planet.

Although the series is carefully thought-out, the programmes seem to suffer from the limits on air-time and budget-restrictions: for example, the animations are crude, and some of the studio and film shots should have been retaken. Among the latter are some shots where slips of the tongue have unfortunately been allowed to survive: a light year is described as "10 million million miles" (rather than kilometres), and Jupiter's 'year' as 13 yr (instead of 11.9 yr), to take just two early examples. In addition, some viewers may find Mitton's presentation slightly patronising. To take one example, a description of 8×30 binoculars as "suitable for sport, and all right for ladies to do astronomy" will not be popular in some quarters. And a half-hour programme without interviews, and with only one presenter, can seem very long. Still, astronomy is an exciting subject, and Mitton's enthusiastic presentation is likely to convince even the casual viewer that astronomy is a subject he should learn more about.

Nigel Henbest

Nigel Henbest, formerly a researcher in radio astronomy, is now a freelance journalist.

obituary

Ralph A. Sawyer

RALPH ALANSON SAWYER, physicist and science administrator, died on 5 December 1978 at the age of 83.

Born in New Hampshire, he graduated valedictorian of his class from Dartmouth College in 1915 and then went to the University of Chicago on a travelling fellowship as a Chamberlain Fellow from Dartmouth. At Chicago he became one of R. A. Millikan's students and, after a brief tour in the US Navy, he received a PhD degree in physics there in 1919. Soon afterward Ralph joined the staff of the physics department at the University of Michigan and, except for the years of World War II, he served the University as a prolific researcher, dedicated teacher and, later, as a gifted administrator until his retirement in 1964.

Before he was called back into active service by the Navy in 1942 Ralph had published more than 50 papers in the field of spectroscopy. His interest in the spectra produced by energetic light sources, which began with his first publication at Chicago on the characteristics of a vacuum spark, continued into his earlier years at Michigan. Using a normal-incidence vacuum spectrograph of his own design, together with an improved hollow-cathode light source, for the extreme ultraviolet, Ralph and his students analysed the first spark spectrum of a considerable number of elements.

In the mid-thirties he became interested in rapid quantitative spectrochemical analysis and together with H. B. Vincent he developed the techniques to the point where a technician could make 'on line' determinations of the composition of samples in the steel-making process fast enough to control the composition of the batch of metal being processed.

From 1942 to 1945, on leave from the University, Sawyer served as Director of the Armor and Projectile Laboratory at the Naval Proving Ground at Dahlgren, Virginia. However, he still maintained an active interest in spectroscopy and managed to finish his book *Experimental Spectroscopy* which was published by Prentice-Hall in 1944 and then, in revised form, in 1951. A third, paperback, edition was published by Dover in 1963.

In 1946 Sawyer was appointed Civilian Director of Technical Operations for the series of atomic bomb tests at Bikini Atoll known as 'Opera-

tion Crossroads'. In view of the fact that he was both a Naval officer and an eminent scientist of proven administrative ability this was an ideal choice. The successful completion of this mission marked the end of Ralph's active service to the Navy and he was awarded their Commendation Ribbon. Later (1951) he was promoted from the rank of Commander to that of Captain in the Naval Reserve.

While the Bikini tests were in progress Sawyer was notified of his appointment as Dean of the Graduate School at Michigan and in the fall returned to Ann Arbor to assume the position which he held until his retirement.

Ralph's talent for administration brought him additional responsibilities. He became the first director of the Michigan Memorial Phoenix project which was devoted to the study of the peaceful uses of atomic energy and then, in 1959, he was appointed to the newly-created post of Vice-President for Research at the University of Michigan. These were times of new and rapidly expanding Federal support of university research and Ralph was in large measure responsible for establishing the University of Michigan as one of the leaders in Federally-sponsored research.

Amid all these activities Sawyer somehow found time for service to various off-campus organisations. He served a term as President of the Optical Society of America and, earlier, was an associate editor of their journal. In 1963 he was awarded the Frederic Ives medal by the Optical Society for 'distinguished work in optics'. Ralph was elected to the Governing Board of the American Institute of Physics in 1954 and served as its chairman from 1959 to 1971. This was a period in which many hard decisions had to be made and Ralph was described by one of the board members as a 'benevolent but firm leader, deeply respected by all'. Upon his retirement from the chairmanship he was awarded the Karl Taylor Compton gold medal by the Institute for 'distinguished statesmanship in science'. Sawyer also served a term as President of the Association of Graduate Schools and was advisor to or member of: the National Research Council, the Goddard Space Flight Centre, the National Bureau of Standards, the International Commission on Optics, and three US Naval Laboratories.

It can truly be said of Ralph Sawyer

that 'he lived a life of large usefulness'. Those of us who knew him well will miss his warm friendship and the entire scientific community will mourn the loss of his leadership.

W. Wallace McCormick

D. I. Blokhintsev

DMITRII IVANOVICH BLOKHINTSEV, the founder of the Soviet nuclear power industry and a prominent theoretical physicist, died suddenly on 27 January 1979. Although 71 years old, he was still actively involved in teaching at Moscow State University—his own alma mater—and in his work as head of the Laboratory of Theoretical Physics at the Joint Nuclear Research Institute at Dubna.

Blokhintsev had formerly been director of the Dubna Institute, from its foundation in 1956, until his official retirement in 1965. It was here that, in 1964, he announced the synthesis of element number 104. The Dubna institute was one of the first major joint efforts of the socialist bloc—the Comecon countries, with the addition of China, North Korea, North Vietnam, and Albania. As Director of the Institute, Blokhintsev received a number of orders and medals from the countries involved, as well as election to several foreign Academies.

Soviet honours, too, were bestowed on him, including the title of Hero of Socialist Labour, the Order of Lenin (four times), the Order of the Red Banner of Labour, the Order of the October Revolution and the Lenin and Stalin (now State) prizes. Many of these awards, including the Lenin Prize, were associated not so much with his academic talents but with his expertise as an organiser of the Soviet Union's nuclear power programme. In 1954, he was in charge of the construction of the Soviet Union's first nuclear power station, a modest 5,000 kW 'semi-pilot' plant at Obninsk, which was to do much, in the following decade, towards the development of Obninsk as a scientific centre with a wide range of research institutes.

Nevertheless, Blokhintsev's achievements in pure physics did not go unnoticed at home. Indeed, in the official *Pravda* obituary, which was signed by numerous high-ranking officials, including Messrs Brezhnev and Kosygin, pride of place is given to these. In the words of this official appreciation 'he enriched world science by his fundamental works in the field of quantum

theory, solid-state semiconductor physics, acoustics of inhomogeneous media, quantum mechanics, the theory of chain reactions in atomic reactors, and the physics of elementary particles. His death, it says, is a serious loss to Soviet science. *Vera Rich*

Peter Bishop

DR PETER MAXWELL FARROW BISHOP, FRCP (Lond.), FRCOG, one of the pioneers of British clinical endocrinology, died on 19 January 1979 at the age of 74. His main achievements were in gynaecological endocrinology, of which he was the prime mover in the United Kingdom.

He was born on 14 August 1904, the son of a doctor, and was educated in Berlin and at Charterhouse, Trinity College, Oxford and Guy's Hospital Medical School, from which he qualified in 1929.

He entered the clinical field from the physiology laboratory at Guy's Hospital Medical School, where he was demonstrator and lecturer from 1930 onwards and where his researches included the modification and application of the Friedman test for pregnancy. With the help of a physician and in liaison with the gynaecological department, he started an endocrine clinic for functional menstrual disorders in 1934. He studied the effects of oestrone in amenorrhoeic women and of the new corpus luteum extract (progestin), soon replaced by synthetic progesterone, in dysfunctional uterine bleeding and recurrent abortion. The clinic later expanded to include testicular and other endocrine disorders.

He was quick to recognise the clinical applications of recent animal experiments and was in close touch with new developments in the pharmaceutical field. Thus he applied the concept of the oestrogen threshold for uterine bleeding developed by Zuckerman in primates and, following the experiments of A. S. Parkes and his colleagues in rats, he was the first to demonstrate the effectiveness of crystalline oestrone implanted subcutaneously in a human female castrate. At the request of the Therapeutic Trials Committee of the Medical Research Council, he undertook the first clinical trial of the earliest synthetic oestrogen, diethylstilboestrol, synthesised by E. C. Dodds and his colleagues in 1938.

His researches were interrupted by the Second World War, during which he was preoccupied with administrative duties at the hospital, although he continued his clinical work and studied with S. J. Folley the rates of absorption of various hormones from subcutaneously implanted pellets.

After the war he was appointed Consultant Endocrinologist to Guy's Hospital and Chelsea Hospital for Women and Senior Lecturer in the Obstetric Department at The Royal Postgraduate Medical School. At Chelsea he made painstaking studies of the comparative potencies of various synthetic oestrogens, as determined by the dosage required to induce withdrawal bleeding in amenorrhoeic women. He also published further studies of the use of implanted progesterone in women with recurrent abortion. At the same time, with his gynaecological colleagues at Chelsea, he established a clinic for the investigation and management of infertility. He also took part in the first British clinical trials of ACTH and cortisone in rheumatoid arthritis in 1950. In later years he and his colleagues were involved in a variety of publications, including the use of dydrogesterone in dysmenorrhoea, induction of ovulation with clomiphene, the use of oral contraceptives, genetic studies in gonadal dysgenesis and the management of Cushing's syndrome.

He was a fine teacher and excelled in his lectures on various endocrine topics and clinical demonstrations of endocrine disorders. Not only undergraduate students but many young physicians and gynaecologists, both at home and from overseas, gained their knowledge and experience of endocrinology under his guidance. His writings were extensive, lucid and stylish. Apart from his original articles he wrote monographs on gynaecological endocrinology and on the chemistry of the sex hormones. He also wrote single-handed the 7th edition of *Recent Advances in Endocrinology* (1954) and contributed numerous chapters to other textbooks and invited articles to various journals for the general reader.

He was at various times President of the Section of Endocrinology of the Royal Society of Medicine and Chairman of the Society for the Study of Fertility and of the Council of Management of the *Journal of Reproduction and Fertility*. He was also Medical Consultant to the Family Planning Association.

Dr Bishop had the unusual distinction of being elected Fellow of both The Royal College of Physicians and The Royal College of Obstetricians and Gynaecologists. He was Sir Arthur Sims' Travelling Professor in 1964. In the year of his retirement from hospital in 1969 he became Master of the Society of Apothecaries.

He retained single-minded dedication to clinical endocrinology throughout his career despite considerable scepticism and opposition from others. This required great determination and

courage: qualities which he likewise displayed in the face of the physical disability which followed his retirement and immobilised him for the last few years of his life, but which never extinguished his interest in his subject nor his zest for living.

Robert R. de Mowbray

Fred Lang

THE death of Dr Fred Lang at the age of 34 in an automobile accident on 8 December 1978, cut short a promising career in neurobiology. He was Associate Professor of Biology in Boston University; he taught in the Boston University Marine Program at Woods Hole, Mass., and was a director of the winter neurobiology course given there. Recently he had received a Career Development Award from NIH to further his research on invertebrate neuromuscular systems, which had reached a flourishing stage and had attracted a large entourage of graduate students at Woods Hole.

Fred Lang worked as a graduate student with Bernard Abbott and Ladd Prosser at the University of Illinois, and as a postdoctoral fellow with Harold Atwood at the University of Toronto. His initial work on the innervation of the heart of the horseshoe crab *Limulus*, and on neuromuscular physiology of crustacean limbs, was extended later to include development of neuromuscular synapses in crustaceans, and in particular, development of the nervous system and muscles in the American lobster, *Homarus*. He and his collaborators described the physiological properties of the differentiated asymmetric 'crusher' and 'cutter' claws of the lobster, and then looked for neural and environmental influences on differentiation. In addition, adaptation of the animal to its environment was examined. A thorough description of physiological and morphological changes occurring in the muscle fibres throughout development led to a correlation of muscle fibre properties with their innervation and to a demonstration that muscle differentiation could be influenced by conditions in the animal's environment. Although this evidence suggests a neural influence on muscle differentiation, the problem remains open and controversial.

His enthusiastic activity, his lively and forthright dialogue, and his ability to generate interest and debate, will be well remembered. He had an important place among invertebrate neurobiologists.

H. L. Atwood

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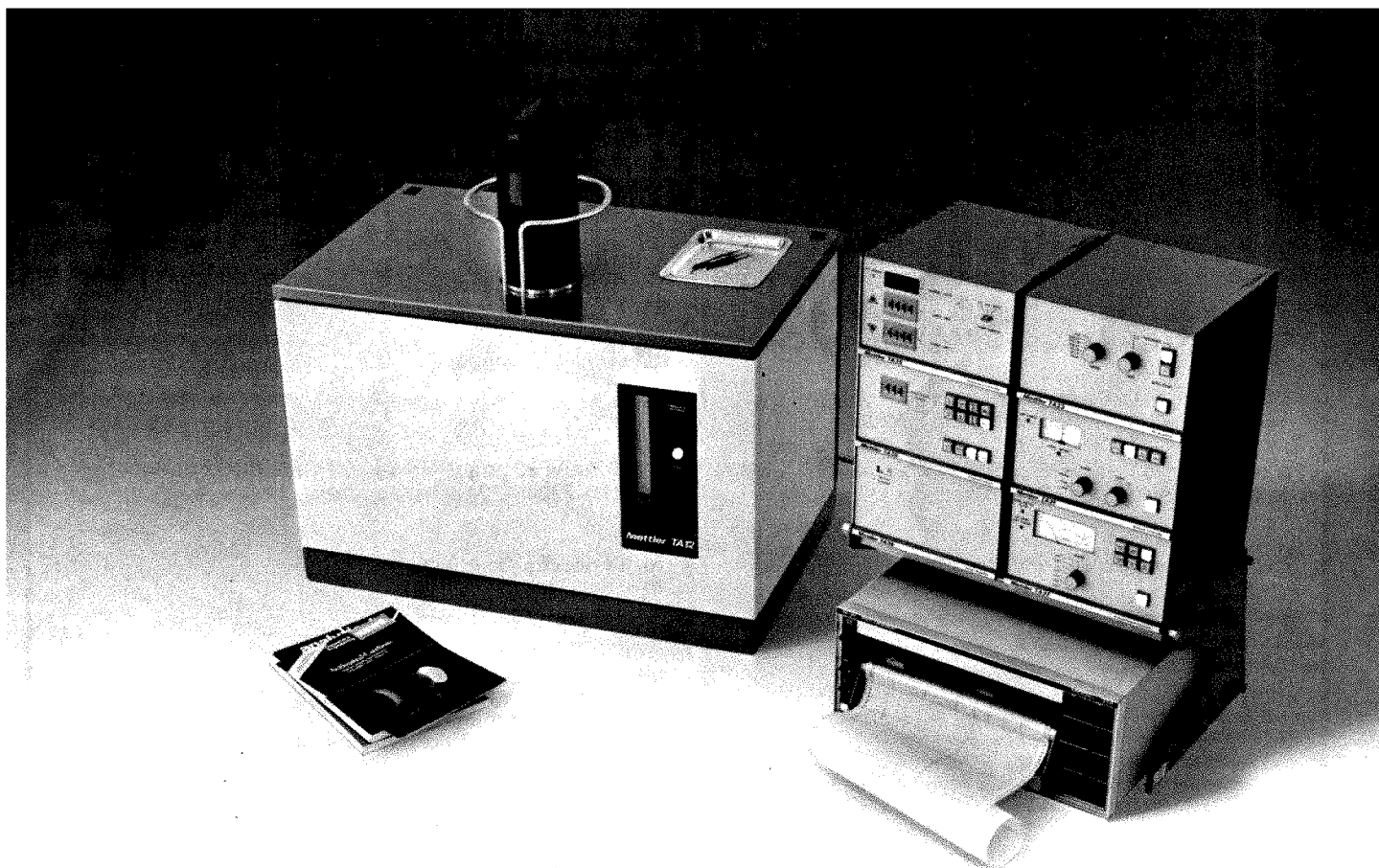
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Simultaneous TG-DSC vs. Guesswork

Thermogravimetry (TG) and heat flow Differential Scanning Calorimetry (DSC) are two important thermoanalytical measuring methods which complement each other in a virtually ideal manner.

The new TA2000C Thermoanalyzer from Mettler supplies simultaneous TG and DSC data in the $+20^{\circ}\text{C}$ to $+1200^{\circ}\text{C}$ temperature range from a single sample. This provides objective information about decomposition reactions, phase transitions, enthalpy and weight changes. All of which can be used to draw objective conclusions about the temperature behavior of substances.

The operation of the TA2000C, however, is very simple. Its freely accessible furnace, the problem-free automatic push-button design of its balance, and the easy operation of its vacuum and temperature programs reduce training time, yet raise the quality of the analyses. As a result, operating errors are almost completely eliminated.

Objectively speaking, these are all very good reasons for using simultaneous TG-DSC. From now on, there is no longer any need for making guesses about the temperature behavior of substances.

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what's new

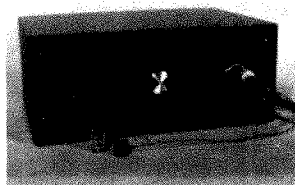
These notes, prepared from material provided by the manufacturers, are intended to give an outline of the range of products on the market. More detailed information may be obtained by circling the appropriate numbers on the Reader Enquiry Card, bound inside the cover.

A feature on spectrophotometers will appear in the 17 May issue.

Temtron

THE Temtron Micro Stirrer is designed to continuously stir micro quantities of aqueous chemical compounds. Previously this has not been possible under certain restrictive operating conditions. But a method is now available to enable scientists to not only stir effectively, but to add further preparations to existing compounds without ever handling the cuvette or disturbing the sensitive, fully enclosed detector system of the analyser. The stirring rate is continuously variable over 0–30 Hz. An 11×11×5 mm energiser capsule is placed under the cuvette and drives a Teflon coated stir bar magnet. By means of a special Temtron development, cuvettes (e.g. 1 ml) having internal physical shape restrictions as small as a 3×10 mm opening, which would normally be impossible to stir, can be accommodated. The Micro Stirrer does not interfere with light sensing detectors.

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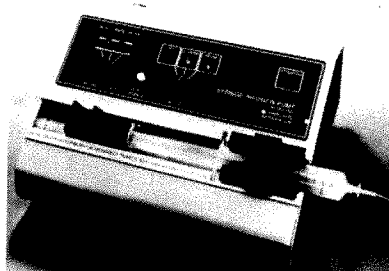


The Temtron Microstirrer

Canning

DETERGENT N22646 is a non-alkaline surfactant which can be used at a concentration of $\frac{1}{2}$ to 1½% in water for the removal of oils and greases. At different concentrations it can also be used for special applications as a scratch brushing fluid; for washing out polished silverware; for barrel finishing; for cleaning prior to plating on plastics and in hot water rinses.

Circle No. 78 on Reader Enquiry Card.



The Model 2616 infusion pump from Harvard

Harvard Apparatus

A low cost infusion pump for critical drug infusion applications has been introduced by Harvard Apparatus. Model 2616 has a reliable solid-state design, allowing a digital infusion rate of anything from 1.0 ml per h to 9.9 ml per h in 0.1 ml per h increments. Its syringe drive, powered by a rugged motor driving a precision lead screw assures dependable operation over prolonged periods of daily usage.

Harvard also offer a wide range of multi-speed pumps for infusion and withdrawal of liquids. These pumps are very accurate and offer a wide choice of flow rates by using different sized syringes (anything from 2 to 200 ml) together with the ability to change the motor speed. Output pressures of over 150 p.s.i. are possible in all pump series. The different model series include single action infusion or withdrawal, reciprocal action, and continuous-automatic operation with solenoid-operated tubing output. Many series offer multiple channel pumping.

Circle No. 79 on Reader Enquiry Card.

LTE

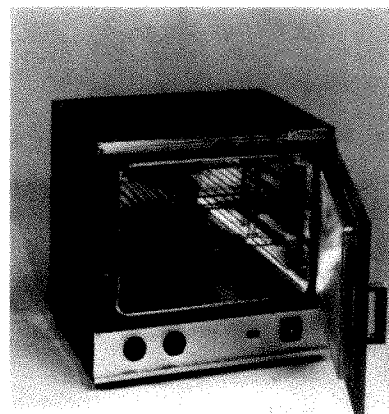
A COMPLETELY new range of ovens and dry heat sterilisers has been designed by LTE conforming to the latest thinking in hot air sterilisation. The Series G models have a modular control panel for maximum flexibility when choosing control options; a solid state controller for accuracy and ease of service; a stainless steel working chamber, and a performance better than the B.S. 2638 and B.S. 3421 requirements for ovens. Both the ovens and sterilisers come in three different sizes ranging from a capacity of 80 to 215 litres.

Circle No. 80 on Reader Enquiry Card.

Prot

VIAL washers, liquid fillers and cappers are now available from Prot. Vial washers come with four different types of working cycles and in various sizes to accommodate from 3,600 to 40,000 pieces per hour. Both conventional and ultrasonic techniques are utilised and cycles may be modified to suit customer requirements. Unloading is automatic and some versions incorporate special regulation of the washing fluid. All machines are built to the highest standards with extensive use of stainless steel. Rotary bottle washers with a capacity of up to 2,160 pieces per hour are available. The range also covers bottle cappers, which have changeable heads to allow handling of closures of differing diameters. Bottle fillers are offered too.

Circle No. 81 on Reader Enquiry Card.



An oven from Electrolux of Sweden

Electrolux

OVENS and incubators from Swedish Electrolux are now available. The smallest model in the range has a capacity of 55 litres and its interior can be either aluminium or stainless steel. The former has a temperature range of 50 to 200 °C and the latter 80 to 300 °C. Both are gravity convected and have temperature variation values (throughout the cabinet) of under ±1.5 °C. Other models include larger sizes of ovens, sterilisers and incubators.

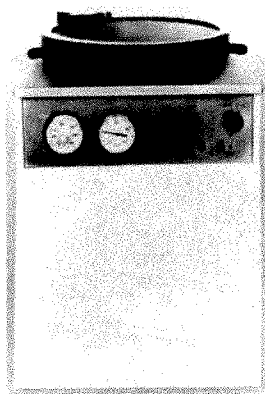
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The 17 May issue of *Nature* will feature colorimeters, spectrophotometers, fluorimeters and related equipment. Manufacturers should send information to the *Nature* office in London before 8 May.

Fedegari Autoclavi Spa

AN extensive range of high quality sterilisation autoclaves is now available and includes both steam and ethylene-oxide models. Strict quality control including exhaustive construction and weld testing ensures that these autoclaves are of the highest standards. Vertical cylinder, semi-automatic models for laboratory use have chamber capacities from 14 to 140 litres. Double wall, horizontal autoclaves can be supplied for pharmaceutical and hospital applications. These autoclaves together with the ethylene-oxide models are all available in six standard chamber sizes ranging from 70 to 1,750 litres capacity. A special super-heated water circulation unit is designed for sterilising plastic or glass vessels containing pharmaceutical solutions for which traditional steam methods are unsuitable. This model is offered in three chamber sizes of capacities from 600 to 11,000 litres. Custom-built autoclaves of any capacity can be supplied.

Circle No. 83 on Reader Enquiry Card.



Astell Hearson Swiftlock autoclave

Astell Hearson

RELIABLE dry heat sterilisation is provided by Astell Hearson's range of fully automatic sterilising ovens. The ovens are designed to give reliable sterilisation at the touch of a button. If a fault should occur such as mains failure, the cycle is automatically terminated thus avoiding the possibility of an incomplete process. The oven features push-button controls, automatic resetting cycle timer, door lock, and electronic strip-chart recorder. Once the door has been shut and the green start button depressed, the oven will heat up to the preset temperature and hold it there for the duration of the cycle. When the cycle is complete,

the heat is cut off but the fan continues to run. The door remains locked until the cooling period is complete.

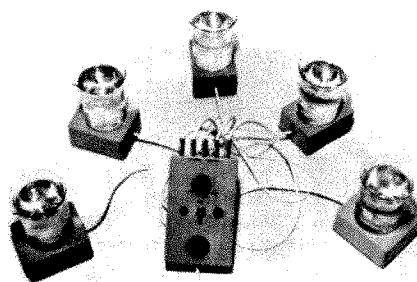
A range of simple to operate laboratory autoclaves meeting all current safety requirements in full is also available from Astell Hearson. Swiftlock 2000 autoclaves are available with a thermal lock with key switch override for the processing of liquids in sealable glass containers. The Swiftlock closure is fundamentally safe and is simple to operate. The lid is easily swung into position, and the outer ring rotated through only 4 inches to close the autoclave. The processing cycle is then started by turning the control knob. This locks the lid, activates the controls and closes off a large bore vent valve. Reversal of this procedure opens the vent valve to safely release any residual steam pressure, and after a short delay, the lock is withdrawn allowing completely safe opening. The Swiftlock 2000 is available for both horizontal or vertical loading, electrically heated or direct steam heated. Sizes range from 460 mm diameter, 610 mm deep, up to 610 mm diameter, 910 mm deep.

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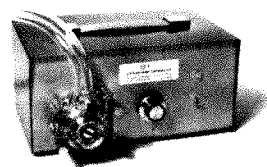
Chandos Intercontinental

A NEW addition to the Chandos range of stirrers is a variation of the Multi Place Unit where a master control unit operates several slave magnetic stirrers on flying leads. These stirrers are operated on low voltage from the master unit and can be placed in hazardous areas for remote operation. Applications also include fume cupboards, glove boxes etc. Most models have, as an alternative to standard motors, a variation using a 'stepping' motor triggered by a pulse generator. In multi station applications these models give identical speeds on all stations, which neither speed or slow with ambient temperature or viscosity change. Several types of pump are offered from high output low voltage d.c. submersible units to multi channel accurate dispensing peristaltic pumps.

Circle No. 85 on Reader Enquiry Card.



Chandos Multi Place Unit showing five slave stirrers operated from the master control unit



Variable-speed peristaltic pump from CP Instruments. Circle No. 76 for more details on the CP range of pumps

Chemlab

A WIDE range of PTFE covered stirring paddles is available from Chemlab. The magnets used in their construction have exceptionally high field strength to ensure excellent stirring even in viscous liquids. All paddles are moulded in one-piece and have a smooth machine finished surface to facilitate cleaning. ChemLab Retriever Rods are available for the recovery of paddles once stirring has been completed. Both the paddles and the rods and their integral magnet are PTFE covered, and may be used at temperatures up to 240 °C in all chemical reagents.

The ChemLab Model SS3H is a variable speed, hotplate magnetic stirrer for a wide variety of chemical and biochemical work. The temperature of the hotplate is controlled by a Simmerstat which regulates it between 50° and 325 °C. A heat baffle plate is fitted between the hotplate and the stirrer to prevent overheating of the internal components. Additional internal cooling is provided by a fan fitted to the stirrer motor. The ChemLab Model SS3 magnetic stirrer with its large, flat, easy-to-clean stainless steel top, powerful magnet and variable operating speed is ideal for large volumes of corrosive liquids that have to be stirred and for when very slow stirring speeds are required. An internal fan ensures efficient cooling of the drive motor.

The ChemLab Mini Stirrer is a compact, inexpensive but reliable magnetic stirrer with variable speed control. It is fitted with an exceptionally powerful magnet enabling it to stir volumes up to 5 litres when suitably supported. The white plastic case is tough, durable, easy to clean and resistant to most chemical reagents.

Circle No. 86 on Reader Enquiry Card.

Alconox

THE Alconox range of detergents includes Alconox powder a general purpose cleaner; Liqui-Nox a phosphate free liquid cleaner; Terg-a-Zyme with protease enzymes; Alcojet specially formulated for washing machines and Alcotabs for pipette rinsers and washers.

Circle No. 87 on Reader Enquiry Card.

**rapid
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easy
field test kit for NPK**



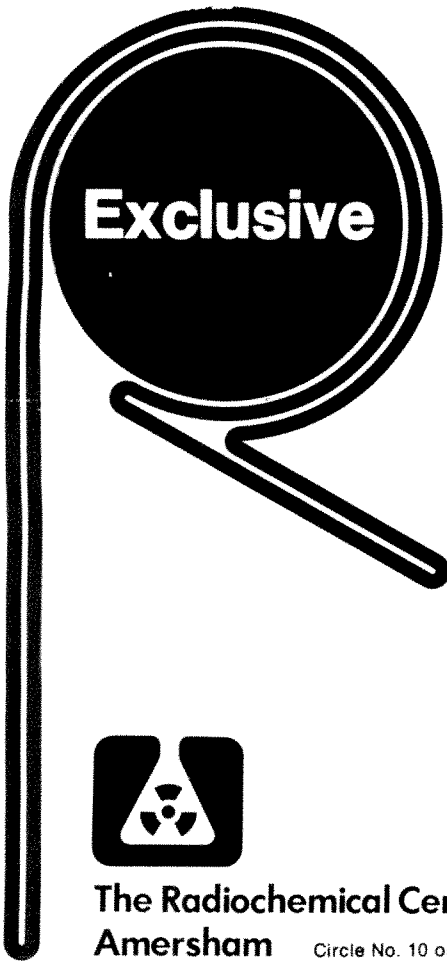
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**The Radiochemical Centre
Amersham**

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Please enquire for further details

Full information is available on request

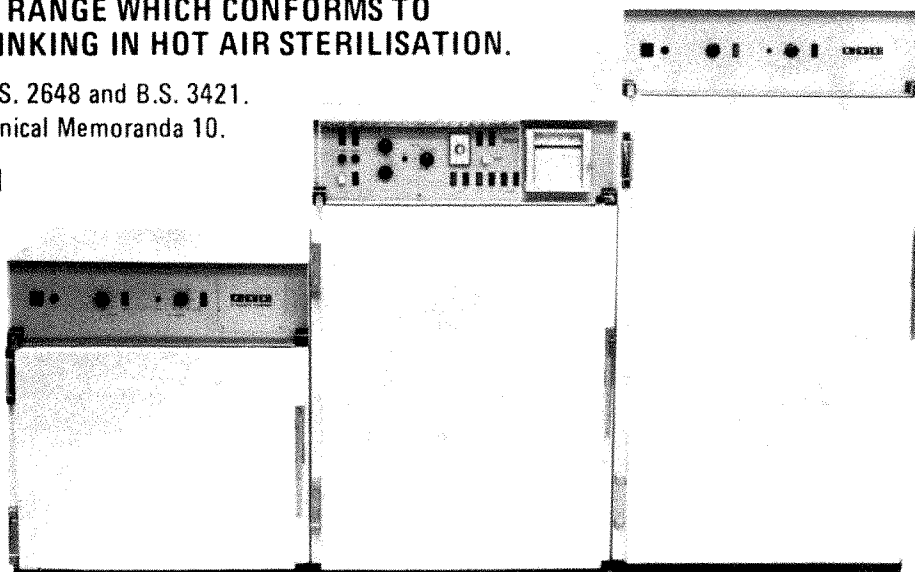
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*The
First
Choice*



Model AA Dohm syphon
1½ gallon per min flow rate

Dohm London

THE simple-to-use Dohm syphon safely and quickly transfers dangerous, corrosive liquids from one vessel to another, without waste, mess or personal hazard often associated with tipping and pouring from one heavy vessel to another. Manufactured from corrosion resistant polyethylene, the Dohm syphon consists simply of a tube with a tap at one end and a concertina-like bellows pump towards the other. In use, the tap is turned to the closed position, the bellows pump is compressed and the open tube end inserted in the vessel from which the liquid is to be taken. When the bellows pump is released the liquid is syphoned into the tube. By opening the tap, the liquid is allowed to flow freely into the container being filled, with the flow always readily under control at the tap. Dohm syphons are made in two sizes—1½ and 3 gal per min flow rate.

Circle No. 88 on Reader Enquiry Card.

Brentchem

THE Brentchem range of 14 specifically developed detergents, sanitisers and ancillary products for use in laboratories has been recently improved. The range includes three liquid and six powder detergents for machine washing of laboratory glassware and other utensils. Hydrosol is especially useful for washing aluminium utensils; Stretonite is a very low foam detergent for animal cages; and Lab-brite is particularly successful on tissue culture containers and similar items. There is a liquid and a powder grade for the manual washing of labware. Task and Septonite are general purpose detergent/sanitisers respectively in a liquid and a powder form, and Sorbitex is an absorbent granular clay for animal bedding and liquid spillage.

Circle No. 89 on Reader Enquiry Card.

L.I.P.

LIPOL liquid is a popular cleaner for laboratory glassware and plastics. It is an extremely active, non-toxic, non-corrosive, phosphate-free, biodegradable surfactant which is safe in use, fully compatible with hypochlorites, inexpensive and free rinsing in cold water to leave a brilliant, grease-free, clean surface. Lipol liquid completely removes biological residues and is an effective radioactive decontaminant.

A recent addition to L.I.P.'s range of small laboratory instruments is a family of magnetic stirrers and hotplates. These range from a fixed speed magnetic stirrer through a variable speed stirrer to a highly efficient hotplate and stirrer. The hotplate is also available unstirred. The fixed speed stirrer is a compact unit stirring at approximately 500 r.p.m. A 25 mm PTFE coated magnetic follower is supplied as standard. The variable speed stirrer has a continuously variable control up to a maximum of 1,300 r.p.m. The silent, long life motor is fitted with a powerful drive magnet and one of each 20 mm and 40 mm PTFE coated followers are supplied as standard. The variable speed stirrer is available fitted with a 13 cm diameter cast aluminium hotplate with a logarithmic energy regulator temperature control up to a maximum of 4000 °C. The hotplate is also available as a separate unit with no stirring facility. 20 mm and 40 mm PTFE coated followers are standard. All the units are made from robust metal and each is fitted with a mains indicator lamp. The smooth white finish provides an easy wipe clean surface.

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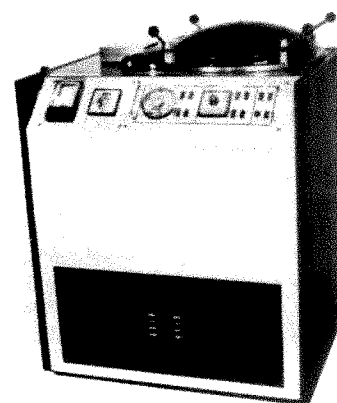
Nusonics

ULTRASONIC cleaning is a necessity in fields where contamination is a danger. Nusonics cleaners are powerful, robust and multipurpose, ranging from light bench models to large free-standing tanks with separate generators, one generator being sufficient to operate as many as three separate and variable tanks. Rapidex is a specially formulated biological surface-active detergent for the cleaning of laboratory and surgical equipment from all pollutants including radioactive contaminants. It is supplied for ease of use in pre-measured sachets ready for mixing with ordinary water in the ultrasonic tank, and two minutes cleaning with this solution followed by a thorough rinsing in fresh water will prepare even the most intricate article for laboratory or other use. Nusonics have formulated liquid detergents and de-greasers for any individual cleaning requirement and will always advise and work with a customer on specific cleaning problems.

Circle No. 91 on Reader Enquiry Card.

Baird and Tatlock

A NEW range of three magnetic stirrers has been produced by B & T. Using standardised casings and components, matched units designed to cope with every laboratory stirring requirement are offered. The simple laboratory stirrer (Cat. no. 333/0024) has a wrap-over top to provide spillage protection to control area and interior. It also dissipates any local heating from the stirring area. 3.2-mm thick aluminium gives a powerful eddy current effect in conjunction with the high flux stirrer magnet and allows speeds to be reliably and continuously controlled between 100 and 2,400 r.p.m. Modification of this design gives the new B & T hotplate magnetic stirrer (Cat. no. 333/0019). This allows a continuous variation of temperature up to 250 °C (230 V). A rectangular hotplate without magnetic stirrer is also available (Cat. no. 312/0066). B & T have also produced a reversible magnetic stirrer (Cat. no. 333/0026). The stirrer can be reversed in one direction only at speeds up to 2,200 r.p.m. or can reverse in an opposite direction on a 7½ second cycling function.



Series 225 autoclave (vertical model)
available from Baird and Tatlock

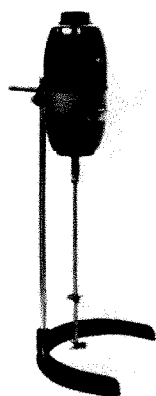
B & T are introducing a new series 225 autoclave. This is available for either direct steam or electrical operation using an integral generator. Control of the operating conditions within the chamber is by steam pressure. The basic unit can cope with all laboratory sterilisation requirements with optional 'plug-in' modules to record temperature, indicate temperature and to control/indicate temperature. The autoclaves are available either as a horizontal or vertical model.

Circle No. 92 on Reader Enquiry Card.

Fluid Equipment (Adelphi)

THREE variable speed laboratory stirrers are marketed by Fluid Equipment. The smallest mixes volumes of aqueous solution up to 1 litre and the largest mixes up to 100 litres. The largest, Model SL4, is a heavy duty laboratory stirrer and can be either stand or clamp mounted. It reaches speeds of up to 3,000 r.p.m. and its thyristor speed control gives greater power at low speeds.

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Adelphi SL4 stirrer

Flow Laboratories

DEVELOPED for use in tissue culture laboratories, 7X is an ideal detergent for glassware and instruments in all laboratories. As 7X is a solution it is simple to prepare the working concentration and there are no problems with undissolved particles. 7X cleans efficiently, gently and without etching and can be efficiently rinsed to give residue-free labware. For use in automatic washers Flow produce 7X O-matic.

Flow now offer three models of the Titertek plate shaker, designed to provide quick and effective re-suspension of cultures, RBCs, etc. Each shaker holds two microtitration plates via a central stud and two springs.

Circle No. 94 on Reader Enquiry Card.

Pool Bioanalysis Italiana

THE Agarster is a bench top agar steriliser unit specifically designed for the preparation and sterilisation of media under closely controlled conditions of time and temperature. Volumes of two to eight litres can be sterilised and cooled to a preset temperature in one hour or less depending upon the sterilisation time. Repeat batches take a shorter time since the unit retains some heat from the previous batch. Sterilisation temperature, sterilisation time and dispensing temperature are each preset at the start of the cycle. A water softening unit is incorporated within the unit to decrease 'furring' of the element and water jacket. Descaling can be carried out if necessary as the outer jacket is fully accessible and replacement of the element can quickly

be carried out. After sterilisation cooling water circulates in the outer jacket to cool the medium to the predetermined temperature. This temperature is maintained by alternately heating and cooling the outer jacket. Emptying of the vessel is achieved by connection of a stainless steel tube through a port on the lid and through a peristaltic pump like that used on the ADD plate pouring unit.

Circle No. 95 on Reader Enquiry Card.

Uniscience

THE new Uniscience Aquavac is a portable water jet vacuum unit which is designed to replace the laboratory tap operated water jet pumps, well known for their high water consumption and poor performance in areas of low water pressure. The Aquavac generates a constant high water pressure, independent of the water mains pressure, thereby giving constant and improved vacuums. The high air displacement up to 80 litres per min at 760 mm. Hg compared to tap operated water jet pumps (1.5 to 5.0 litres per min) provides rapid evacuation even for large volumes up to 50 litres. Recirculation of water provides a considerable saving of water. Only a small flow of water is required to the Aquavac to maintain the continuous removal of condensed products. The unit can safely handle the extraction of acid fumes and solvent vapours without the use of filters or freezing traps. It is available with 2 or 4 vacuum outlets and is ideal for laboratory and industrial scale processes including vacuum filtration and distillation, rotary evaporation, evacuation of drying ovens and dessicators, etc.

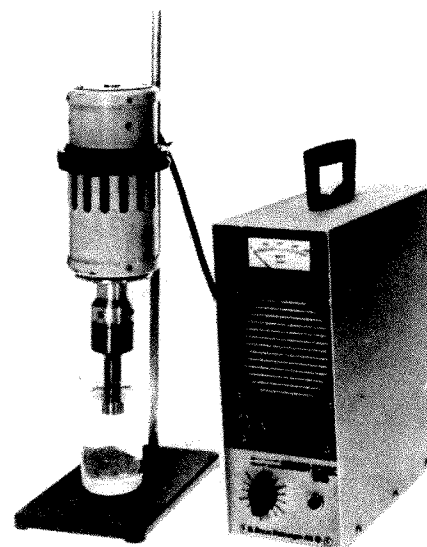
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B. Braun Melsungen

THE Potter-S tissue homogeniser ensures excellent reproducibility of results by the use of an electronically controlled drive unit operating between 150 and 1,500 r.p.m. and simple manual control of the plunger stroke length and frequency. Vessels with capacities from 2 to 60 ml are automatically aligned in a cooling jacket which has the important advantage of ensuring that there is no risk of breakage. Cell-free extracts from single-cell suspensions such as yeasts, bacteria and algae are obtained with the MSK cell disintegrator. The cells are mixed with glass micro-beads and oscillated at high speed while being cooled with liquid CO₂. High fragmentation yields of up to 99% are obtained with short homogenisation times. A high-output ultrasonic disintegrator, the Labsonic 1510, permits reproducible disintegration of all cell material such as mitochondria, chloroplasts and cell nuclei. A wide range of probes and cooling jackets

enable the Labsonic to be used for a wide variety of applications. Homogenisation of very tough materials such as connective tissue, cartilage, cancerous tissue, bone and dental enamel can be carried out using the Braun Microdismembrator.

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Braun Labsonic 1510 ultrasonic disintegrator

Newamatic

THE latest model F 570 Newamatic laboratory glassware and instrument washer is a compact bench top or plinth mounting machine offering all the facilities required in the busy modern laboratory. This sturdy machine is simple to load and operate. Apparatus to be washed is accommodated in baskets. 'Open' glassware and instruments may be loaded in simple baskets while narrower neck vessels, flasks, bottles, etc. are supported upright on racks with spigots. An exceptionally reliable pump and a patented spray and turntable system—the Jet system—ensures that water is projected on to surfaces to be cleaned with high velocity. This action, combined with fresh (not recycled) water rinses, adjustable wash temperature, the provision of up to two distilled water rinses and a built-in drier provides efficient cleaning at a competitive price.

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Italgas

A GLASSWARE washing machine specifically designed and constructed for the laboratory has been produced by Italgas. It efficiently washes all types of laboratory glassware, and although automatic it allows the operator to monitor and regulate all parameters in the easily operated, versatile washing procedure. Six interchangeable baskets cope with all types of glassware, and the push button control system allows unlimited programming.

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Applications are invited for the post of

SENIOR LECTURER/LECTURER IN THE DEPARTMENT OF BIOLOGY

Preference may be given to a person with experience and interest in animal and/or plant physiology. The appointee will be expected to assume duties by August 1, 1979, and not later than October 1, 1979. Salary Scales: Senior Lecturer BDS\$25,827 to BDS\$34,017 per annum. Lecturer BDS\$19,071 to BDS\$29,799 per annum (£1 sterling equals BDS\$4.107). F.S.S.U. Unfurnished accommodation will be let by the University at a rental of 10 per cent of salary. A housing allowance of 20 per cent of salary is payable to staff who make their own housing arrangements. Family passages. Study and Travel Grant. Detailed applications (three copies) giving full particulars of qualifications and experience, date of birth, marital status and the names and addresses of three referees should be sent as soon as possible to the Secretary, University of the West Indies, Cave Hill, P.O. Box 64, Bridgetown, Barbados. Applicants resident in UK should also send one copy to Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further particulars are obtainable from either address 478(A).

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

CANADA
DEPARTMENT OF BIOLOGY

INSECT ULTRASTRUCTURE BACTERIAL TAXONOMY/ ECOLOGY

Postdoctoral position available September 1, 1979. The position is initially for one year to study the structure of the peritrophic membrane and gut wall of selected forest pests and renewable for a second year. The second year will involve a taxonomic and spatial analysis of the insect gut bacterial flora. Initial salary \$12,900. Send curriculum vitae and names of three referees to Dr R. A. Nolan, Department of Biology, Memorial University of Newfoundland, St John's, Newfoundland, Canada A1B 3X9 490(A).

UNIVERSITY OF LONDON KING'S COLLEGE RESEARCH TECHNICIAN GRADE 5

Required to work with Solid State Physics Group. Main responsibilities will be the maintenance, development and setting up of research equipment and preparation of samples. Experience in electronic desirable. Salary on scale £3,989 rising to £4,580 per annum. Inclusive Five weeks annual holiday. Contributory pension scheme. Interest free advance for annual season ticket. Apply in writing with full details to The Head Clerk (Ref 214729/N), King's College London, Strand WC2R 2LS. 492(A).

ROTHAMSTED EXPERIMENTAL STATION HARPENDEN, HERTS AL5 2JQ PLANT PHYSIOLOGIST

to work in the Soils and Plant Nutrition Department on investigations into the distribution and properties of crop roots in relation to their absorption of nutrients and water, as part of a group working on root-soil relationships.

Qualifications. First or upper second class honours degree in botany or biology, with good background in chemistry, and at least two years' relevant post qualifying experience. Preference will be given to candidates with a doctorate or other post graduate experience in the field of plant physiology or ecology.

Appointment in grade of Higher Scientific Officer (£4,101 to £5,488) or Senior Scientific Officer (£5,154 to £6,898). At least four years' post-graduate research or other approved experience is required for appointment as S.S.O. Non-contributory superannuation.

Apply in writing to the Secretary giving names and addresses of two referees and quoting Ref 388 by May 18, 1979. Further details on request 457(A).

UNIVERSITY COLLEGE LONDON LECTURER IN PHYSIOLOGY

Applications are invited for a Lectureship in Physiology. Candidates will generally be expected to have a Ph.D. degree or/and a medical, veterinary or dental qualification. They should have at least three years' postdoctoral experience in research and preferably should have experience of working outside the U.K. Salary will be in the range £3,909 to £7,754 (under review) plus £502 London Allowance. Applications should include the names of three referees and should be submitted to the Assistant Secretary (Personnel), University College London, Gower Street, London WC1E 6BT by May 10, 1979, from whom further particulars may be obtained. 447(A).

UNIVERSITY OF LIVERPOOL DEPARTMENT OF MARINE BIOLOGY Port Erin, Isle of Man LECTURESHP IN MARINE BIOLOGY

Applications are invited for the above post tenable at the University's Marine Biological Station, Port Erin, Isle of Man. Applicants should have postgraduate experience in experimental marine biology, preferably in biochemical aspects of the physiology of marine animals, and will be expected to extend departmental teaching of courses in the physiology of marine invertebrates and fish.

The post is tenable from October 1, 1979 at an initial salary within the range £3,883 to £4,882 per annum on a scale rising to £7,754 per annum.

Applications, together with the names of three referees, should be received not later than May 18, 1979, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref RV/563/N 465(A).

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF CHEMICAL ENGINEERING LECTURESHP

A lecturer in Chemical Engineering is required, preferably with expertise in Biological Chemical Engineering. Some experience of Chemical Engineering drawing would be an advantage, but is not essential. The appointment will be from October 1, 1979 (or earlier by arrangement).

Salary will be on the scale £3,883 to £7,754 (under review), plus superannuation. Maximum starting salary will depend on age, experience and qualifications.

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (three copies) containing a full curriculum vitae and naming three referees should be sent by Monday, May 7, 1979.

Please quote ref. NT7 444(A)

Houghton Poultry Research Station LIAISON OFFICER

Applications are invited for the post of Liaison Officer, the duties of which include liaison with, and an information service for, the poultry industry, veterinary profession, Agricultural Research Council and Ministry, the Press, journals, educational organisations, and the preparation of publications and exhibits.

Applicants should possess a degree H.N.C. or equivalent in a science subject, with at least five years' relevant experience. Experience in public relations and editorial work would be an advantage.

Salary in Higher Scientific Officer grade (£4,101 by 8 to £5,448).

Further particulars from the Secretary, Houghton Poultry Research Station, Houghton, Huntingdon Cambs. PE17 2DA. Closing date for applications: May 11, 1979 446(A).

Wyeth Institute of Medical Research

Gastroenterology

We are seeking to appoint a graduate physiologist or pharmacologist with two or more years experience of research on drugs which affect gastric secretion.

This post could be suitable for someone completing a second degree. The successful applicant will join an existing team which is committed to the discovery and development of new treatments for peptic ulcers. We are looking for someone who will be able to make an immediate contribution to the work of the section.

Please apply to:

Mrs. J. Andrews, Personnel Officer,
Wyeth Laboratories,
Huntercombe Lane South, Taplow,
Maldenhead, Berks. Tel. Slough 28311.



Wyeth Laboratories

Chemical Mutagenesis

Shell Toxicology Laboratory (Tunstall)

The Cytogenetics and Mutagenesis section of the Pathology Division at our Sittingbourne Research Centre has a vacancy for someone experienced in mammalian cytogenetics. You will be studying the cytogenetic effects of agricultural and industrial chemicals including chromosome studies on mammals, cell cultures and human lymphocytes. Your work will be divided between conventional metaphase analysis and the development of new techniques for studying the effects of chemicals on meiotic and mitotic chromosomes. Emphasis is placed on the application of automated methods of chromosome analysis using an image analyser for metaphase finding.

You should have at least a good honours degree in a subject involving genetics, a knowledge of mammalian cytogenetics and about three years post graduate experience. Preference will be given to those possessing a Ph.D. in an area oriented towards mammalian cytogenetics, or who have experience of automated cytogenetic procedures.

We offer a competitive salary and assistance will be given with relocation expenses where appropriate. You will be able to join our pension fund, enjoy flexible working hours and work in well-equipped laboratories which are situated on a 450 acre farmland site. There are also excellent facilities for sport and social activities. Please write, giving all relevant details about yourself and your career, or telephone for an application form to.

Shell Research Limited, Recruitment Division, (N1) PNE/11, Shell Centre, London SE1 7NA. Telephone. 01-934 2948.

476(A)



Pharmacologist

Miles Laboratories Ltd. are an international pharmaceutical company based in an attractive part of South Buckinghamshire.

We are currently seeking a Pharmacologist to join a team working on the therapeutic role of prostaglandins in the respiratory tract. The person would be directly concerned with studies on the classification/nature of receptors associated with the varied actions of prostaglandins in the respiratory tract and other systems. Ideally we are seeking someone with a Ph.D. or alternatively, an Honours Graduate with post-graduate research experience in pharmacology.

In return we offer excellent conditions of employment, four weeks holidays, subsidised staff restaurant, free life and private health insurance, contributory pension scheme, flexible working hours.

For an application form please write or ring my secretary on Farnham Common 2151

Mrs. Nancy Morgans
Personnel Manager
Miles Laboratories Limited
Stoke Court, Stoke Poges
SLOUGH.

467(A)

UNIVERSITY OF GLASGOW DEPARTMENT OF ELECTRONICS AND ELECTRICAL ENGINEERING

THREE RESEARCH APPOINTMENTS

Acousto-optical Devices

Three posts, financed by a Ministry of Defence Research Contract for three years, are available immediately for research on an integrated acousto-optical device for microwave spectrum analysis.

The posts are

(A) One senior engineer or physicist with an optical physics, electronics or systems research background. Starting salary up to £6,793 on Range II of the salary scales for Research and Analogous Staff.

(B) One senior Research Technologist with a background in optical fabrication techniques such as polishing and grinding. Starting salary up to £6,793 on Range II of the salary scales for Research and Analogous Staff.

(C) A graduate assistant (possibly a recent graduate), with some background in optics or optical electronics. Starting salary up to £4,631 on Range IA of the salary scales for Research and Analogous Staff.

The above posts are superannuated

For further particulars, write to Professor John Lamb, Department of Electronics and Electrical Engineering, The University, Glasgow G12 8QQ, to whom applications (two copies) should be addressed, giving the names and addresses of at least TWO referees. State for which post the application is made. Closing date May 12, 1979.

In reply please quote Ref No 4431M

499(A)

UNIVERSITY OF MALAWI CHANCELLOR COLLEGE

Applications are invited for the post of

PROFESSOR OF MATHEMATICS

Applicants should have a good first degree and a higher degree plus considerable experience in university teaching and research with some administrative research. In addition to being Head of Department, the appointee will be expected to teach third and fourth year courses in his/her specialism. At present, third and fourth year courses are offered in algebraic structures, analysis, differential equations, linear algebra, number theory, numerical methods and statistics.

Salary scale: K7,900 to K10,300 per annum (£1 sterling equals K1.65) plus either a University addition of between K1,820 and K1,920 (taxable in Malawi) or the British Government may supplement salary by £5,310 per annum (sterling) for married appointee or £4,134 per annum (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages, superannuation scheme transferable with FSSU: Gratuity 15%-25%, biennial overseas leave, housing, various allowances.

Detailed applications (two copies) with curriculum vitae and naming three referees should be sent by air-mail to Registrar, University of Malawi, P.O. Box 278, Zomba, Malawi, by June 9, 1979. Applicants resident in UK should send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

479(A)

Biochemical Technician

Shell Research Limited is looking for a technician to join the Biochemical Pathology section of its Toxicology Laboratory at Sittingbourne in Kent. The purpose of the work is to assist with investigations into the effects of foreign compounds in animal tissues. You will be involved in dissecting, working with the subcellular fractionation of animal tissues, and enzyme activity measurements.

You should possess an HNC/D in Applied Biology and some experience in the above areas including the use of spectrophotometric, radiochemical and chromatographic techniques.

We offer a competitive salary and assistance will be given with relocation expenses where appropriate. You will be able to join our pension scheme, enjoy flexible working hours and make use of the excellent sports and social facilities. Please write giving all the relevant details about yourself and your career, or telephone for an application form to:

Mrs I Broodbank, Personnel Division, (N1), Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG. Telephone: Sittingbourne 24444 ext. 203.



435(A)

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified persons for appointment to the following position

The John Curtin School of Medical Research

DEPARTMENT OF MICROBIOLOGY

RESEARCH FELLOW

A position is available to work on the molecular biology of influenza viruses. In particular studies on the structural differences between variants selected with monoclonal anti-bodies. The major interests are (1) nucleic acid sequencing, using recombinant DNA technology, (2) immunochemical characterisation of the antigenic determinants of the haemagglutinin.

The appointment, which is funded by a grant from the National Institutes of Health, USA, is for one year in the first instance with probable extensions for a further two years. It is hoped that the successful applicant would be able to take up the appointment in the near future.

Salary: Commencing salary will be in accordance with qualifications within the range \$A15,786 to \$A17,716. Current exchange rates are \$A1 56p \$US1 13.

Other Conditions: A limited grant towards travel and removal expenses may be made. Assistance with housing is provided for an appointee from outside Canberra.

Applications should be sent to the Registrar, Australian National University P.O. Box 4, Canberra ACT 2600, Australia as soon as possible.

496(A)

UNIVERSITY OF BRISTOL POSTDOCTORAL RESEARCH ASSISTANT

Required from October 1, 1979 for a period of three years to join a group working on the pathogenesis of herpes virus infections. The project will be funded by the MRC and will be concerned with the interaction of herpes simplex virus with cells in culture at different stages of growth and differentiation. Experience of virology is desirable but applicants with a background of cell biology, biochemistry or pathology may be suitable. Starting salary up to £4,382 per annum (scale under review).

Further information from Dr T J Hill, Department of Bacteriology, Medical School, University Walk, Bristol BS8 1TD to whom applications, including a curriculum vitae and names of two referees 484(A)

UNIVERSITY OF GLASGOW DEPARTMENT OF CHEMISTRY POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Postdoctoral Research Assistantship, supported by the Science Research Council, to undertake research into the use of chiral lithium dialkylamides in organic synthesis. The post is tenable for one year from October, 1979. Salary will be within Range 1A of the salary scales for Research and Analogous Staff (£3,975 to £4,505), according to age and experience, with USS.

Applications enclosing a curriculum vitae and the names of two referees should be sent to Dr E W Colvin, Department of Chemistry, University of Glasgow, Glasgow G12 8QQ.

In reply please quote Ref No 4428M.

486(A)

UNIVERSITY OF OXFORD MICROBIOLOGY UNIT DEPARTMENT OF BIOCHEMISTRY

There is a vacancy for a postdoctoral assistant to work on either the genetics or the biochemistry of bacterial sporulation. The appointment, which will be for one year in the first instance, on the scale £3,883 to £6,555 (under review), may be extended for a further period of two years and there is provision for USS membership.

Application, which should include relevant details and the names of two referees, should be sent before May 30, 1979 to Professor J Mandelstam, Microbiology Unit, Department of Biochemistry, South Parks Road, Oxford OX1 3QU 472(A)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF VETERINARY PHYSIOLOGY

Applications are invited for the post of Lecturer in the Department of Veterinary Physiology. Preference will be given to candidates with experience in teaching physiology and with proven ability to carry out independent research.

Initial salary on the scale £3,883 to £7,754 per annum.

Applications together with the names of three referees, should be received not later than May 31, 1979, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref RV/559/N 438(A)

THE DISTILLERS COMPANY LIMITED Yeast and Food Division

MICROBIAL BIOCHEMISTRY

The Yeast and Food Division of The Distillers Company Limited require a graduate to work in areas of Microbial Biochemistry in their research and development laboratories, Glenochil Technical Centre, Menstrie, Clackmannanshire.

Applicants, who should be under 28 years old, must have a degree in microbiology, biochemistry or closely related subjects and preferably have postgraduate experience.

Further details together with an application form can be obtained by writing to: The Yeast Research Manager, The Distillers Company Limited, Yeast and Food Division, Glenochil Technical Centre, MENSTRIE, Clackmannanshire FK11 7ES.

305(A)

Plant Breeding in the tropics

Papua New Guinea is a fascinating country to take up a challenge and a fertile field in which to test your skills, knowledge, adaptability and above all, experience.

Your expertise can add weight to the growing cocoa industry which extends to a number of islands as well as the mainland of Papua New Guinea.

The main role of the post is to carry out a cocoa plant breeding programme including the introduction and development of new disease resistant material, selection, crossing and evaluation on behalf of the Cocoa Industry Board of Papua New Guinea.

Candidates should be honours graduates with a first degree in plant science and relevant post-graduate qualifications and/or several years experience in breeding of tree crops in the tropics. Experience in cocoa breeding would be a very considerable advantage.

The financial rewards are attractive. The basic annual salary will be around £11,500 plus generous gratuities based on length of service starting at 24% of basic salary. Taxation is low, a furnished house and transport are provided and a significant education allowance for children between 9 and 19 is available together with generous leave and air fare provisions.

Applications giving full details of qualifications, career to date and relevant personal details should be addressed in the first instance to Austin Knight Limited, Tricorn House, 51-53 Hagley Road, Edgbaston, Birmingham B16 8TP. Please mark your envelope "Cocoa Industry".

469(A)

UNIVERSITY OF EDINBURGH DEPARTMENT OF ZOOLOGY

Applications are invited for the post of Lecturer in the Department of Zoology. Salary scale £3,883 to £7,754 per annum (under review), with placement according to qualifications and age, and with superannuation benefit.

The successful candidate will be expected to take up duty on October 1, 1979.

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, with whom applications (six copies), giving the names of two referees, should be lodged not later than May 5, 1979. Please quote Reference 1032.

400(A)

PROFESSORSHIP IN MOLECULAR GENETICS

Seeking individual for Professorship in Molecular Genetics. Individual must be established investigator for research and teaching in molecular genetics.

Position in Cancer Center with broad laboratory research programmes including cellular genetics and development, tumour immunology, viral oncology, and chemical carcinogenesis.

Salary negotiable dependent on level of experience and academic rank of applicant. Send curriculum vitae and inquiries to: Chairman, Molecular Genetics Professor Search Committee, Cancer Center/Institute of Cancer Research, Columbia University, 701 West 168th Street, New York, New York 10032.

An Equal Opportunity Employer. W60(A)

SCOTTISH INSTITUTE OF AGRICULTURAL ENGINEERING

Head of Basic Cultivations Section
SENIOR SCIENTIFIC OFFICER/PRINCIPAL
SCIENTIFIC OFFICER

(Ref. AG/20)

A vacancy exists for a leader to head the Basic Cultivations Section at the Scottish Institute of Agricultural Engineering, and to be responsible for its day-to-day running. The work involves fundamental research, the design and development of machinery, field trials, and liaison with potential manufacturers on a number of projects in the fields of Basic Cultivation and Farm Transport. Some engineering aids to the sheep farmer may be included. Extensive support services are available (drawing office, workshops, field station, electronics and instrumentation, computing, photography, statistics). The section leader is expected to carry out a personal research programme and will report directly to the Head of the Agricultural Department.

Candidates should have a 1st or Upper 2nd class honours degree in engineering or an equivalent qualification, together with experience of engineering research. Agricultural experience or background and membership of an appropriate engineering institution are also desirable.

Appointment in the Grade of Senior Scientific Officer (£5,154 to £6,898) or Principal Scientific Officer (£6,609 to £8,461) according to experience. Non-contributory superannuation scheme.

Applications and further particulars from the Secretary, Scottish Institute of Agricultural Engineering, Bush Estate, Penicuik, Midlothian, EH26 0PH, to whom applications should be submitted by May 8, 1979.

434(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF PHYSICS

Applications are invited from suitably qualified candidates for a temporary post of RESEARCH FELLOW in the above Department under the auspices of the ICI Joint Research Scheme. The successful applicant would undertake research on molecular conformations and orientation in an active research group studying the properties and microstructure of polymers. The appointment will be made for a fixed period of three years. Applications are encouraged from those who will be available to start on or before October 1, 1979.

Salary on the IA scale for Research and Analogous Staff (£3,883 to £6,555) (under review), according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 52/4/D. Closing date for applications May 21, 1979.

463(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL (University of London) Denmark Hill, London SE5 8RX THROMBOSIS RESEARCH UNIT RESEARCH MEDICAL LABORATORY SCIENTIFIC OFFICER

Applications are invited from recently qualified graduates or candidates holding H.N.D./M.I. Biol or equivalent qualification for the post of Research Medical Laboratory Scientific Officer.

Work will involve investigation of platelet vessel-walled interaction using a variety of *in vitro* techniques. Experience in a biochemical or pharmacological laboratory would be an advantage.

Salary c £3,500 according to qualifications and experience. For further information please contact Dr J Westwick on 01-274 6222, Ext 2913.

Applications, giving full curriculum vitae and the names and addresses of two referees should be sent to the Secretary of the Medical School as soon as possible.

448(A)

UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF SOIL SCIENCE RESEARCH ASSOCIATE

Applications are invited for the post of Research Associate in the Department of Soil Science to work on chemical and selected physical problems of using soil covers in the reclamation of colliery spoil. The post, which is supported jointly by the Department of the Environment and the European Economic Community, has assured tenure until December 1980.

The appointment will be made at the appropriate point on the Range IA scale for research and analogous staff: £3,883 to £6,555 per annum, according to age, qualifications and experience. Membership of the appropriate university superannuation scheme will be required.

Further particulars may be obtained from the Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU, with whom applications (two copies) with brief curriculum vitae and the names and addresses of two referees, should be lodged not later than May 12, 1979. (Please quote reference Agric. 167.)

481(A)

UNIVERSITY OF CAMBRIDGE

Applications are invited for office of University Demonstrator (equivalent to Assistant Lecturer) in PHYSICAL CHEMISTRY as soon as possible. Person appointed, apart from undertaking normal teaching duties, expected to initiate research in solid state chemistry. Appointment for three years, with possibility of reappointment for two years.

Salary in scale from £4,133 to £5,127 per annum. Higher scales, forming the second part of the rectification of the anomaly in the remuneration of University teachers, have been agreed for introduction with effect from October 1, 1979. Negotiations are also at present proceeding for a cost-of-living increase to have effect from October 1, 1978.

Further information from Secretary of Appointments Committee, Faculty of Physics and Chemistry, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE, to whom candidates should send applications (three copies) together with names and addresses of not more than three referees, to reach him not later than May 9, 1979.

500(A)

UNIVERSITY OF SOUTHAMPTON SCHOOL OF BIOCHEMICAL AND PHYSIOLOGICAL SCIENCES GRADUATE RESEARCH ASSISTANT

Applications are invited for appointment as a Graduate Research Assistant in the Neurophysiology Group of this School, to work with Dr H V Wheal on aspects of the physiology and pharmacology of the crustacean neuromuscular junction. Applicants should have a good honours degree in physiology, pharmacology or related discipline and have an interest in neurophysiology.

The salary will be within £3,384 to £3,883 p.a. (award pending) on scale 1B for research and analogous staff, U.S.S. benefits.

Applications giving date of birth, curriculum vitae and the names and addresses of two referees, should be sent to Mrs P. Vaughan-Smith, The University, Southampton SO9 5NH by May 3, 1979, quoting reference 1063/R/Na.

482(A)

ROTHAMSTED EXPERIMENTAL STATION HARPENDEN, HERTS AL5 2JQ CHEMIST or PLANT SCIENTIST

with strong interest in plant nutrition required in Soils and Plant Nutrition Department, for work on soil-plant relationships of trace metals, in relation to both deficiencies and pollution problems.

Qualifications: 1st or upper 2nd class honours degree in agricultural chemistry, soil science or a plant science, and preferably postgraduate experience in plant nutrition.

Appointment in grade of Scientific Officer (£2,839 to £4,415) or Higher Scientific Officer (£4,101 to £5,448). At least two years' relevant postgraduate experience is required for appointment as H.S.O. Non-contributory superannuation.

Apply in writing to the Secretary giving names and addresses of two referees and quoting Ref. 392 by May 18. Further details on request.

458(A)

INSTITUTE OF ORTHOPAEDICS (University of London). RESEARCH ASSISTANT, Honorary Graduate in Biology, to participate in research into human hip disease. Suitable for M.Phil/Ph.D. Three-year Arthritis & Rheumatism Council grant. Information from and applications to the Secretary, Institute of Orthopaedics, Brockley Hill, Stanmore, Middlesex HA7 4LP, with names of two referees.

487(A)

An investigatory and advisory role for Pharmacists

£4,850-£7,585

These posts within the Scientific and Technical Branch of Supply Division offer the opportunity to join a professional, London-based team providing advice, information and consultancy services to pharmaceutical and related departments in hospitals, to the pharmaceutical and medical supplies industries, and to government organisations (both at home and abroad).

The wide range of work undertaken includes participating in the development of national and international standards, promoting research and development of new and improved pharmaceuticals and medical equipment, advising hospitals on the selection and use of such products, and liaising between industry and the N.H.S. on users' needs and problems. Presentation of papers at professional conferences is encouraged.

The current vacancies are concerned with pharmaceutical supplies and sterile single-use medical

devices including renal dialysis and blood transfusion equipment. The work involves the inspection of manufacturing facilities and quality control procedure both in the UK and abroad. Assessment of products includes suitability for intended use, the biocompatibility of materials, stability and mechanical safety, and packaging and sterilisation methods.

For more information about the work, telephone Dr Kay (ext 3379) or Miss Robotham (ext 3124) on 01-636 6811.

Candidates must have a degree (or equivalent qualification) in Pharmacy or a closely related field, and relevant professional experience preferably including some in the manufacture or control of pharmaceutical or medical products or in their procurement in the N.H.S.

Starting salary between £4,850 and £7,585 depending on qualifications and experience. Salary under review. Promotion prospects: Non-contributory pension scheme.

For full details and an application form (to be returned by 17 May 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68651 (answering service operates outside office hours). Please quote 7/5060/2. 433(A)

Department of Health and Social Security

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY RESEARCH OFFICER— ANALYTICAL CHEMIST

We are seeking an energetic Analytical Chemist with a sound background in trace metal analysis and laboratory management experience. Duties include training and some teaching in analytical procedures, method research and development.

Minimum qualifications B.Sc. (Chemistry) or equivalent. Commencing salary, in the range £3,883 to £6,555 plus London Allowance, depending on age and experience.

For details and application form contact Dr I. Thornton, Applied Geochemistry Research Group, Geology Department, Imperial College, London SW7 2BP. 491(A)

QUEEN MARY COLLEGE University of London ASTRONOMY—APPLIED MATHEMATICS

Applications are invited for posts with Professor I. W. Roxburgh on the following S.R.C. supported projects:

(a) POSTDOCTORAL RESEARCH ASSISTANT for three years, using the Distributed Array Processor to study problems of convection and turbulence in the sun and stars.

(b) RESEARCH ASSISTANT/RESEARCH PROGRAMMER for two years to work on (1) Numerical Models of the Solar Wind, and (2) Numerical Simulation of space experiments to test theories of gravity. Initial salary in range (under review) £3,883 to £4,382 per annum plus £502 London Allowance.

Applications, including age, qualifications and experience and names of two referees as soon as possible to The Registrar, (N) Queen Mary College, Mile End Road, London E1 4NS. 494(A)

SHRINERS HOSPITAL FOR CRIPPLED CHILDREN Montreal, Canada POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the post of postdoctoral research assistant in the Joint Diseases Laboratory. The applicant should possess a Ph.D. in Biochemistry or a closely related subject, and preferably have gained experience in techniques for studying the structure of biological macromolecules. The project will be concerned with investigating the structure of the complex protein-polysaccharides (proteoglycans) present in hyaline cartilage.

The post is available for an initial period of two years, with a starting salary of \$14,000. Applications, with curriculum vitae, should be sent to Dr P. Roughley, Joint Diseases Laboratory, Shriners Hospital for Crippled Children, 1529 Cedar Avenue, Montreal, Quebec, Canada. W68(A)

UNIVERSITY OF CAMBRIDGE LUCASIAN PROFESSORSHIP OF MATHEMATICS

Applications invited for the above Chair which will become vacant on September 1, 1979, by the resignation of Professor Sir James Lighthill. Candidature is limited to persons whose work is connected with some aspect of Applied Mathematics and Theoretical Physics and the Professor will be accommodated in the Department of Applied Mathematics and Theoretical Physics.

Present pensionable stipend (under review) £10,939.

Applications (10 copies) marked 'Confidential' should be sent to the Secretary General of the Faculties, from whom further information may be obtained, at the General Board Office, The Old Schools, Cambridge CB2 1TT. Names of two referees may be submitted if desired. Closing date, May 25, 1979. 475(A)

Athrofa Gogledd-dd Cymru The North E Wales Institute of Higher Education

POSTDOCTORAL SCIENTIST

Applications are invited for a postdoctoral research post to work on Lipid Oxidation in Milk Products. The appointment will be for at least two years, with salary in the range £3,910 to £4,408 per annum plus superannuation. The work is sponsored by the Dairy Industry and will be undertaken at the North East Wales Institute, under the guidance of Dr J. C. Allen.

Applications should be made before May 11, 1979, to The Institute Registrar, North East Wales Institute of Higher Education, Keirsterton College, Connah's Quay, Deeside, Clwyd, giving full details of qualifications and experience, also the names of two referees. 485(A)

TECHNICIAN/RESEARCH OFFICER

required for Tumour Virology Laboratory, for work involving tissue culture (preparation of primary cultures and handling of normal and virus-infected cell lines), virus assays, cytological techniques (cell fusion, karyology) and nucleic acid biochemistry (DNA extraction, enzyme assays, hybridisation). The technician will share routine laboratory duties but would also work on research projects without day-to-day supervision.

Degree/HNC required plus at least two years relevant laboratory experience. Salary range £3,582 to £4,902 plus L.W. Appointment available May 1979. For application form write or telephone Miss S. M. Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, W.C.2 on 01-242 0200 ext 305. 412(A)

SCIENCE MUSEUM ASTRONOMY

Assistant Keeper

... to take charge of the Astronomy Collection and either the Meteorology or Geodesy and Surveying Collection.

The work includes the acquisition of new material to fill gaps in the existing collections and to expand the modern astronomy section; preparing various publications; assisting the serious student of the collections; maintaining and expanding liaison with university and government departments and with industry. The successful candidate will be expected to become a recognised authority on aspects of the collection. Opportunity to present papers to conferences, at home and abroad.

Candidates must have a degree with first or second class honours (or equivalent) which includes astronomy and either meteorology or surveying. They should have a sound knowledge of modern astronomy, an imaginative approach and the ability to communicate clearly and concisely with non-specialists. Postgraduate degree, and working knowledge of French, German or Latin, advantageous.

Salary: as AK First Class £5,915—£8,875; AK Second Class £3,635—£5,365. Level of appointment and starting salary according to age, qualifications and experience. *Salaries under review.* Non-contributory pension scheme.

For full details and application form (to be returned by 10 May 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants. RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote G(22)382.* 436(A)

KENSINGTON AND CHELSEA AND
WESTMINSTER AREA HEALTH AUTHORITY (T)
North West District

Medical Laboratory Scientific Officer/ Junior Medical Laboratory Scientific Officer

Biochemistry Department

The laboratory has a specialist responsibility for hormone radio-immunoassay but full training is available within the District.

Applicants should be state registered although a junior medical laboratory scientific officer studying for HNC (Clinical Chemistry) will be considered.

For further details telephone Mr. Vermont, Senior Chief Medical Laboratory Scientific Officer, extension 357. Application forms available from Personnel Department, St. Charles Hospital, Exmoor Street, London W10 6DZ, telephone: 01-969 2488 extension 354. (468(A))

**st
Charles'
hospital**

UNIVERSITY OF ABERDEEN DEPARTMENT OF BACTERIOLOGY LECTURESHP

Applications are invited for the above post from suitably qualified graduates in medicine or science. The Department has interests and facilities in Bacteriology, Virology and Immunology. The successful candidate will participate in the diagnostic service, undertake research, and take part in teaching of medical and science students.

Salary scales (under review): Clinical £4,298 to £7,696; Non-clinical £3,883 to £7,754. Appropriate Honorary Hospital Status would be sought.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by 18 May, 1979. 452(A)

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF BOTANY POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a post-doctoral research assistantship to work on the synthesis of enzymes of nitrogen assimilation in wheat. The post, which is supported by the Rank Prize Funds, is for a period of three years from October 1, 1979. The salary will be on the scale 1A, starting at £3,883 p.a.

Applications with curriculum vitae and the names and addresses of two referees should be sent as soon as possible to Dr J. C. Gray, Department of Botany, University of Cambridge, Cambridge CB2 3EA, from whom further particulars may be obtained. 453(A)

UNIVERSITY OF QUEENSLAND Brisbane, Australia DEPARTMENT OF MINING AND METALLURGICAL ENGINEERING POSTDOCTORAL RESEARCH ASSOCIATE

To study phase transformations in inorganic materials with special reference to reactions of gases with metal oxides. Research, to be carried out under the supervision of Dr P. Hayes, will involve studies of both reaction kinetics and product morphology.

Applicants should hold or be about to complete a Ph.D. in Metallurgy or Physical Chemistry; experience in computing and electron microscopy desirable. Starting date as soon as possible.

Salary SA13,083 to SA14,987. Assistance with travel is available for successful overseas applicant.

Applications, quoting reference No. 15179, with curriculum vitae and the names of two referees, should be sent as soon as possible to The Staff Officer, University of Queensland, St Lucia, Queensland, 4067, Australia. 451(A)

UNIVERSITY OF LIVERPOOL ORTHOPAEDIC AND ACCIDENT SURGERY TECHNICIAN

for research project concerning the biological approach to replacement of arthritic joints. Facilities are available for cartilage and bone cell culture. Previous experience of immunological techniques desirable, but training given. Candidates must possess H.N.C. or an appropriate equivalent qualification and have had several years' laboratory experience. Salary within a range up to £4,056 per annum according to qualifications and experience. Application forms available from the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref: RV/558/N. 439(A)

MEDICAL RESEARCH COUNCIL TOXICOLOGY UNIT NEUROPHYSIOLOGIST

A neurophysiologist is required to initiate a research programme on the mechanisms of action of toxic substances on the central nervous system. Experience in electrophysiological recording in rodents would be an advantage.

The appointment will be temporary but for a term of at least three years. Salary on M.R.C. Scientific Staff scales depending on age and experience. Applications, together with a full curriculum vitae and the names of two professional referees, should be lodged with The Director, M.R.C. Toxicology Unit, Woodmansterne Road, Carshalton, Surrey SM5 4EF. 474(A)

UNIVERSITY OF OXFORD DEPARTMENT OF PSYCHIATRY MEDICAL LABORATORY SCIENTIFIC OFFICER

A Medical Laboratory Scientific Officer is required for two years to assist with research in clinical pharmacology of psychotropic drugs. The work involves analysis of drugs and hormones in biological fluids. The techniques used include radioimmunoassay, GLC, and HPLC.

The person appointed should have a degree, and H.N.C. or an equivalent qualification preferably in chemistry or a related subject. Salary on Whitley Council M.L.S.O. scale £3,261 to £4,680 per annum.

Application with Curriculum Vitae and the names and addresses of two referees to Mr M. Franklin, Department of Psychiatry Research Unit, Littlemore Hospital, Oxford OX4 4XN. 473(A)

UNIVERSITY OF LEICESTER DEPARTMENT OF PHYSICS RESEARCH DEMONSTRATOR

Applications are invited for the post of Research Demonstrator in the Department of Physics. He/she will be required to undertake, under the direction of the Head of Department, tutorial or demonstrating work within the Department up to a maximum of twelve hours a week; he/she is not expected to give lecture courses. The successful applicant will be required to work for the degree of Doctor of Philosophy. Preference will be given to candidates who wish to do research in the field of disordered matter or metal surfaces but those with related interests would also be considered.

Salary within the range £3,215 to £3,384 to £3,634.

Further particulars and application forms obtainable from the Registrar. 466(A)

UNIVERSITY OF OXFORD POSTDOCTORAL SCIENTIFIC ASSISTANT

Applications are invited for a post-doctoral scientific assistant position (awarded to January 31, 1982) on an M.R.C. Programme Grant concerned with studies on the endocrine control of parturition in sheep and man. Previous experience of separation techniques for protein and steroid hormones would be an advantage but is not essential. Salary on the scale £3,383 to £6,555.

Applications giving details of qualifications and experience, together with the names and addresses of two referees should be sent to Professor A. C. Turnbull, Nuffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital, Headington, Oxford OX3 9DU. Closing date for applications May 12, 1979. 471(A)

The Research Division of Synthelabo located in
PARIS
requires a
SENIOR CARDIOVASCULAR PHARMACOLOGIST
to join its Department of Biology

The high success rate and rapid growth of this pharmaceutical group is related to the quality of people they employ and their continued commitment to research and development.

For this key position in the Cardiovascular Group we require a professional and highly motivated scientist who will be responsible for various projects within the group and should also be able to conduct independent research programmes.

Applicants can be either male or female and should have relevant postdoctoral experience (a maximum of five years). It is essential that the candidate should have experience of the pharmaceutical industry.

A realistic salary can be expected and relocation assistance will be available with other financial benefits.

All applications will be treated in the strictest confidence and should be sent directly to:

"NATURE"
c/o Box No. W67(A) 3 DYERS BUILDINGS
HOLBORN, LONDON EC1N 2NR

W67(A)

QUEEN MARY COLLEGE
University of London
ZOOLOGY AND COMPARATIVE
PHYSIOLOGY DEPARTMENT

Applications are invited for a
**TEMPORARY
LECTURESHIP**

for one year from October 1 (possibility of extension for second year), to undertake teaching of general ecology courses with a special interest in freshwater biology. Willingness to participate in existing research project on stream ecology an advantage. Salary in range (under review) £3,975 to £6,095 per annum plus £502 London Allowance. Please apply by letter, enclosing curriculum vitae, list of publications and names of three referees, to The Registrar (N), Queen Mary College, Mile End Road, London E1 4NS, not later than May 15 450(A)

**UNIVERSITY OF
BRISTOL**
SCHOOL OF CHEMISTRY
**POSTDOCTORAL
RESEARCH ASSISTANT**

Applications are invited for an S.R.C. supported Postdoctoral Research Assistantship (Range 1A) for work on the synthesis of medium-ring bridged bicyclic compounds with functionalised carbon and heteroatom (particularly boron) bridgeheads. The successful applicant must have experience in organic or organometallic synthesis and preferably be familiar with the handling of air sensitive materials.

Commencing salary up to £4,382 per annum (scale under review).

Applications should be sent to Dr R. W. Alder, School of Chemistry, University of Bristol, Bristol BS8 1TS and should be accompanied by a brief curriculum vitae and the names of two referees 483(A)

**ROTHAMSTED
EXPERIMENTAL STATION**
HARPENDEN, HERTS AL5 2JQ
STATISTICIAN

to join a group within the Statistics Department engaged in the design and analysis of sample surveys. Applicants will be expected to have some knowledge of sampling theory, an interest in survey methods and preferably also in agriculture, and the work will involve use of a general programme for survey analysis.

Qualification: Honours degree or equivalent in Statistics, preferably with some interest in and knowledge of agriculture, or an ordinary degree with relevant experience.

Appointment in grade of Scientific Officer (£2,839 to £4,415 per annum). Starting salary according to qualifications and experience. Non-contributory superannuation.

Apply in writing to the Secretary giving names and addresses of two referees and quoting Ref 393 by May 10, 1979. Further details on request 459(A)

**UNIVERSITY OF
LIVERPOOL**
DEPARTMENT OF GEOLOGY

Applications are invited for the post of TEMPORARY LECTURER in the Department of Geology. The appointment will be for twelve months only. The successful candidate will be required to teach sedimentology throughout the three-year Honours course in Geology, to teach some elementary geology, to assist with field classes and to supervise the work of two research students.

Salary according to qualifications and experience within the range £3,883 to £4,382 per annum.

Applications, together with the names of three referees, should be received not later than 7th May, 1979, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/562/N. 437(A)

THE UNIVERSITY OF CALGARY

DEPARTMENT OF BIOLOGY
**PLANT TAXONOMY
AND BIOSYSTEMATICS**

Applications are invited for a faculty position at the assistant professor level, starting August 1, 1979. The successful candidate will be expected to teach in junior undergraduate courses in the taxonomy of lower and higher plants and in advanced courses in experimental angiosperm systematics. He/she will also be expected to develop an active research program, and to supervise graduate students.

A Ph.D. degree is required and postdoctoral and/or teaching experience desirable. Send curriculum vitae, a brief statement describing your research interests, and the names and addresses of three referees to Dr. D. Parkinson, Biology Department, University of Calgary, Calgary, Alberta, Canada T2N 1N4. Closing date for application is May 15, 1979.

W67(A)



Agriculture Development and Advisory Service PLANT PATHOLOGISTS

... to diagnose plant diseases caused by fungi, bacteria and viruses, and to assess the damage and losses caused by plant diseases, as well as seeking preventive and control measures.

Candidates (normally aged under 35) must have a good honours degree or equivalent qualification in agricultural or natural science, or applied biology that has included a specialised study of plant pathology or mycology. They must also have at least 3 years' relevant post graduate experience.

Salary starting between £4,175 and £5,770, rises to £6,895, according to age, qualifications and experience. *Salaries under review.* Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 14 May 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote ref: S(N)906.*

497(A)

MINISTRY OF AGRICULTURE
FISHERIES AND FOOD



THE WORLD HEALTH ORGANIZATION

invites applications for a post of STATISTICIAN to assist the Onchocerciasis Control Programme operating in West Africa, with duty station in Geneva.

Duties will involve the preparation of schemes for planning statistical services to epidemiological and entomological activities of the Programme, the statistical processing of data and the development of appropriate methods for its analysis, and the preparation of evaluation reports. Visits to the Programme area will be made as required. Applicants should have a university degree or equivalent, with emphasis on mathematical statistics, and several years' experience in the analysis and handling of statistical data relating to biological, medical and public health problems. Experience in computer application required, and an excellent knowledge of English or French with a working knowledge of the other language.

Applications, quoting reference VN/OCP P79/17 should be addressed to:

World Health Organization
Personnel
CH-1211 Genève 27

Only candidates under serious consideration will be contacted.
W65(A)

UNIVERSITY OF WESTERN AUSTRALIA Perth TUTORSHIP IN MICROBIOLOGY

Applications are invited for the above appointment which will be for a period of three years. The minimum qualifications are a B.Sc. degree with Honours. Duties include the responsibility of demonstrating for Science, Agriculture and Dental Science undergraduate laboratory classes, as well as the co-ordination of these programmes and tutorials within the total teaching programme. Current total student numbers are approximately 160 from 2nd and 3rd year levels. Current salary range: \$A11,220 to \$A13,210 per annum. The appointee will be entitled to fares to Perth for self and fully dependent family and to a removal allowance, and to similar return benefits to place of engagement on completion of three years' service.

Applications in duplicate stating full personal particulars, qualifications and experience should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia, 6009, by May 12, 1979. Candidates should request three referees to write immediately to the Staffing Officer.

480(A)

JOHN INNES INSTITUTE DEPARTMENT OF APPLIED GENETICS

Applications are invited from geneticists with interests in the development and physiology of higher plants to work on the control of the determinate habit in peas.

This project will be of direct relevance to the Institute's programmes on the production of new varieties of leafless peas and Rhizobium genetics, and will interrelate with other studies on the physiology and seed protein biochemistry of peas. Further particulars may be obtained from Professor D. Roy Davies.

Appointment will be as Higher Scientific Officer (£4,101 to £5,448) or Senior Scientific Officer (£5,154 to £6,898), grade and starting salary depending on experience. At least two years' relevant postgraduate experience is required for appointment as Higher Scientific Officer, with four years for Senior Scientific Officer. Preference will be given to candidates with a Ph.D. in Genetics. Non-contributory superannuation.

Applications, quoting reference AG/35, giving full particulars and the names of two referees, should be sent to the Secretary, John Innes Institute, Colney Lane, Norwich NR4 7UH, not later than May 11, 1979.

488(A)

ROTHAMSTED EXPERIMENTAL STATION HARPENDEN, HERTS. AL5 2JQ

Applications are invited from suitably qualified scientists for the post of Head of the Insecticides and Fungicides Department to succeed Dr I. J. Graham-Bryce, who has been appointed Director of East Malling Research Station, with effect from August 1, 1979.

The Insecticides and Fungicides Department has over 50 scientific staff and receives many visiting workers. The scientist appointed will be expected to lead a multidisciplinary research programme seeking to make the chemical control of pests and diseases more effective and safer.

Appointment in the grade of S.P.S.O. £10,043 by two increments to a maximum of £11,300. Non-contributory superannuation.

Apply in writing to the Secretary, giving names and addresses of two referees, and quoting Ref. 385 by June 1, 1979. Further details on request.

456(A)

ROTHAMSTED EXPERIMENTAL STATION HARPENDEN, HERTS. AL5 2JQ AGRONOMIST/CHEMIST

required in the Soils and Plant Nutrition Department to join a small team working on a varied programme of field experimentation and associated laboratory work, mainly concerned with fertilisers and plant nutrition.

Degree, H.N.C. or equivalent qualification in soil science, agricultural chemistry or agricultural science, experience of field experimentation would be an advantage, and a driving licence is essential.

Appointment in grade of Scientific Officer (£2,839 to £4,415), or Higher Scientific Officer (£4,101 to £5,448). Acceptable post-qualifying experience is required for appointment to the higher grade. Starting salary according to qualifications and experience. Non-contributory superannuation.

Apply in writing to the Secretary, giving names and addresses of two referees, and quoting Ref. 381 by May 11, 1979. Further details on request.

455(A)

UNIVERSITY OF GLASGOW DEPARTMENT OF ELECTRONICS AND ELECTRICAL ENGINEERING RESEARCH POST IN INTEGRATED OPTICS

A post financed by an S.R.C. Research Grant, tenable until September 30, 1981, is now available for a person with a background in experimental solid-state chemistry or materials science to work with a large integrated optics group on electro- and acousto-optic devices. Starting salary up to £5,129 (interim) within Range 1A of the salary scale for Research and Analogous Staff.

For further particulars write to Professor John Lamb, Department of Electronics and Electrical Engineering, University of Glasgow G12 8QQ, to whom applications (two copies) should be addressed, giving the names and addresses of at least two Referees. Closing date May 25, 1979.

In reply please quote Ref. No. 4429M.

462(A)

QUEEN MARY COLLEGE University of London FLUID MECHANICS ON THE DISTRIBUTED ARRAY PROCESSOR

The Distributed Array Processor, a fundamental development in computer architecture, is particularly suited to solving the Navier-Stokes equations for fluid motion. S.R.C. is funding research here into the Large Eddy Simulation of turbulence and has awarded a grant for application of the DAP to this work. Applications for this post are invited from those with relevant experience of large programmes (DAP uses a variant of FORTRAN); preference will be given to those with a knowledge of fluid mechanics.

Initial salary in range (under review) £3,883 to £4,382 per annum plus £502 London Allowance. Further details obtainable from Professor D. C. Leslie, Nuclear Engineering Department, while applications, with curriculum vitae, publications and names of two referees, should be sent to The Registrar (N), Queen Mary College, Mile End Road, London E1 4NS.

493(A)

MAX-PLANCK-INSTITUT FÜR BIOCHEMIE AT MARTINSRIED NEAR MUNICH

The
Deutsche Stiftung für Krebsforschung,
together with the
Max-Planck-Gesellschaft zur Förderung der
Wissenschaften e.V.,

offers

A YOUNG SCIENTIST

the opportunity to set up a research group in the above institute in the field of basic, medically oriented tumor research and to conduct his/her research programme independently.

Fields of activity of the institute are biochemistry, physical biochemistry, molecular biology, structure research, genetics, cellular biology, and virus research. There also exist good opportunities for cooperative links with clinics.

Applicants should already have accomplished internationally recognised research; their special field of research should fit into the frame of the institute's activities.

Work should start at the earliest possible date. The appointment salary will be made for an initial period of five years. The salary will be calculated according to group AH3 of the Niedersächsische Besoldungsgesetz.

The group will be provided with up to two positions for German or foreign scientific co-workers and with up to three positions for technical staff. It will be given its own laboratory, appropriate equipment and adequate operating funds.

Applications should include a tabular curriculum vitae, a list of publications, and a short description of scientific work, both achieved and intended, and should be sent to

Dr. Mildred Scheel — Deutsche Stiftung für Krebsforschung
— Villa Hammerschmid — D-5300 Bonn-Bad Godesberg — no
later than May 31, 1979. W64(A)

Universiteit van Amsterdam

Applications are invited for a teaching and research post

lecturer (m/f)

(wetenschappelijk medewerk(st)er)

in the Department
of Structural Geology.

Duties will include: (1) courses of lectures and practicals in the microstructural interpretation of the relationship between deformation and metamorphism, and of deformational mechanisms, (2) research in the field of structural geology, and (3) help with general teaching and administrative duties in the Department.

Salary in accordance with background and experience, Ph.D. required.

Applications, with curriculum vitae, precise research interests and the names of at least two referees, should be sent to
Dr. O. J. Simon, Geologisch Instituut,
Nieuwe Prinsengracht 130,
1018 VZ Amsterdam, The Netherlands,
before June 1st 1979.

W63(A)

FELLOWSHIPS

UNIVERSITY OF SUSSEX

SCHOOL OF MATHEMATICAL AND PHYSICAL SCIENCES ASTRONOMY DIVISION RESEARCH FELLOWSHIP IN OBSERVATIONAL EXTRAGALACTIC ASTRONOMY

Applications are invited for a Postdoctoral Research Fellowship in Observational Extragalactic Astronomy, to commence as soon as possible after October 1, 1979. The post will be for two years, with possible extension to a third.

The Research Fellow (M/F) will be expected to collaborate with the other observational Research Fellows at the Astronomy Centre, and with visiting faculty members based at the Royal Greenwich Observatory in carrying out spectrometric and photometric observations of extragalactic radio and X-ray sources and peculiar galaxies in general in the Southern Hemisphere.

Commencing salary will be in the range £3,883 to £6,555 per annum (under review), with U.S.S. benefits.

Further information can be obtained from Professor L. Meisel, Astronomy Centre, Physics Building, University of Sussex, Falmer, Brighton BN1 9QH, to whom applications, and the names of three referees, should be sent by May 31, 1979. 445(E)

UNIVERSITY OF SOUTHAMPTON DEPARTMENT OF PHYSICS RESEARCH FELLOWSHIPS IN CRYOGENICS

Applications are invited for Postdoctoral Fellowships to work on the following projects:

1. Heat transfer in the reboiler/condensers of cryogenic plant, using model rigs and a full length test section.
2. Complex convective flow in stratified cryogenic vapours and liquids using Laser Doppler Anemometry.
3. Heat transfer and fluid behaviour of helium at 3,000 r.p.m. for the development of large superconducting machines.

Applicants should have experience in mechanical/chemical engineering or low temperature physics.

Salary in range up to £5,367 plus U.S.S. benefits.

Applications with names of two referees should be sent to Mrs E. C. P. Sears, Staffing Section, The University, Highfield, Southampton SO9 5NH. Please quote reference number 1607/R/N. 441(E)

BRANDEIS UNIVERSITY Rosenstiel Center

A postdoctoral fellowship is available for the study of structure and assembly of tobacco mosaic virus by X-ray diffraction from oriented gels. Extension and refinement of present 4 Å structure, study of other helical TMV protein aggregates and of related viruses. Project is related to larger program of Dr D. L. D. Casper on virus structure. Computing ability is desirable and preferably crystallography, protein chemistry or biological structure experience. Available immediately, for up to two years \$12,500-\$15,000.

Contact Dr G. Stubbs, Rosenstiel Center, Brandeis University, Waltham, MA. 02154, USA. (617)-647-2467 W55(E)

THE ROYAL SOCIETY The Smith & Nephew Foundation SENIOR RESEARCH FELLOWSHIP

Applications are invited by the Council of the Royal Society for The Royal Society Smith & Nephew Foundation Senior Research Fellowship, established by means of a five-year grant from the Smith & Nephew Foundation for research in organic chemistry or biochemistry. The appointment will be tenable in any appropriate university department or research institution in the United Kingdom approved by the Council of the Royal Society. Candidates, who must be citizens of the United Kingdom or the Commonwealth, must supply the usual personal details and give two referees. Testimonials will not be considered. The subject of the proposed research, and where it would be done, together with the name of the head of the department, whose consent should first be obtained, must be stated.

The appointment, which will be subject to the Society's general regulations governing research appointments, will be tenable for five years from October 1, 1979 (or another date to be arranged). Applicants should be under 40 on October 1, 1979. The present stipend scale is £8,031 by £252 to £8,787 by £249 to £9,036 per annum, and the point of entry will be determined by Council. Superannuation benefits will be provided. Some provision for research expenses will be available.

Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, and returned by June 4, 1979. 443(E)

FLINDERS UNIVERSITY OF SOUTH AUSTRALIA SCHOOL OF BIOLOGICAL SCIENCES POSTDOCTORAL RESEARCH FELLOWSHIP IN VIROLOGY

Applications are invited for a Postdoctoral Research Fellowship in the Microbiology Group of the School of Biological Sciences. The successful applicant will work with a small group studying the biochemistry of the anti-tumour action of interferon, with particular reference to the role of 2'-5' polyadenosine oligonucleotides in mediating the growth-inhibitory properties of interferon. Experience with interferon biochemistry, nucleotide chemistry or cell-cycle biology, while not essential, would be an advantage.

The Fellowship (funded by the Anti-Cancer Foundation) is available immediately and is renewable on an annual basis until December 1981. Further particulars may be obtained from Professor E. M. Martin of the School of Biological Sciences.

Salary: \$A14,400 to \$A15,586 per annum.

Applications, which should include a full curriculum vitae, list of publications, and the names of at least two referees, should be addressed to The Registrar, The Flinders University of South Australia, Bedford Park, South Australia, 5042; they should reach Australia by April 27, 1979, but will be accepted for a further seven days. 477(E)

COURSES

HAZARD ANALYSIS

A Post Experience Course will be held at Imperial College from June 25 to 28, 1979. Details from Dr D. H. Napier, Chem. Eng. and Chem. Tech., Imperial College, London SW7 2BY. Tel 01-589 5111, Ext. 1906 448(D)

STUDENTSHIPS

HILL FARMING
RESEARCH
ORGANISATION
A.R.C. RESEARCH
STUDENTSHIP

Applications are invited for an Agricultural Research Council Studentship tenable for 3 years from October 1, 1979 at the Hill Farming Research Organisation for research leading to a higher degree. The research topic to be investigated concerns the nutritional and animal performance consequences of the type, composition and amount of supplementary feeding given to pregnant sheep grazing hill vegetation in winter.

Applicants should possess, or expect to obtain, a 1st or Upper 2nd class honours degree in Agricultural Science or a related subject.

The award which is under review comprises a tax free maintenance allowance according to circumstances (£1,610 per annum for a single person living in rented accommodation) approved University Fees, some travelling expenses and dependent allowances where appropriate.

Further details and application forms can be obtained from the Secretary, Hill Farming Research Organisation, Bush Estate, Penicuik, Midlothian EH26 0PY. Applications should be received by May 11, 1979. 432(F)

LANCHESTER
POLYTECHNIC
AT
COVENTRY
FACULTY OF
APPLIED SCIENCE
DEPARTMENT OF
BIOLOGICAL SCIENCES
N.E.R.C. C.A.S.E. RESEARCH
STUDENTSHIP

THE JOB, to work on the respiratory, physiology and energetics of Antarctic protozoa. The project will be jointly supervised by the Department of Biological Sciences at the Polytechnic and the Life Sciences Division of the British Antarctic Survey.

REQUIRED applicants should have, or expect to obtain, a first or upper second class honours degree in biological science and an interest in ecology, soil microbiology or invertebrate zoology.

For application form and job specification please apply in writing enclosing a large stamped and addressed envelope to the Personnel Officer, Lanchester Polytechnic, Priory Street, Coventry. (Informal enquiries to Dr H. G. Smith, telephone (0203) 24166, Ext. 629) returnable by Friday, May 4, 1979. 470(F)

UNIVERSITY OF
NEWCASTLE UPON TYNE
M.R.C. RESEARCH
STUDENTSHIP

Applications are invited from good honours graduates in a biological science and from students expecting to graduate in 1979 for an M.R.C. research studentship to study myofibre regeneration in muscular dystrophy in man. The work will involve quantitative techniques of electron microscopy, light microscopy and, possibly, tissue culture, and will be carried out at the Muscular Dystrophy Research Laboratories at Newcastle General Hospital in association with Dr M. J. Cullen.

The studentship (value £1,610 per annum) is tenable for three years and the successful candidate will be registered for the degree of Ph.D.

Applicants should write to Dr M. J. Cullen, Muscular Dystrophy Research Laboratories, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, enclosing a curriculum vitae and the names and addresses of two referees, by May 14, 1979. 460(F)

Agricultural Research Council
FOOD RESEARCH INSTITUTE
A.R.C. RESEARCH
STUDENTSHIP

Applications are invited for an Agricultural Research Council Research Studentship, tenable for up to three years from October 1979, in the Nutrition and Food Quality Division of the A.R.C. Food Research Institute.

The research will be conducted under the supervision of Dr D. A. T. Southgate on one of the following topics.

(a) The behaviour of complex polysaccharides in the small intestine with special reference to the effects of the unavailable carbohydrates (dietary fibre) on digestion and adsorption.

(b) The characterisation of the microbial fermentation of dietary fibre in human foods.

Candidates should hold or expect to gain this summer a first or upper second class Honours degree in a relevant discipline and the successful candidate will be expected to register with the University of East Anglia for a higher degree.

Further details and application forms (to be returned not later than June 1, 1979) are available from the Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA. 449(F)

QUEEN MARY COLLEGE
University of London
CHEMISTRY DEPARTMENT
ORGANIC
ELECTROSYNTHESIS

Applications are invited for the following positions, both supported by S.R.C. for work in collaboration with Dr J. H. P. Utley.

C.A.S.E. Postgraduate Studentship—jointly with Phizers Central Research (Sandwich) and involving electrochemical synthesis of pharmaceutically active amines. Usual S.R.C. conditions apply. Candidates should be graduates, or expecting to graduate in 1979, with 1st or upper 2nd class honours.

Postdoctoral Fellowship—for work on enhancing selectivity in a variety of electro-organic syntheses. Previous experience of organic electrochemistry an advantage but good background in organic synthesis would suffice. Appointment for one year in first instance, with possibility of renewal for second year. Initial salary in range (under review) £3,883 to £4,382 per annum plus £502 London Allowance.

Applications, including age, qualifications and experience and names of two referees, as soon as possible to The Registrar (N), Queen Mary College, Mile End Road, London E1 4NS. 495(F)

S.R.C. C.A.S.E.
RESEARCH STUDENTSHIP
UNIVERSITY OF
NEWCASTLE UPON TYNE
SCHOOL OF PHYSICS

Applications are invited from persons holding, or expecting to obtain, a good honours degree in physics for a C.A.S.E. studentship in theoretical solid state physics concerning electronic properties of defects in semiconductors, in collaboration with the Royal Signals and Radar Establishment, Malvern.

Further details are available from Dr M. Jaros, School of Physics, The University, Newcastle upon Tyne NE1 7RU, to whom applications should be sent along with curriculum vitae and the names of two referees. 442(F)

UNIVERSITY OF DURHAM
DEPARTMENT OF BOTANY

Applications are invited for an S.R.C. Studentship and Case Studentship available from October 1979 for suitable candidates interested in recombinant D.N.A. methods or the physiology and biochemistry of crop improvement.

Applications giving brief curriculum vitae and naming two referees should be sent by 11 May, 1979, to Professor D. Boulter, Department of Botany, Science Laboratories, South Road, Durham DH1 3LE. 440(F)

UNIVERSITY OF
CAMBRIDGE
DEPARTMENT OF APPLIED
BIOLOGY
M.A.F.F. RESEARCH
STUDENTSHIP

Applications invited from graduates or those expecting to graduate this summer for a Studentship to study the ecology of the carrot fly (*Pila roseae*) with view to establishing a pest management system for its control.

Further details from the Secretary, Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX. 498(F)

ASSISTANTSHIPS

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DEPARTMENT OF
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This project seeks to define the parameters which control anti-inflammatory activity, probably by the use of principal component and discriminant analysis. The post is suitable for a pharmacist or pharmacologist with an interest in data analysis, or a chemist or physical scientist with an interest in the life sciences.

(Informal enquiries to Dr J. C. Deardon, on 051-207-3581, Ext. 45)
B *The metabolism, disposition and activity of some potent phenyl-piperidine analgesics in animal and human subjects*

A post financed by the local education authority is available for a graduate in pharmacy or related fields. (Informal enquiries to Dr K. Chan, on 051-207-3581, Ext. 47)

Applicants for posts A and B should possess or expect to gain a good honours degree and the appointee will be expected to register for a higher degree of the C.N.A.A.

Application forms and further particulars from the Personnel Office, Liverpool Polytechnic, Richmond House, 1 Rumford Place, Liverpool L3 9RH Tel 051-227-5581, Ext. 43. Please quote reference LP/281. 464(P)

UNIVERSITY OF DURHAM
DEPARTMENT OF PHYSICS

Applications are invited from an electronics engineer or physicist with an electronics background for a senior research assistantship tenable for two years, from as soon as possible. The work will involve the development of silicon diode arrays for low light level imaging in astronomy and their associated data acquisition systems. Experience with minicomputer systems would be an advantage.

Initial salary in the range £3,883 to £5,129 (under review) on range 1A plus superannuation.

Applications (three copies) naming three referees should be sent by May 11, 1979 to the registrar and secretary, Science Laboratories South Road, Durham DH1 3LE, from whom further particulars may be obtained. 410(A)

UNIVERSITY OF DURHAM
DEPARTMENT OF
CHEMISTRY

Applications are invited from chemists, materials or poly scientists, or solid state physicists an S.R.C. funded postdoctoral Science Research Assistantship tenable for years from October 1, 1979 (or earlier by arrangement) for an interdisciplinary investigation of the formation and properties of thin films of structure and reactivity of polymers, systems, and composites in general primarily by ESCA. The appointee will assist in setting up a cur designed spectrometer dedicated to research.

Salary £3,883 to £4,133 on range 1A plus superannuation.

Applications (three copies) naming three referees should be sent by 1.1.1979 to the Registrar and Secret Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. 411(C)

UNIVERSITY OF
CAMBRIDGE
DEPARTMENT OF BOTANY
GRADUATE RESEARCH
ASSISTANT

Applications are invited for a graduate research assistantship to work the synthesis of components of photosynthetic electron transfer in higher plants. The post, which is supported by the Science Research Council, is for a period of three years from 1 October, 1979.

The salary will be on the scale starting at £3,384 per annum. A suitable applicant may be able to register for the degree of Ph.D.

Applications with curriculum vitae and the names and addresses of referees should be sent as soon as possible to Dr J. C. Gray, Department of Botany, University of Cambridge, Cambridge CB2 3EA, from whom further particulars may be obtained. 454(C)

SCHOLARSHIPS

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Applications are invited from graduates of universities that members of the Association Commonwealth Universities or graduates of any university who are citizens of a Commonwealth country. Candidates are expected to have a degree or postgraduate research experience.

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The Scholarship at senior level valued at \$A14,400 to \$A14,797 is tenable for one year but may be renewed for a second.

Application forms are available from the Registrar, University of Sydney, N.S.W., 2006, Australia, to whom applications close on June 1979. 485(F)

WORKSHOPS

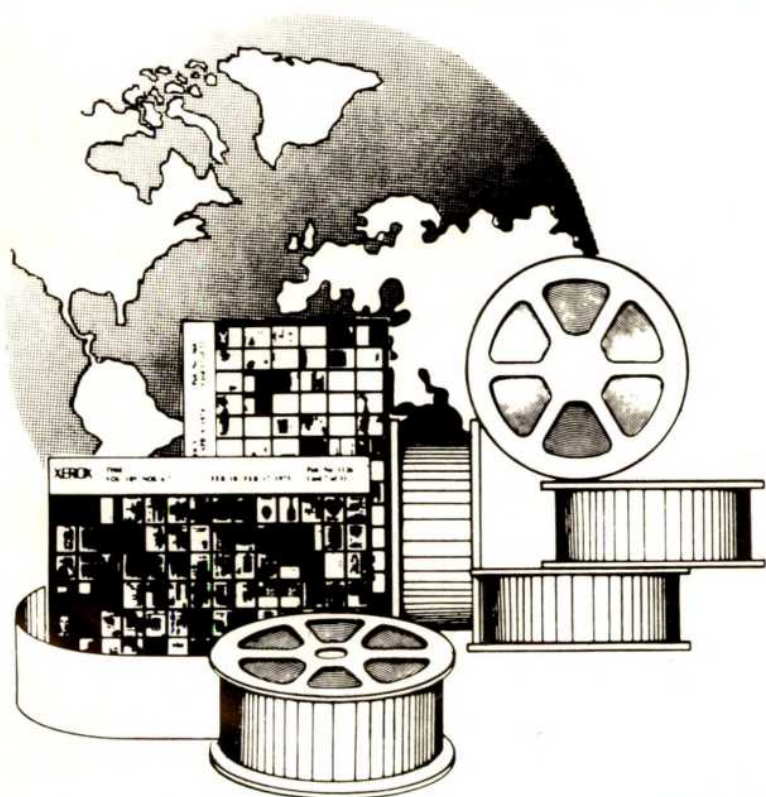
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Applications to Dr Julian Gottfried, Friedrich-Miescher-Institut, P.O. 273, CH-4002 Basel, Switzerland. 466(C)

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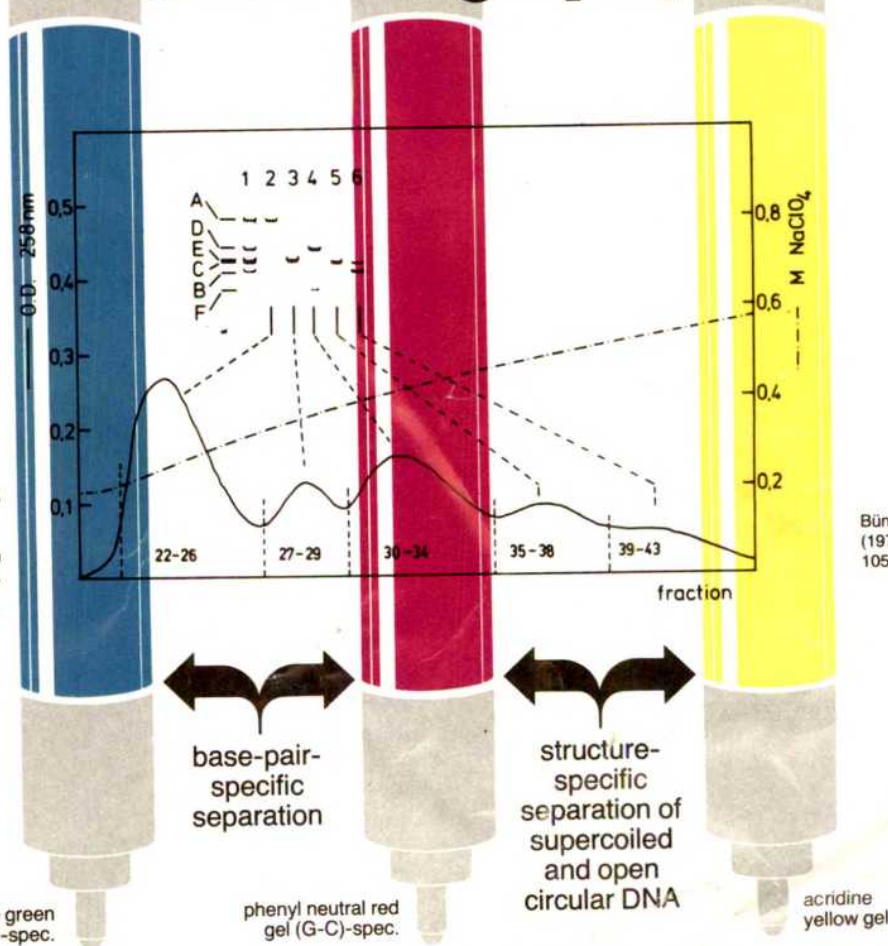


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R. A. Fisher (right), the subject of the lead review in this year's Spring Books Supplement (pages 784-824), talking to C. D. Darlington (himself a reviewer in this issue) at a genetics conference in Stockholm, 1948.

[Photo: Courtesy C. D. Darlington]

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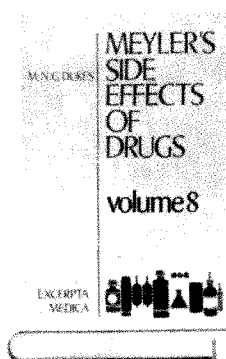
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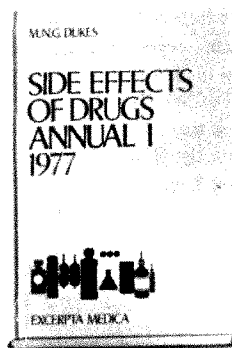
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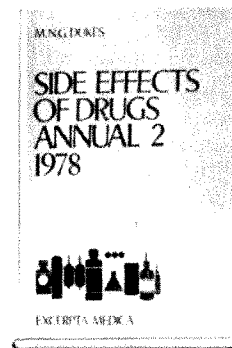
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With the moving of many of *Nature's* American activities to our New York office, we lose the services of Mary Wade, our Washington Office Manager, after fifteen years with *Nature*. She spent seven years in London before moving to Washington. Mary has done much more than simply run an office cheerfully and effectively; she has kept the London office in close touch with literally thousands of American scientists and has ensured that *Nature* has responded efficiently to their concerns.

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Mixture as before—to 1984?

IN A week's time the British electorate will instal a new government which could, if returned with a sufficient majority, see the nation through to 1984. The three major political parties, Labour, Conservative and Liberal, each publish brief but comprehensive manifestos regrettably padded out with a tedious mixture of boasts and insults. Somewhere in their midst, however, we ought to be able to discern clear, separate strands of political thinking which differentiate the parties and which when viewed against the current economic and social background give a clear indication of the most appropriate party for the next five years.

What is this background? In the 1970s Britain's industrial production has barely risen at all, whilst that of other major industrialised countries has climbed—in difficult circumstances—by amounts ranging from 15% to 30%. In terms of gross domestic product per capita, 15 years ago Britain was closely on a par with many European countries and nearly a factor three ahead of Japan. Now most of Europe has left us far behind, and Japan is fast doing so too. Only in the inflation league has Britain shown its competitors (with the exception of Italy) a clean pair of heels. All of this has happened to a country not notably short of material and energy resources, and certainly not lacking in human and intellectual resources.

Many argue, quite reasonably, that Britain is still an agreeable country in which to live, that the people are civilised and not driven by the materialist urges that allegedly are rife in other countries. Why, it is said, worry about international growth, productivity and efficiency leagues in such a green and pleasant land? The answer to this must surely be that unless we exert ourselves to the full, competition from other countries will not allow us just to stand still—it will rapidly bring on irreversible decline and the emigration of large numbers of talented people.

With the coming of post-industrial society, in which a diminishing number of people are involved in factory-style production and a growing number in some sort of 'information activity'—teaching, research, entertainment, printing, health, journalism, banking and so on, it is particularly important that flexibility be emphasised. It is almost impossible to envisage what the world will be like in 10 years' time, but one thing is certain: that it will have undergone rapid change, and that unless strong action is taken, a lot of people—indeed whole nations—are going to be left behind.

The strong action must take two forms—first, to permit or even encourage declining industries to die peacefully without deceiving those associated with them into thinking that better times are just around the corner; second, to take much more vigorous steps to promote lifelong education and retraining, so that going back to school or college is seen not as a humiliating thing of last resort, but as a perfectly natural thing to do.

How much of this is hoisted in by the two main political parties—and how much are they prepared to act on it? The Conservatives are probably more capable than Labour of being ruthless with declining industries. But they do not show much sign of recognising the changes that need to be wrought in our educational system beyond stating "we shall review the relationship between school, further education and training to see how better use can be made of existing resources"—which could mean anything. On the whole Labour seems, in its manifesto, to appreciate the changes that are necessary—even putting in a reference to the impact of the silicon chip—but the party has largely presided over the years of industrial decline without doing much to arrest it.

The real source of Britain's dilatory industrial performance cannot be blamed on a scarcity of resources, nor on a lack of intellectual skills. It must be placed squarely on our apparent inability to work together without breaking up into isolated groupings—'workers', 'skilled workers', 'management', 'civil servants', 'academics' and so on, each finding difficulty in understanding the other and each either busy exploiting the others or accusing the others of exploitation. To the extent that the British are naturally sceptical of authority, lack of homogeneity is fine. But the divisions are much more bitter and unproductive than that. Both major political parties exist, indeed thrive on these dichotomies, and so are themselves part of the problem they should be trying to eliminate. This, then, should make the Liberal party an attractive alternative. Certainly the Liberal manifesto touches on many of the matters discussed above, and the party even makes a play for being regarded as the environmental party. Traditionally a vote for the Liberals has been regarded as wasted in the British system of representation. That must not be allowed to deter thoughtful people, worried about an ever-declining, ever-confrontational Britain, from looking very seriously at the Liberals. □

University challenged over 'agribusiness' connections

David Dickson contributes his third weekly article on science in the American West with a report on farm workers in California, who are suing university officials over the social consequences of agricultural research

EARLY next month a county court in California will begin hearings on charges that have been brought against the University of California, claiming that it has carried out agricultural research leading to a variety of undesirable social consequences. These include the disappearance of harvest-time jobs as a result of increased automation, the shift from small family farms to large-scale 'agribusinesses', and the enrichment of corporate stock-holders out of public pockets.

A suit filed by a publically-financed group, California Rural Legal Assistance, on behalf of a number of farmworkers, concentrates in particular on the way that university research has contributed to the growth of mechanised agriculture in the state, resulting, the group claims, in extensive job dislocation and unemployment.

The suit demands that such research be stopped. And it also accuses senior members of the university and its Board of Regents of a conflict of interest over their connections with large food-producing corporations which, it is claimed, have been the principal beneficiaries of state- and federally-financed research.

The university, in response, has strongly denied any impropriety. In a statement issued at the end of last week, university counsel Mr Donald C. Reidhaar said that if the suit succeeded, it would prevent the university from carrying out research which might result in harm to certain people and in help to others.

"A basic mission of the university is research and the creation of new knowledge. Acceptance of the plaintiff's proposition would require the elimination of all research with any potential practical application," Mr Reidhaar said.

Research workers at the University of California have been engaged for more than a hundred years in developing techniques to raise agricultural production; ironically one of the university's first major political crises involved the claim that it was not doing enough of practical assistance to local agriculture.

Much of this research has been related to mechanising traditional agricultural practices, often with state support. In the mid-1960s, for example, the university was awarded an annual grant of \$150,000 by the state legisla-

ture to carry out research into automated farm machinery, following the phasing-out of a programme to bring farm labourers across the border from Mexico at peak harvesting time.

Yet not everyone has appreciated—or benefitted from—these changes. In tomato growing, for example, research at the university's Davis campus has led to developments ranging from automated picking and sorting machines, to a strain of "square tomato" whose thick skin protects it during mechanical processing; but many small farmers who could not afford the expensive new equipment have been forced out of business, while tomato harvesting no longer provides summer-time work for many thousands of migratory workers.

Strong opposition to the increasing automation of agriculture in California—as well as to the university's role in encouraging this process—has come from the United Farm Workers, the union established in the early 1970s after several bloody confrontations with growers. The union is concerned, for example, that many farm owners have used the elimination of jobs to offset the increased wages that the union has been able to negotiate for its members.

"We are not against mechanisation, but do not think that the taxpayer should pay twice, first for the univer-

sity research and then for the increased unemployment, welfare and social service costs" says union official Mack Lyons. Last year the union's convention passed a resolution demanding a moratorium on future research in agricultural mechanisation until it had been assured that the interests of farm workers would be protected.

The legal challenge, however, has come not from the union, but from a group of attorneys working for an organisation set up on state and federal funds to protect the rights of farm workers. In a suit filed in the Alameda County Court, it is demanded that the university stop all research on any agricultural mechanisation process that conveys has "a special economic benefit to narrow, private agribusiness interests at the expense of farmworkers, small family farmers, consumers, taxpayers and the quality of rural life".

By using tax money to benefit a small group of private corporations and by acting out of individual economic self-interest, the plaintiffs claim that the university and various named members of its Board of Regents have acted contrary to the state constitution, which requires that the university be administered as a public trust for the benefit of all Californians, free of political influence.

"We are questioning who benefits from the technological changes resulting from this research, who gets hurt—and who takes the decisions. In this type of situation we feel that there should be an open decision-making pro-



"The machine won't strike, it will work when the growers want it to work"

cess before money is spent on research," says Mr Al H Meyerhoff, one of the attorneys who have filed the suit on behalf of nineteen farmworkers and the California Agrarian Action Project.

"The increasing monopoly over agricultural production of large food-producing corporations is being facilitated by research and development work at public universities initially established, under the land grants colleges legislation, to help small farmers. We feel these institutions should not be contributing to the problems that these farmers face."

The university strongly denies charges that the results of its agricultural research programmes have been socially detrimental. In particular, according to university staff.

- although mechanisation has resulted in declining employment in some areas, this has been largely compensated for both by the introduction of new jobs in other areas, and by other employment opportunities brought about by general increases in agricultural productivity;

- rather than merely benefitting large corporate producers, the technological developments arising from university research have been of general benefit to the community, the advantages of increased productivity for example being passed on through lower food prices,

- and the university is also challenging whether it should have any particular responsibility for the social consequences of its research programmes, or whether this responsibility should not be shared by the whole community.

"The university's responsibility is to create new knowledge or information, to develop new ways to produce food as efficiently as possible, and to be aware of new developments, and so forth. But in terms of the conflict of social goals, that's not only *our* job, but the job of society, of the legislature." Professor Charles Hess, dean of the college of agricultural and environmental sciences, said in a recent interview.

Others at the university strongly support this view, although many admit that the automated machinery that they have developed has been made particularly attractive to local growers by the increasing strength and militant tactics of unionised farmworkers.

"Automatic lettuce harvesters, for example, developed at the university have been available for some time, but have not been widely taken up for a number of reasons, in particular cost. But I don't know how many more lettuce strikes we will have before something happens," says Dr William Chancellor, professor of agricultural engineering at the university of Cali-

fornia's Davis campus

From the union's standpoint, increasing mechanisation is a direct threat to its bargaining capabilities. One tomato grower near Sacramento, the target of an unsuccessful UFW campaign in 1975, subsequently bought an electronic tomato sorter for \$200,000, and was able to reduce his work force from 100 to 28, thus getting rid of "all the troublemakers". As one university staff member has been quoted as saying, "the machine won't strike, it will work when the growers want it to work".

The farmworkers have already received considerable support in their fight against mechanisation from members of the state legislature. At the request of one state representative, for example, the state accounting office is already carrying out an audit of the university's research activities to see if it reveals any "improprieties".

But neither has the university been totally insensitive to its criticisms. In addition to publicising the social value of its research, the university points out that the amount of research into agricultural mechanisation is being decreased, with emphasis shifting, for example, to methods for improving the biological productivity of crops.

The university is also both carrying out research and offering retraining courses aimed at the problems faced by farmworkers who lose their jobs as a result of automation. "We have been accused about not caring about the problems that mechanisation causes, but we are now looking at these too," says Dr Chancellor.

In responding to the charges made by the legal aid group, however, the university has denied that there is anything improper in the close links that it has established with private industry: claims that such links result in an "inordinate influence" on research policy are, it says, subjective assessments based on a particular political viewpoint.

Critics remain unconvinced. They blame the major food producers for the social problems of US agricultural workers—as well as the declining flavour of US food—and accuse the University of California (as well as universities playing similar roles in other states) of direct collaboration in this process.

"It belongs to society as a whole to decide what help people affected by agricultural developments should get, and how much. We should not be expected to do this on our own," says one university spokesman. "We believe that it is a travesty for the government to use tax money, in the form of research grants, to force people out of work and drive small family farmers off the land," says Mr. Meyerhoff. □

Professional bodies lobby to protect US science budget

QUOTING a 25% drop in the proportion of the federal budget devoted to basic research between 1968 and 1978, 40 US scientific societies and higher education associations last week issued a joint statement supporting President Carter's bid for a significant increase in support for basic research in the fiscal year 1980.

The statement is critical of the administration's decision to request virtually no increase in funding for biomedical research through the National Institutes of Health, pointing out that this will mean a decrease of almost 50% in the number of new competitive research grants available.

Apart from this, however, the various organisations put their voices solidly behind President Carter's request for a 9% increase in basic research funding—even accepting that this will be barely sufficient to keep up with inflation—and urges Congress to do the same.

So far, the Congressional response to the budget request submitted in January has been relatively good. The Senate budget committee, for example, having taken a detailed look at the requested science budget, has recommended that it be accepted almost in full, although suggesting cuts in virtually all other areas of public spending.

But there may well be stormy weather ahead. The House of Representatives, for example, in authorising a budget for the National Science Foundation close to the \$1,000 million requested, accepted by 219 votes to 174 an amendment reducing funds for biological, behavioural and social sciences research (and aimed primarily at the last of these) by \$14 million; last year a comparable amendment was rejected 174 to 229.

Immediate cause for concern are imminent floor debates on broad budget resolutions in both the Senate and the House, with various proposals that could affect science funding. A further test will come when key appropriations subcommittees meet to decide on agency budgets later next month.

Keen to prevent a repeat of last year, when a substantial increase in funding for basic research requested by President Carter was cut back by Congressional committees to a level—apart from the NIH—scarcely above inflation, the research community has been busy putting its lobbying act together in Washington.

In issuing a joint statement, the

various higher education, scientific and technological societies said they were taking an "unprecedented step" in presenting to Congress a unified position on the needs of science. They expressed concern at "the lack of a congressional policy in science which would embrace the principles of stable, balanced and controlled investment in basic research".

At a press conference in Washington to coincide with the publication of the joint statement, Dr Derek Bok, president of Harvard University, said that there was no contradiction between President Carter's aims to achieve a balanced budget, and to increase the federal investment in basic research.

"These positions are inextricably related, for out of basic research, if history is any judge, will evolve industrial innovation and growth, more efficient and thus less costly uses of labour, energy and equipment, and better health and security for all," Dr Bok said.

Dr David Saxon, president of the University of California, pointed to the present energy crisis as a "very real example" of what could happen if investment in basic research was allowed to fall behind. "Congress used

foresight in 1954 when it amended that Atomic Energy Act to provide for research and development of alternative energy sources. However a review of the funding record for programmes of basic research reveals a history of peaks and valleys. This lack of sustained commitment has discouraged outstanding talent and blunted national progress."

The need to maintain stability in funding is, in particular, being used to justify demands for further increases in funding for the National Institutes of Health. In announcing that it was requesting a level of funding virtually the same as the current year (the Office of Management and Budget is even said to have suggested a decrease) the administration pointed out that Congress voted last year for a 23% increase in biomedical research support—and that even if this was spread over two years, it would still be comparable to planned increases in other areas over the same period.

Supporters of more funds for biomedical research, however, argue that things are not that simple, and that a stationary NIH budget would introduce instabilities by drastically cutting back on new research starts. "Analysis of

the budget by mechanism reveals a disconcerting and unacceptable reduction in the funds available for young investigators," says Dr Suzanne Oparil of the University of Alabama, chairwoman of the American Federation for Clinical Research.

A marked increase in obligated non-competing research projects supported by NIH would, she says, result in a 43% decrease in funds available for competing research projects. "Because young biomedical investigators are supported primarily by investigator-initiated competing research grants, this reduction in funds for competing grants must be vigorously opposed."

Biomedical research workers are particularly concerned that although in previous years Congressional committees have traditionally provided significant increases to medical research, the current mood against further public spending—together with the loss of several key Congressmen, such as Senator Edward Brooke and Representative Paul Rogers, who had previously championed the medical research cause—mean that a comparable increase this year is far from guaranteed.

David Dickson

US accused of banning foreign scientists

FOREIGN scientists invited to lecture or participate in scientific meetings in the US continue on occasion to suffer the embarrassment of being denied entry to a country "proud of its right to freedom of speech", according to a senior member of the American Association for the Advancement of Science.

This situation arises because those taking part in scholarly and cultural exchanges whose political beliefs fall into particular categories—and in particular past or present members of the Communist party—must obtain a special waiver from the State Department to obtain a temporary entry visa.

"In general this policy has been implemented in a liberal way so as to remove automatic exclusion of foreign scientists and others who might express beliefs or support for political doctrines previously restricted by our immigration laws," Professor John Edsall, chairman of the Committee on Scientific Freedom and Responsibility, told a Congressional committee studying US compliance with the Helsinki Agreement earlier this month.

"However in some cases the policy has remained unchanged, or foreign visitors have been excluded on grounds other than political beliefs, thus making the waiver procedure more difficult to implement. What is most desirable from the view of scientists is the removal of these restrictions from

the US immigration laws."

Professor Edsall, who is professor emeritus of biochemistry at Harvard University, listed a number of cases which, he said, appeared to violate the principles of free travel as stated at Helsinki, instances in which political or administrative decisions had impeded the free circulation of scientists. These included:

- The refusal of a visa application—later granted—to Dr Sylvia Berman, an Argentine psychologist now living in exile in Mexico city who is alleged to have previously expressed Marxist beliefs, to participate in a meeting of the American Public Health Association in Washington DC, last year.

- The denial of a visa in 1975 to Dr Joseph Needham, the British science historian. Although the state Department informally acknowledged that this was because of his allegedly Marxist beliefs, it refused to provide him with the reason for the denial. Dr Needham subsequently refused to apply for a waiver. On subsequent visits, Dr Needham has been granted a visa without having to apply for a waiver.

- Late last year, delays in reviewing the visa application of Professor Jean Pierre Vigier, a French physicist who had been invited to address the AAAS annual meeting in Houston, Texas, made it impossible for him to attend the conference.

- Dr Andre Frank, professor in the

school of development studies at the University of East Anglia, although previously a US resident, has consistently been denied a visa on various grounds, both regarding his political affiliations and unspecified evidence—which Dr Frank denies—that he may wish to take up residence in the US again.

Professor Edsall said that other areas of concern involved the controls placed on the travels of foreign scientists while visiting the US, since the State Department has divided the country into open and closed areas as part of our diplomatic relations with the Soviet Union and East European countries.

"It appears questionable at the present time whether such a travel restriction policy will also be applied to the Chinese scientists, who are currently seeking expanded scientific exchange with the US. In the interests of science it is important to promote free access to all individuals who wish to exchange information, without arbitrarily assigning the need for screening or political controls", he said.

"Our violations are, I would agree, a good deal less serious than those that have occurred and still occur, in countries with Communist governments; but let us do away with them and show that we truly honour the spirit of the Helsinki accords."

David Dickson

Soviets boost their own . . .

LAST week, the Soviet Union celebrated its new "Science Day" (the third Sunday in April). To mark the occasion, *Pravda* published an extensive article by Academician Anatolii P. Aleksandrov, President of the Academy of Sciences, which, while praising, naturally, the achievement of Soviet science, did so largely at the expense of the West.

The new Soviet "Science Day", of course, has considerable political overtones. The date was chosen to commemorate 12 April, 1918, when the Soviet of People's Commissars directed the Academy of Sciences to solve, as an "important and urgent" problem, the "correct distribution of industry in the country and the best rational use of its economic forces".

Accordingly while tracing the source of Soviet science from its early days to the present work-force of 1.3 million scientists ("the largest scientific team in the world—its potentials are enormous") Aleksandrov found it necessary to devote almost a sixth of the article to the Cold-War.

Partly, it is clear, he wanted to exculpate Soviet scientists involved in armaments production.

Then, after a brief paragraph on Brezhnev's peace initiatives, Aleksandrov moved the attack to the economic front. The Soviet Union "carrying out Lenin's directives has created a reliable fuel-energy basis of its own, a diverse basis of raw materials, and all forms of industry. . . . "There is no scientific-technical problem" he concludes triumphantly "that would not be within our power".

Imports of such technological goods as gas pipelines, he explains, are simply a way of receiving payment for exports of "excess production", and allow "advances in the necessary sectors" to be made; they are never, he averred, "vitally necessary".

To ram his point home, he then described the US economy, on the brink of energy shortages and major economic crisis, following this by a review of the benefits of Soviet science, "not only for our country, but for mankind as a whole"—the first nuclear power station, the first nuclear ice-breaker, the start of space research. Other Soviet successes, he maintained, had "given the impetus for corresponding work to be done in the West"—lasers, Cerenkov radiation, "certain principles of thermonuclear synthesis", and "the production of relativistic electrons".

The final column is quieter in tone; an allusion is made to Mr Brezhnev's enthusiasm for basic research, the instrument-construction and computer sectors receive a slight censure, the RATAN-600 telescope and Baksansk neutrino observatory are particularly praised.

And that is all. The feast-day of Soviet science, as portrayed by Aleksandrov, is an entirely Soviet celebration. The outside world is cited only to make the Soviet picture shine more brightly by contrast.

Perhaps most significant, although the politicians regularly mention scientific and technical cooperation as one of the benefits of the Helsinki accords, Aleksandrov does not reciprocate the compliment. In what must, by its length and occasion, count as a major statement on Soviet science policy, the word "detente" is never mentioned.

. . . but bring the Bulgarians down with a bump

ABORTING the Soyuz-33 flight with the Soviet and Bulgarian cosmonauts must have proved particularly embarrassing to the Soviet space planners. Not only was it a premature return, but it took place on "Cosmonautics Day" (12 April, the anniversary of Gagarin's flight).

Bulgaria's space research programme has always been particularly closely linked to that of the Soviet Union. It was initiated in 1956 following a Soviet suggestion that the Bulgarians should explore the possibilities of carrying out ionospheric research and monitoring future Soviet satellites. The Bulgarian Council of Ministers established a Centre for Radio-Observation and Ionospheric Research, which was ready to go into operation when Sputnik-I was launched in October 1957.

At the same time, other Comecon countries—Czechoslovakia, Poland, East Germany and Hungary, were making a firm commitment to space research. This, however, was a stepping

up of existing efforts, not, as in Bulgaria's case, an entirely new initiative.

By 1966, when the *Interkosmos* programme was initiated, Bulgaria had caught up considerably. From the beginning, there was a major Bulgarian participation in the *Interkosmos* working groups on space physics, satellite communications, space meteorology and space biology. On the hardware side, Bulgaria was still somewhat behind although *Interkosmos-2* included a joint Soviet-Bulgarian Langmuir probe experiment. By December, 1972, Bulgaria was able to provide a complete experiment, monitoring electronic and ionic concentrations and temperatures. Updated versions were later carried by the *Interkosmos-12* and -14 satellites, and the *Vertikal-4* and -5 geophysical rockets.

In addition, Bulgarian scientists have been involved in the equipping and processing of experiments carried by several Soviet *Kosmos* satellites. Throughout, Bulgaria's main interest has been directed towards particle and field distribution, including some interesting results on magnetosphere shock-waves. It is to such experiments that Bulgaria's own satellite, *Bulgaria-1300*, will largely be devoted.

This satellite is intended to celebrate the forthcoming 1300th anniversary of Bulgarian statehood, and will presumably engage the entire launch power of an *Interkosmos* blast-off, resembling the Polish *Copernicus-500* jubilee satellite of 1973, rather than the small Czechoslovak *Magion* satellite, which was put into orbit as part of *Interkosmos-18* last autumn.

Bulgaria would then become the second non-Soviet Comecon country to have a satellite of its own—which should compensate considerably for the loss of *amour-propre* over the *Soyuz-33* abort. Indeed, the Bulgarian media have already done an excellent PR job on *Soyuz-33*—backed up by the Soviet and Bulgarian governments which have awarded Ivanov and his Russian commander Rekavishnikov the usual orders and titles. The return to Earth, using the back-up engine for re-entry, and the reactions of the cosmonauts to this "short but difficult" flight were presented as valuable lessons in space dynamics and psychology.

As for the Bulgarian experiments designed for the mission—which were to include a unique spectroscopic survey of the Earth—these will almost certainly be carried out by a repeat mission in the near future. So, when the current programme of *Interkosmos* manned flights comes to an end, Bulgaria may well have two manned flights (and possibly two cosmonauts) to her credit, while the other fraternal allies have only one apiece.



Ivanov: awarded the usual medals

Vera Rich

Union steps up lab safety campaign

UNION action at UK university and public health laboratories which use dangerous pathogens is to be stepped up in further attempts to improve safety. The Association of Scientific Technical and Managerial Staffs (ASTMS) has warned that it is now to launch a campaign to enforce greater safety at laboratories dealing with category B pathogens—such as those responsible for botulism, typhoid, paratyphoid and the plague. These are organisms less deadly than the category A smallpox virus which caused the death of medical photographer Janet Parker at Birmingham University last year but they still pose special hazards for workers.

The general secretary of ASTMS, Mr Clive Jenkins, said last week that the union would be telling its members: "Do it cleanly. Do it safely. Wear masks when necessary. Use the autoclaves. Use the fume cupboards. Demand things be made safe from the beginning". And he added: "We still have a situation in about 60% of our laboratories where mouth-pipetting is still permitted. That is disgraceful, awful. We will certainly be urging our members to stop mouth-pipetting at these category B laboratories."

"I think heads of departments are being dirty, careless and slovenly. There is no excuse for doing things incompetently, inefficiently and in a dirty fashion".

Mr Jenkins said the Birmingham smallpox outbreak and the subsequent revelations of the Shooter report had spurred ASTMS to take a fresh look at the 29,000 people in the laboratory population of universities and the health service. "These people are being ravaged by disease and are contracting all sorts of things like brucellosis, psittacosis, beryllium contamination, TB, hepatitis, and about eight or nine other things as well", he added.

The union now has 2,000 legal cases pending on behalf of its members—of which about 60% are related to diseases with industrial or laboratory sources. More staff have been taken on to help cope with this load and ASTMS has also rejigged its entire legal department to be investigative rather than retaliatory, Mr Jenkins added. "Our members are flooding us with calls now they realise that we are passionately interested in this area", he claimed.

ASTMS is now negotiating a new pay package for its technicians and is expecting a 9% rise backdated to last



Clive Jenkins: mouth pipetting "is disgraceful"

October which is to be followed by three other increases over the next 18 months. But Mr Jenkins stressed that although this claim had led to several disputes at departments, the union was trying to rein in its members. "One can damage a university without having any effect at all upon politicians or upon the economy and therefore we feel guilty because in the private sector we have been getting quite sensible agreements. But the universities are a real problem for us".

"We want to abolish the concept of danger money", Mr Jenkins said. "The arguments that we would use are that this is a very hard-working, productive, active and well-qualified group. Discussions about disease are separate. University technicians should be properly rewarded for their qualifications and work but we are not going to be bought off for more money to have them working in slovenly laboratories".

At the same time, ASTMS is still pressing for a public inquiry into the Birmingham outbreak with a legally qualified chairman and some form of union representation. Indeed this latter approach is a particularly important one for the union which wants far greater union representation on all bodies administering laboratories.

And Mr Jenkins reacted bitterly to the suggestion reported in *Nature* (29 March, p. 387) of Mark Carlisle, Conservative education and science spokesman, that there should be no lay or union members on the Dangerous Pathogens Advisory Group, the body responsible for screening biological laboratories such as the one at Birmingham.

"I would have thought that that was a scandalously reactionary suggestion. DPAG has been a disaster. We want it disbanded. Everyone inside it should be sacked and the body reconstituted within the Health and Safety Executive. I don't know why he wants the Tories to be the party of germs and danger".

Robin McKie

UK taxonomy is healthy, says report

A REVIEW group of the UK Advisory Board for the Research Councils (ABRC) has recommended that the major support in Britain for taxonomy—the classification of plants and animals—should go to studies of organisms of economic and social importance. Most in need of study it says, are the nematodes, tiny and prolific worms that attack crops and livestock throughout the world. This reflects the concern of the European Science Foundation Group on Biological Recording, Systematics and Taxonomy, which in 1977 instituted a survey of European plant-parasitic nematodes.

The classification of plants and animals may not be one of the more glamorous branches of biology, but the accurate identification of species, both past and present, and the study of their relationships is basic to both fundamental and applied biological progress. Reawakened interest in the field has inspired the official ABRC report, *Taxonomy in Britain* (HMSO, £3.50). The report has been produced by the ABRC's Review Group on Taxonomy, set up in 1974 under the chairmanship of Sir Eric Smith, formerly director of the Laboratory of the Marine Biological Association of the United Kingdom.

The state of British taxonomy seems on the whole to be healthy, the report concludes, although the group would like to see some changes, particularly to ensure proper care and use of the scattered national collections of biological specimens, and to encourage young people to take up taxonomy.

Also, the British collections of living material including, as well as the plants in the botanic gardens, cultures of algae, protozoa, yeast, bacteria, fungi and viruses, have special needs not shared by dried specimens. The report expresses particular concern about uncertainties in the funding of the collection of living fungi at the Commonwealth Mycological Institute and of the National Collection of Yeast Cultures and recommends that the ABRC review the funding of all such taxonomic reference collections.

To encourage recruitment and training of taxonomists, the report recommends that the study of taxonomy should be fostered as part of the general educational exposure to science and natural history. Field courses are vital, says the report. To build on these foundations there should be a modest increase in the small number of post-graduate studentships for taxonomic research.

Mary Lindley

Planners backtrack after German nuclear inquiry

Most of the plans for a reprocessing plant at Gorleben are only drafts after all. **Klaus Höpfner** reports on surprise developments at the hearing into West Germany's 'Windscale'

DURING the last big debate on nuclear energy in the West German Bundestag in December 1978, speakers described the present situation over the country's atomic waste as "precarious" and "critical". They placed their hopes in the swift completion of the central nuclear disposal centre at Gorleben in Lower Saxony.

Since then, the storage ponds for burnt-out fuel elements in the German nuclear power stations have filled up still more, the construction of a larger intermediate storage facility at Ahaus in North Rhine-Westphalia has been further delayed, and there are still problems with the temporary dumping of used fuel elements at La Hague in France.

Moreover, plans for the reprocessing and final disposal installations in Gorleben have run into trouble. Severe criticism has been made by 25 international scientists called in by the local *Land* government of Lower Saxony (the authority responsible for plant licensing) to assess the plans. Their 2,200-page report set down little that was encouraging for the pro-nuclear lobby.

During a six-day hearing in Hanover from 28 March to 3 April, these expert critics argued with the Gorleben planners and also explained the plans to a wider public. Initial fears that the press and public would be excluded and denied sensitive information were not borne out. Although the scientists' report has not yet been published, notes of the sessions were distributed and the hearing was open to detailed reporting by the media. Neither has there been any substance so far in the fear that the hearing would merely be a front, and that it would have no influence at all on the decision whether or not to build the plant. On the contrary it seems as if doubts raised about safety and the incompleteness of the existing plans have already begun to have an effect. The Minister-President of Lower Saxony, Ernst Albrecht, made it known, while the hearings were still going on, that the project in its present form would not get his government's approval and would have to be altered. DWK, the German nuclear fuel reprocessing company which is designing and building Gorleben, reacted by declaring that, contrary to all assumptions, large portions of its plans were only preliminary and open to alteration. Only the first phase of construction—the entry storage ponds of

the plant—was decided and needed to be licensed now. Plans for the other phases—reprocessing plant, uranium and plutonium fabrication, vitrification of wastes and final processing—were simply drafts (which nevertheless enabled a decision in principle on technical safety to be made).

The pro-nuclear case at the Hanover hearing was put by representatives from the industrial interests concerned, from nuclear research institutes and other scientific establishments in Germany and elsewhere. On the first day of the hearing, they described the plan as an "optimal draft", for which no alternative existed in the present state of technology. For safety reasons, a fully integrated disposal centre was the best solution. Transport of dangerous loads would be minimised, control of fissionable materials would be made easier, and the costs of guarding and protecting it from attack would be kept relatively small.

Of the three methods for the treatment of used fuel elements which were feasible in principle—namely: reprocessing, long-term intermediate storage with a view to possible reprocessing later on, and final storage in specially built facilities, the decision had been made in favour of reprocessing. This was on ecological grounds (reduction of dangerous materials to a minimum by treatment and re-use as reactor fuel) and on economic grounds (recycling, sparing of scarce uranium resources). Moreover, according to DWK, there was "no technically proven process that would permit highly burnt-out fuel elements to be stored safely over very long periods". Linnemann (Radiation Management Corp., Philadelphia) said it was a "logical, progressive and necessary programme" that would be recognised by many countries and by the International Atomic Energy Agency.

However, the battle over reprocessing must be seen in the context of the commercial use of breeder technology which the German electricity industry is striving for. This is meeting strenuous opposition, as much from the political parties (whose leaders are still leaving the decision open) as from the environmentalists and pressure groups which have achieved considerable success lately at local elections.

Technically, reprocessing is to be by the PUREX process, which, says DWK, "has held good for over 30 years in a large number of treatment plants",

including a small experimental plant in Karlsruhe. Even its advocates admit that the PUREX process, which was developed in the 1940s for military nuclear programmes, still has important technical problems to overcome with the much higher radioactivity of the fuel elements from power reactors (especially in the first stages of the reprocessing, when dealing with the radioactive gases given off). They also concede that many of the steps in the process still have to be adapted for the major technical requirements of a plant with a capacity of 1,500 tonnes a year. Nevertheless, the pro-nuclear supporters in Hanover appeared firmly convinced that all the problems could be solved, and spoke of "normal steps" such as are usual with every advance in technical development.

The general question of the expediency and safety of reprocessing plants, and the technical problem of how far experience with small reprocessing plants can be applied to plants many times bigger became increasingly important during the hearings. The sharpest critics flatly denied that the large-scale reprocessing of reactor fuel elements would ever be feasible. Reprocessing made one problem (the final storage of used fuel elements) into a whole series of problems whose solutions "have never been successfully demonstrated on an industrial scale" according to Walt Patterson (London). It was ecologically senseless because it did not reduce radioactive waste, but increased it, and did so in an uneconomic way, with much higher costs than returns from the nuclear fuels recovered. Scarce uranium stocks could be eked out better in other ways, eg. in enrichment and in the production of the fuel elements. Professor Lindstrom (Stockholm) tried to quantify the dangers: the problems of reprocessing were "more difficult to the order of magnitude of seven" than those of designing a nuclear reactor, and the risks were correspondingly greater. Mr Schapira (Institut Physique Nucléaire, Orsay), supported this with a long list of only the "most important unsolved problems".

Amory Lovins (London), Professor Barnaby (Stockholm) and others saw the greatest problem in the 14 tonnes of plutonium which would be obtained in the plant each year and would have to be guarded against misuse. The huge capacity of the plant could possibly also be used to treat nuclear fuels for countries which were not a party to the agreement limiting atomic weapons and, in practical terms, to start an export trade in plutonium.



Ghostly garb for anti-nuclear demonstrators in Hanover

Minister-President Albrecht disagreed with the critics on this. The federal government kept strictly to the terms of the non-proliferation treaty. In any case, the treaty ought not to lead to discrimination against countries with no nuclear weapons by denying them access to a closed nuclear fuel cycle—that is, to reprocessing and production of plutonium fuel. From the designers' side, it was added that the plant would not have any extra capacity. That would be to assume that—after clearing up the stocks of atomic waste—there would eventually be 50 nuclear power stations in West Germany. At present there are only 12 (excluding experimental plants) and resistance is growing with every licence that is granted.

Serious misgivings were also raised during the hearings about the safety of the planned entry storage facility, which has been conceived as a "wet store" (water pools in massive concrete structures). A number of scientists warned of the catastrophic consequences of a breakdown in the coolant supply. To this, astonishingly, the designers conceded that the draft, commissioned in 1977, was in fact out of date. They were now thinking of dry storage in metal containers in which the fuel elements would lose heat naturally to the surrounding air (similarly to when they are transported).

Another controversy was about the best way of treating highly active wastes (more than 1,000 cubic metres of concentrated highly active solutions per year), and final storage in the salt domes of Gorleben or in other places with a similar geological formation. Three variants of highly active waste vitrification have been developed in Germany. It is now a question of putting one of them into practice.

For the final storage of radioactive wastes, the *Land* government of Lower Saxony chose the salt dome at Gorleben, near the East German border. But, geologists at Hanover said the geological conditions there were by no means the best. There were sure to be carnallite deposits there and probably anhydrite layers. In storing highly active wastes, special problems would have to be anticipated because of their heat radiation. Building a disposal centre on top of the salt dome would add to the hazard.

Immediately after the six-day hearing, which evoked great public response, particularly in light of the events at Harrisburg, it was clear that there was no basis, either scientifically or politically, for a comprehensive decision in principle.

DWK is now trying to exclude from discussion, for the time being, the most explosive part of the draft—the reprocessing plant. They argue that there is no pressure of time about the reprocessing; that it is not expected before the beginning of the 1990s, and breeder reactors are indeed a matter for the

21st century. There is no hurry either, they say, for the final storage facility, which is scheduled for 1995. Only the further steps for licensing the entry storage facility need to be hurried. Even this, though, should not be too pressing because the major intermediate storage facility at Ahaus should be ready before then.

Meanwhile, there is speculation that Minister-President Albrecht will reduce the decision to one small first step—further investigations into the entry storage facility—and for the rest avoid any commitment on future action. Not only would this gain time to test the feasibility of the plans, but alternatives could be more fully investigated. This applies principally to the option of long-term underground storage with the possibility of retrieval, which has been gaining more and more supporters. In theory at least, this has two advantages: it solves the problem for today and keeps the options open for the future. Its disadvantage is that there has been hardly any experience with it.

Minister-President Albrecht could find himself in a political dilemma in making his decision. He is a member of the executive committee of a political party (the CDU) which is still in favour of nuclear energy. At the same time, Gorleben is anything but popular, and Albrecht governs Lower Saxony with only a bare majority. His opposite number, the former Federal Minister of Building, Ravens (SPD), is only waiting for one wrong move. So Albrecht has now sought an interview with federal Chancellor Schmidt. Without powerful backing from Bonn (where federal Research Minister Hauff has already mounted a big information operation to take the disposal plan to the people), he will be reluctant to give the go-ahead. Since Harrisburg, though, Bonn is even more divided over the nuclear energy question than before. □

Klaus Höpfner is a writer with the Institut für Systemtechnik und Innovationsforschung, Karlsruhe.

'Splash-over' caused Windscale leak

FIGURES on the Windscale leak discovered by borehole sampling (29 March) have finally been released by British Nuclear Fuels. A spokesman said preliminary estimates of the total activity in the leak are from 10,000 to 100,000 curies, the total volume of escaped radioactive liquid is 200 gallons, and the dosage in the borehole is 300 rems per hour. (The International Commission on Radiological Protection calls a dosage of 400 rems lethal.

The leak has been located in an out-

of-use storage site for waste analysis where active liquid from the processing chain was diverted into unused piping to the storage shed due to a "splash-over at a junction point". The maximum activity is 15 to 20 feet under ground and is separated from ground water by a layer of clay. The route of the leak from the storage shed to the borehole is unknown.

BNFL has told police, fire, health service and county council official that there is no hazard to the community or the workforce. □

correspondence

DPAG needs public interest representatives

SIR.—I refer to your article (29 March, page 387) "Britain's shadow science minister believes in experts."

If the substance of your article really does represent the views of Mr Carlisle, perhaps the headline would have more accurately read "believes in non-trade union experts" or even "anti-trade union experts."

The plain fact of the matter is that the Dangerous Pathogens Advisory Group (DPAG), which does not have representatives from the trade unions or from the community, and consists solely of experts in the sense that the Conservative shadow minister uses the word, has in safety terms been a disaster. GMAG, which does include representatives of the public and trade unions, has been able to exercise a positive, constructive and effective role in the area in which it operates. The comparison is dramatic.

Of twelve laboratories cleared by the non-statutory "leave it to the experts" DPAG (which excludes trade union representatives) as safe to handle Category A pathogens, the Health and Safety Executive has now served prohibition/improvement notices on three. No such problems have arisen in laboratories working in the field of genetic manipulation experimentation! These have been controlled from the outset by close cooperation between the Health and Safety Executive and the Genetic Manipulation Advisory Group which does, of course, include the Trades' Union Congress (TUC) nominees and representatives of the community. This is because trade unions' organising staff doing such work can provide experts as good as any, with the added advantage of links with democratic organisations possessing economic and political clout. This makes it possible to defend the interests of the public and employees to a degree DPAG has either been unwilling or unable to do. The consequences have been tragic as we well know from the smallpox occurrence at Birmingham, and the laboratory now closed as unsafe was cleared by a DPAG system. Mr Carlisle evidently holds in such high regard

Would it be fair to conclude that Mr Carlisle is moved by fossilised Conservative policies, rather than the health and safety of the community and of the work people concerned.

Yours faithfully,

R. A. BIRD

Association of Scientific, Technical and Managerial Staffs,
London

International energy programmes need coordinating

SIR.—In the face of the approaching threat of a world energy shortage Professor Rotblat's "Call for world

(non-nuclear) energy organisations" (1 March, page 4), to coordinate and stimulate the development of non-nuclear sources of energy seems a positive step. But will this include oil, natural gas and coal? If Professor Rotblat is referring only to the so-called renewable energy sources any means of increasing their contribution beyond the 2% of world energy supply by the year 2000 suggested in a recent report to the Club of Rome deserves encouragement.

Something is, however, already being done. the World Bank Group has lent \$10 billion since 1948 for building power plants. In 1978 alone it lent \$1,146 million for energy and power projects in developing countries—all non-nuclear (almost 20 times the 1978 budget of the IAEA). Furthermore the Centre for Natural Resources, Energy and Transport at the United Nations, as well as the UN Regional Commissions, actively promote all non-nuclear forms of energy, as do UNESCO, UNIDO and UNEP and the US/AID. What is needed is a greater concentration and coordination of the programmes of existing agencies—not a new one.

If the world is to avoid a damaging energy crisis with widespread unemployment, protectionism and even armed conflict over limited energy supplies, all available energy sources—including nuclear power—must be developed, and energy conservation programmes increased. Professor Rotblat is then misguided in attacking what he calls the "promotional" activities of the International Atomic Energy Agency.

In fact a major role of the IAEA is in developing an institutional regime within which nuclear power can be used safely and securely. IAEA's 1979 'assessed' budget provides approximately \$20 million for regulatory activities (of which \$15.6 million are for safeguards against proliferation) compared with \$3.4 million for the division that deals with nuclear power and reactors.

The other promotional activities of the IAEA are in subjects like theoretical physics and fusion or for application of radio-isotopes in biology, medicine, food and agriculture. They are designed to help the great majority of the developing countries for which nuclear power is hardly, if at all, in prospect.

In addition to the "assessed" budget there is a voluntary technical assistance budget. In 1977, 21.9% of this went to nuclear engineering and technology (chiefly training in the safe operation of nuclear plants) compared with about 40% for application of isotopes in agriculture, hydrology, medicine, etc.

Professor Rotblat's main contention that a large expansion of nuclear power would greatly increase the chance of nuclear war, since almost every nation would have access to plutonium, flies in the face of the facts of history, economics and technological development. All nuclear weapon programmes have been based on series of special facilities dedicated solely to

military purposes and quite independent of the civil power programmes of the countries concerned. This separation becomes even more marked as time goes on. It is far cheaper, easier and less conspicuous to build and operate the small purpose-built plants needed for a modest weapon programme than to engage in commercial nuclear power. The difference in cost is of the order of twenty to one. Stopping nuclear power would not cause a moment's pause in the nuclear armament and submarine programmes of the weapon states.

A more immediate threat to world peace comes from competition over limited supplies of oil. According to a report in the *Financial Times*, 26 February, 1979, the US Defence Secretary has said that in protection of vital Middle East oil supplies "we will take any action that is appropriate, including military force". By increasing energy supplies and reducing the pressure of demand over scarce resources nuclear power will prove to be a stabilising factor in international affairs.

Professor Rotblat may not think much of treaties and international safeguards, but the fact is that the rate of proliferation of nuclear weapons has declined each decade since 1944. At the same time almost all the industrial and many of the leading developing countries of the world have benefitted from the peaceful use of nuclear technology. The danger of nuclear war comes from the arsenals of the great powers and not the power plants of the small.

Yours faithfully,

G. GREENHALGH

London, SW15.

Recombinant DNA dangers and wild *E. coli*

SIR.—Many scientists engaged in recombinant DNA research now expound the doctrine, referred to by Eleanor Lawrence in her report on the COGENE meeting at Wye (12 April, page 590), that the dangers that might arise during the course of their experiments cannot be greater than the dangers posed by working with the most dangerous organisms employed in those experiments. This might well be so when the recipients of genetic material are 'enfeebled' organisms such as *E. coli* K12, but it could be far from true if other recipients were employed, deliberately or accidentally. For example, to my mind, the implantation of efficiently expressible genes for botulinum toxin production in wild *E. coli* or other organisms that can multiply relatively freely in the food or in the intestinal tract of man or animals represents a much greater danger than the presence of these genes in *Clostridium botulinum* itself.

Yours faithfully,

H. WILLIAMS SMITH

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news and views

A pioneer's view of Venus

from Garry E. Hunt

VENUS, our nearest planetary neighbour, has long been thought to be the Earth's sister, because of its similarity in size and mass. But the rapidly advancing knowledge of Venus gained from Earth-based telescope studies, and the measurements taken during space missions, has shown that the planets are actually quite different. Without doubt, the most dramatic development in our understanding of Venus, and one that has also changed our theories of how the Solar System was formed, has come from the recent Pioneer multiprobe orbiter mission, whose initial results were described recently in *Science* (203, 743; 1979). (Except where stated all following references are to that issue).

Descending through the thick atmosphere, the neutral mass spectrometer (Hoffman *et al.*) and the gas chromatograph (Oyama *et al.*) showed surprisingly large concentrations of ^{36}Ar , ^{38}Ar and ^{20}Ne . The volume mixing ratio of ^{36}Ar in the Venusian atmosphere is approximately 90 times greater than that of the Earth's. It follows that the absolute abundance of ^{36}Ar in the Venusian atmosphere must be approximately 200 to 300 times larger than the Earth's. The abundance of ^{20}Ne is also higher in the Venusian atmosphere and comparable to that of ^{36}Ar . The ratio $^{36}\text{Ar}/^{38}\text{Ar}$ is similar to values observed for the Earth, meteorites and the Moon.

The ^{36}Ar measurements have a profound effect on theories of the formation of the Solar System. Taking the Viking measurements for Mars into account (see for example Anders & Owen *Science* 198, 453; 1977) we now find that the amount of argon decreases from Venus to Mars. This suggests that the solar nebula, out of which the planets were formed, had an inhomogeneous distribution of material. The inert gases, such as argon, were trapped in the grains which formed the planets and settled as a veneer on these bodies as they condensed out of the nebula. These discoveries by Pioneer Venus have clearly reshaped our thinking on this important

subject. To obtain a more detailed picture we need similar measurements of other Solar System objects, and in the foreseeable future the probe measurements of Jupiter and the studies of cometary atmospheres could yield invaluable data.

The multiprobe system on the Pioneer spacecraft consisted of a bus transporting a large probe and three small probes designed to land at a northern latitude, on the day side and night side of the planet. In this way a global picture may be developed from this set of measurements. The contrast of the atmospheric structure below an altitude of 50 km at the widely differing locations was found by Seiff *et al.* to be no more than a few degrees Kelvin, with slightly warmer temperatures at 30°S than at 5° or 60°N . The atmosphere was stably stratified above 15–20 km, suggesting that near adiabatic lapse rate is maintained by the atmospheric motions. The profiles move from near adiabatic toward radiative equilibrium, at altitudes above 40 km. There seems to be a region of vertical convection above the dense cloud deck, in the altitude range of 47.9 to 49 km, where the temperature is approximately 360 K.

Venus is covered with a ubiquitous layer(s) of cloud, whose structure is intimately associated with the observed temperature structures. From their orbiter measurements, Travis *et al.* have detected a substantial layer of sub-micron particles of thickness 0.05–0.1 in the lower stratosphere, well above the main sulphuric acid haze layer. Stewart *et al.* find evidence of albedo features at 2068 Å which corresponds to the bright and dark features seen in the Mariner 10 images. The dark regions may therefore be locations where SO_2 is strongly absorbing. Taylor *et al.* find temperature contrasts of 50 K between a cold circumpolar band. Beneath these visible clouds, the layers of material have now been examined in detail by the Pioneer instruments.

A middle cloud region exists between 51 and 56 km and shows a decrease in concentration to about 100 cm^{-3} according to Knollenberg and Hunter. This layer appears to contain both liquid particles and solid grains of

radius 10–20 μm . The dense layer lies between 49 and 52 km with the visibility restricted to less than 1 km. This is the region of largest particle size observed. Just beneath this thick layer is a distinct thin layer of somewhat reduced opacity.

The region from 32 to 48 km below these clouds possesses a thin haze of particles nearly $1\text{ }\mu\text{m}$ in size with concentrations of 1 to 20 cm^{-3} . The upper part from 47–49 km is most dense and corresponds to the region of convective activity observed by Seiff *et al.* Below the lower boundary the atmosphere appears to be free of particles all the way to the surface. However, this does not mean that the visibility is unlimited. The Venusian atmosphere is so dense that the scattering of light by gas molecules alone will hide the surface from view.

The general behaviour of the measurements reported by Knollenberg and Hunter, and Ragert and Blamont indicate a concentration of particle independent of height or decreasing at lower altitudes. This behaviour is consistent with a source at the top and sink at the bottom, and a downward flow by mixing and by gravitational settling. This suggests there is a source of sulphur and H_2SO_4 at or about 57 km, which is the top of the middle cloud region. The source is presumably photochemical in nature, requiring ultraviolet radiation that does not penetrate far into the sulphur cloud. The H_2SO_4 droplets lose water on the way down, but they encounter increased H_2O and perhaps SO_2 and SO_3 to form the lower cloud region of dilute acid and the densest layer. Most important, the large particle size ($>10\text{ }\mu\text{m}$) encourage the possibility of precipitation on Venus either in the dilute H_2SO_4 lower cloud region, or initiated by sulphur from the middle cloud region, which would pick up dilute H_2SO_4 when falling below 51 km.

Several substances, besides water, are almost nonexistent above the clouds but well represented below them. The abundance of SO_2 varies in this way by a factor of 240 and oxygen by 61 (Oyama *et al.*). These higher abundances arise when sulphuric acid and sulphur rain out of the clouds, vapour

ise and even dissociate in the heat below and then chemically attack gases found in the lower atmosphere.

The presence of SO_2 and not COS (Prinn *Science* **182**, 1132; 1977) as the major sulphur-bearing compound in the lower atmosphere, is a great surprise. (Oyama *et al.*). Previously it was thought that the abundance of COS indicated that the amount of CO in the atmosphere is buffered by certain minerals at the surface, and its value is representative of the amount below the clouds (Lewis *Earth. Planet. Sci. Lett.* **10**, 73; 1973). Now these new measurements suggest that the CO (approximately 50 p.p.m.) is generated almost entirely by photochemical processes and that no significant amounts of COS should be expected.

The Pioneer measurements confirmed the surface temperature at $\sim 737\text{ K}$ and reasonably uniform, at the base of this huge CO_2 atmosphere. Since Venus and the Earth are nearly planetary twins, why are their atmospheres so different? This is not simply an academic question. The planets would have had some basic similarities in their initial state and the processes that have transformed Venus into its current inhospitable state might still occur on Earth.

Venus is much nearer the Sun and receives twice the incident sunlight that reaches the Earth. The additional heat would have quickly produced an efficient 'greenhouse' effect, increasing the atmospheric CO_2 opacity and raising the surface temperature to a level which would be too hot for any water vapour to condense into oceans. It is therefore important that we fully understand the evolutionary process of Venus to gain a better perspective of the possible perturbations that may affect the Earth's atmosphere.

Now, Tomosko *et al.* have found that only 2% of the incident sunlight reaches the surface. However, for the greenhouse effect to be maintained, Bolse *et al.* find that the contribution from the water vapour, whose mixing ratio is only 10^{-3} , is essential to provide the additional opacity which combines with the contributions from CO_2 and the cloud particles.

What is the origin of the present-day Venusian atmosphere? Observations of the surface of the planet are essential to address this problem, but this is obviously difficult because of the extensive cloud layers. Radar maps of the surface are being produced from measurements made from the orbiter (Pettengill *et al.*). Although the measurements are restricted to the region bounded by 74°N and 63°S , the high resolution observations show evidence of a rift valley having vertical relief of up to 7 km. There is almost the suggestion of volcanic features, which may indeed be responsible for the creation of the

current atmosphere. The surface of Venus may therefore have some similarities with other terrestrial planets.

One of the most startling observations was made as the spacecraft descended toward the surface, where in the altitude range of 6–14 km, lightning storms were encountered. At least 1,000 impulses of radio noise were measured. Lightning on Venus is not unreasonable, since the atmosphere is electrically active. By way of comparison, there are typically 100 lightning strokes every second scattered all over the Earth. But on Venus there may be several times that number in a localised area. Instead of being illuminated for a brief instant by a dazzling flash of lightning, as on Earth, the Venusians may well be glowing from the nearly continuous discharges of those frequent lightning strokes. We have also gained a bonus measurement from the day probe which unexpectedly survived on the surface for more than an hour. It impacted on a surface composed of loosely compacted material so that a small amount of fine dust was ejected from the surface into the atmosphere in the vicinity of the probe (Ragen & Blamont). The dust subsequently settled out on to the surface in a few minutes.

The orbiting spacecraft travels on an eccentric path with a periapsis altitude of $\sim 200\text{ km}$ and apoapsis of $\sim 67,000\text{ km}$, and carries a range of instruments to investigate the properties of the upper atmosphere. Taylor *et al.* find that in the 60–140 km region of the atmosphere, there are small diurnal temperature differences near the cloud tops which increase with altitude. The pole-to-equator tempera-

ture contrasts are much larger. An isothermal layer of at least two scale heights in the vertical is found near the 100-kilometre altitude, where the temperature is $\sim 175\text{ K}$. The monitoring of this region above and in the neighbourhood of the cloud top is important in determining the mechanisms of the meteorology of this region.

In spite of the absence of any appreciable magnetic field, the ionosphere of Venus seems to repel the solar wind at least as well as the terrestrial magnetosphere (Wolfe *et al.*; Scarf *et al.*). At Venus, streams of particles and radiation from the Sun confine the ionosphere below a well defined boundary. This is called the ionopause, and is not found around the Earth. One of the major surprises is the presence of the ionosphere on the night side of Venus. The ions and electrons that produce this region should recombine when the Sun is removed as an excitation source. On Venus, the night lasts for 8 weeks, so it was at first thought that the ionosphere should disappear (Taylor *et al.*). Perhaps the atmosphere at these levels rotates more rapidly than the planet and therefore prevents the charged particles from neutralising.

The Pioneer Venus mission, with the orbiter and sophisticated probe systems has provided a great deal of information on our nearest neighbour. The *in situ* measurements of the atmosphere have produced surprising information that has changed our understanding of the formation of the Solar System. This is an amazing accomplishment. Detailed exploration of the planets always yields surprises and gives a new perspective on the Earth. □

Nerves, molecules and embryos

by Miranda Robertson

THE interactions of nerve cells with one another and with their environment at any stage are incompletely understood; the least understood of all are those that take place during embryonic development. Two philosophies can be discerned among biologists in this difficult field. One is that developing animals are so complicated that experiments on them cannot possibly yield interpretable information on the molecular mechanisms underlying development, and the only reasonable strategy is to investigate selected developing cells *in vitro*. The other is that because development is so complex

and the topology of the developing animal so important, nothing interesting can be learned about its mechanisms from cells, or even tissues, in culture.

They are probably both right; and a recent meeting* largely devoted to neural interactions in development therefore started propitiously with a talk from N.M. Le Douarin (CNRS Nogent-sur-Marne) whose work on neurochemical differentiation in chicks has recently run in reassuring parallel with that of the Harvard group, represented at the meeting by L. Chun and D. Potter, on neurochemical differentiation in Petri dishes.

The issue is what determines whether a sympathetic ganglion cell will become noradrenergic or cholinergic. Le

*A meeting on Cell-Cell Interactions in Neurobiology was held on 12–15 February, in San Diego. It was partly sponsored by the National Foundation March of Dimes.

Douarin's contributions to this question are based on the use of tissue grafts from quail embryos into chick: the grafted cells participate in the embryonic development of the chick, where their progeny can be identified by a stable nucleolar marker peculiar to quail. By grafting small pieces of quail neural crest into more anterior or more posterior positions in chick neural crest, she has been able to show that the migration pattern of the cells to the sympathetic ganglia and other structures is determined by their position on the antero-posterior axis, their neurochemical differentiation probably being induced by tissues at their destination.

Investigations by the Harvard group have suggested that the inducing influence is a soluble factor released by the target organs of the neurones: adrenergic neurones dissociated from the superior cervical ganglion of the rat are induced to express cholinergic functions instead if they are cultured with (for example) heart cells, or medium in which heart cells have been growing ('conditioned medium'). The existence of soluble factors would imply that direct cell contact is not necessary, but there are indications from preliminary experiments by E. Hawrot on ganglion cells cocultured with dead heart tissue that a cell surface factor may also exist (L. Chun, Harvard University Medical School).

The discovery that ganglion cell neurochemistry can be manipulated in this way has raised the question of how stable the differentiation of neural crest cells is even after they have reached their destinations. This stimulated Le Douarin to show that a similar switch can be induced *in vivo* by transplanting cholinergic ganglion cells from 4-6-day quail embryos to a potentially adrenergic region in the neural crest of chick embryos at an earlier stage of development (Le Douarin *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 2030; 1978). It has yet to be established how late in development ganglion cell differentiation remains reversible, but there is now some evidence that activity induced by presynaptic fibres may be what stabilises transmitter choice. This was originally suggested by some experiments of Black *et al. (Brain Res.* **34**, 229; 1971), and the Harvard group has been able to show that depolarisation induced by various means can prevent the effects of conditioned medium on cultured ganglion cells (Walicke *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 5767; 1977). The importance of the soluble factor in conditioned medium is that it specifically induces differentiation, without affecting growth or survival (by contrast with nerve growth factor, for example, which enhances growth and survival without influencing

differentiation). It is therefore crucial to establish clearly that conditioned medium truly causes phenotypic switching and not merely the differential survival of cholinergic cells in an already heterogeneous population. The Harvard group now has persuasive evidence for switching, from the results of experiments in which one or at most two cervical ganglion neurones are grown on a bed of heart tissue with which they can synapse. By monitoring the effects on heart cell contraction of stimulating the individual neurones, they have been able to show that neurones which by all available criteria were adrenergic at the start, become dual function (presumably in the process of switching) or cholinergic in such culture conditions.

Soluble factors

One consequence of Petri-dish embryology is that developmental neurobiology is beginning seriously to rival immunology in the proliferation of uncharacterised or semi-characterised 'factors' which are identified by their important but ill-understood actions on cells *in vitro*. G. Fischbach (Harvard Medical School) has made a start on the characterisation of a factor from spinal cord explants which will induce within hours an increase in both the density and the clustering of acetylcholine receptors on cultured chick myotube. From its molecular weight and sensitivity to tryptic digestion, the factor seems to be a small peptide, possibly related to the activity reported by Podleski *et al. (Proc. natn. Acad. Sci. U.S.A.* **75**, 2035; 1978) in nerve extracts and which has similar effects on cultured rat muscle. Its relationship to those described by Anderson *et al. (J. Physiol. Lond.* **268**, 731 & 757; 1977) and Christian *et al. (Proc. natn. Acad. Sci. U.S.A.* **75**, 4011; 1978) from neural tube and neuroblastoma/glioma hybrids respectively is more problematical, since in both these cases there was receptor clustering without any significant increase in receptor density. The importance of factors influencing receptor distribution is that they may play a part in neuromuscular synaptogenesis. A recent controversy about whether the receptor clusters that form at the site of the synapse are intrinsic to the muscle or induced by the nerve has recently been settled in favour of the nerve (see *News & Views* **278**, 599; 1979); but the mechanism remains unknown.

Another factor was discussed by J. Brockes (California Institute of Technology), whose long-term aim is to find out what determines the interactions of

Schwann cells with the neurones the myelinate. Schwann cells can be induced to proliferate in culture either by agents which elevate intracellular cyclic AMP, or by a factor which is present in extracts of bovine pituitary and brain but which does not seem to be any of the more obvious hormone or growth factors, which have no effect on Schwann cell division. The factor is not found in non-neural tissue and is a basic protein of molecular weight about 50,000. While the signal that induces proliferation is thus partly characterised, the signal for myelination is so far detectable only operationally. Schwann cells distinguish between small-diameter axons, which they envelop without myelinating them, and larger ones around which they wrap their membrane to generate the myelin sheath. Brockes reported that Schwann cells make detectable amounts of the major peripheral myelin proteins only when stimulated to do so by contact with appropriate axons, and interact with artificial fibres in the way that they do with unmyelinated axons. This suggests that Schwann cells are induced to myelinate by a chemical signal from certain axons, rather than simply by the geometry of large-diameter fibres. Considerably more elusive than factors influencing differentiation have been those that influence morphogenesis; but it is increasingly believed that differential stickiness of cell surfaces may account for much specific contact-making, both between individual cells and between sheets of tissue. Dennis Bray (Washington University Medical School, St. Louis), investigating the general question of the mechanism of neurite outgrowth, presented results which may suggest how differential adhesion could direct the growth of nerve fibres. Growing neurites end in a specialised growth cone, from which filopodia extend symmetrically to attach to the substrate. By pulling the neurite gently to one side or the other, Bray has shown that the direction of growth follows the main axis of tension.

The importance of stickiness

So far, there has been no attempt to test theories of differential adhesiveness on actual embryos; but it became clear at the meeting that this could and should be done, specifically in the case of the adhesive molecule that G. M. Edelman and U. Rutishauser (Rockefeller University) have identified in cultures of chick retinal and tectal cells. It now seems possible that adhesive molecules on the surface of retinal cells may be the basis for the strict ordering of their connections with the optic tectum which it is now believed may be determined simply by the preservation of neighbour relations between

growing axons. This notion has recently been checked anatomically by J. Scholes (*Nature*, **278**, 620; 1979) against the optic nerve of Cichlid fish, which is ideal for such studies because it takes the form of a ribbon in which the radial coordinates of the retina can be shown by retrograde degeneration to map across its width, and the circumferential coordinates across its thickness. (Much the easiest way to envisage this geometry is to see the retina as a fully opened circular fan: close the fan and you have the beginning of a ribbon.) Scholes has established that neighbour relations are preserved with very little deviation for the whole length of the nerve almost up to the tectum, when discrete bundles of fibres (within which neighbour relations continue to be preserved) undergo a complex rearrangement before spreading out on the tectal surface.

Stickness, clearly, could explain this kind of ordering, particularly since the retina grows in successive rings from centre to periphery, and new axons could thus follow the adhesive surface of the neighbouring older axon. The work of the Rockefeller group on a cell adhesion molecule (CAM) in cultures of chick retinal and tectal cells (see *J. Cell Biol.* **79**, 371, 382; 1978) may provide a biochemical test for the nearest-neighbour hypothesis. They have used antibodies to CAM to disrupt such ordering as occurs in cultures of retinal cell aggregates and spinal ganglia in a way that suggests that CAM mediates the sorting out of cells and reunions to form tissue layers, and the antibody can inhibit the side-to-side adhesion between neurites that produces nerve bundles. It may thus be possible directly to test the nearest neighbour hypothesis by injecting anti-CAM into embryos during optic nerve development and looking to see if the retino-tectal map is scrambled.

While there is now some hope of discovering the molecular basis for short-range effects such as the induction of differentiation and the guidance of growing axons, the long-range or global control of morphogenesis remains as mysterious as ever. What, for example, causes the optic nerve fibres to undergo a systematic piecemeal rearrangement at the tectum (Scholes *op. cit.*)? Indeed, what in general determines the orientation of developing systems? This question was raised in J. Cooke's (National Institute for Medical Research, London) discussion of his recent work, which is concerned with the determination of orientation in the tadpole eye. If a tadpole's eye is rotated at any stage after the first few hours after spawning, the growing nerve fibres will turn before they reach the tectum and make the connections they would have made from a normal

eye, thus giving the frog a rotated visual map. According to some classical experiments by M. Jacobson (*Science* **155**, 1106; 1967; *Devl Biol.* **17**, 202; 1968), eyes rotated at earlier stages become respecified, making connections appropriate to the rotated eye and producing a normal visual map in the adult frog. Cooke has failed to repeat this finding and in the course of seeking the reasons for this failure he has touched on the kind of developmental phenomenon that is most resistant of all to molecular explanation.

Cooke and his colleagues never find normal maps from rotated tadpole eyes, no matter how early the rotation: they find either rotated maps, or compound maps in which half the visual field is rotated and half normal. By using albino tadpoles as the hosts and grafting rotated eyes from wild-type tadpoles, they have been able to establish from the histology that the compound maps are due to the partial regrowth of the host eye. They suspect that Jacobson's results may therefore have been due to the regeneration of normally oriented eye tissue, the absence of compound maps being due either to the death of the rotated tissue or to a gradual takeover by the regenerating tissue in the course of growth—since many of Jacobson's animals were mapped when relatively mature.

These experiments led Cooke to the question of whether in fact it is the rotation itself that induces the regeneration of host eye tissue. This question arises from the attempts of French *et al.* (*Science* **193**, 969; 1976) to explain the occasional duplication of regenerating structures. Their proposal which has become known as the 'clockface' model, is that growing structures are specified according to circular coordinates and that rotated structures can give rise to duplications at the site of the graft because of intercalary regeneration between discrepant clock-face values. To test whether rotation is thus causing the regenerative duplication of the retina in tadpoles, Cooke has experimented with grafts of unrotated eyes; and preliminary results suggest that in these animals regeneration does not occur.

This kind of interaction is particularly difficult to explain by any imaginable molecular mechanism. Not that any actual molecular mechanism has yet been found to account for the relatively approachable problem of Cartesian positional gradients, which are generally believed to account for most morphogenetic signalling. Scholes has pointed out that the retinal circular fan is a way of transforming circular to Cartesian coordinates. This may not help with the identification of a morphogenetic model; but it could point the way to a unified theory. □

Is DNA really a double helix?

from Struther Arnott

ALTHOUGH it is 26 years since the announcement of the base-paired double-helical structure for DNA, there are still attempts to answer questions about DNA without using the Watson-Crick paradigm. For example, Rodley *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **73**, 2959; 1976), Cyrax and G  th (*Naturwissenschaften* **65**, 106; 1978), and Sasisekharan *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75**, 4092; 1978) have all proposed that the two strands of DNA do not coil plectonemically round one another. Thus, the problem of unwinding extensive lengths of cohelical DNA for replication is abolished at a stroke and new roles will have to be postulated for relaxase, gyrase, helicase and so on.

Rodley's structure is typical. It retains the antiparallel, complementary base-paired chains of the Watson-Crick structure and the 10-base pair axial repeat of B-DNA. Lack of mutual coiling is contrived by irregular chain conformations in which blocks of about five nucleotides have right- and left-handed twistings alternately. The resulting model is analogous to a warped zipper. Since the phosphate groups lie more or less on the surface of a cylinder with radius about 9 Å and the base-pairs are stacked 3.4 Å apart along the long axis of the molecule, it has many features in common with the double-helical model of B-DNA. The calculated diffraction pattern is therefore encouragingly similar to that observed from DNA fibres. Had such a model been available in 1953, it would have been regarded as a serious competitor for the original bihelical model.

Since 1953, the original DNA model has been substantially modified by Wilkin's school and refined to fit the >100 X-ray diffraction intensities which can be obtained from oriented and polycrystalline fibres of B-DNA. Recently, R. Chandrasekaran and I remeasured these intensities and re-refined the double-helical model of B-DNA which now provides a reliability index $R=0.28$, which is reasonable when one considers that fixed standard bond lengths and angles have been imposed on the model as well as specific base and furanose ring shapes leaving the six conformation angles of the generating nucleotide as the only variables. Poorer models have higher values for R . For example, a double-helical

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model with an unacceptable alternative base-pairing scheme has $R=0.45$. For the Rodley model $R=0.48$. These less regular warped zipper models have 10 times the degrees of freedom allowed to regular helices and would have to have $R<0.20$ to be regarded as being significantly superior to the best bihelical model according to Hamilton's test (*Acta Cryst.* **18**, 502; 1965).

It is not unthinkable to me that a warped zipper model could be contrived with R rather less than the present value of 0.48. The effort required would be considerable and, in the circumstances, one is inclined to look for more decisive experiments not requiring accurate knowledge of the atomic positions in DNA. Crick *et al.* (*J. molec. Biol.* **129**, 449; 1979) believe that studies of closed circular DNA molecules will suffice. When a closed circular DNA with N base pairs is unwound by known amounts, one can calculate the net number of times each chain is coiled round the other in relaxed DNA. This linkage number (α) turns out to be approximately $N/10$ as would be expected from the classical bihelical model rather than near zero as predicted for the warped zipper models. (By retaining a modest amount of coiling as a result of failing to balance exactly the right- and left-handed twistings, the proponents of the warped zipper structures have contrived that $\alpha \neq 0$ and avoided having to explain why closed circular DNA duplexes cannot be melted to give two separable classes of single-stranded circles.) Crick *et al.* argue also that the quantised migration of supercoiled DNA molecules in gel electrophoresis can arise only from the fact that all the molecules in one band have the same linkage number which differs by unity from that of molecules in adjacent bands, and that this can be explained only by assuming that the molecular secondary structure is double helical.

There is also crystallographic evidence that nucleic acid duplexes are regular helices rather than asymmetric zippers which does not require accurate knowledge of atomic coordinates. Intuitively, one might expect that molecules with fourfold screw symmetry (like the eightfold helices of *D*-DNA) would crystallise with tetragonal symmetry. They do. Similarly, one is not surprised to find that the 12-fold helices of *A'*-RNA crystallise with rhombohedral symmetry. The crystalline arrangements which would permit 11- or 10-fold helices to exploit the equivalence of many residues and maximise the number of favourable contacts between molecules are less intuitively obvious and might be expected to be rather peculiar. On the other hand, if such peculiar packing arrangements are indeed observed in

polynucleotide crystals, it would be difficult to escape the conclusion that the crystals contained 10- or 11-fold helices. *A*-RNA duplexes which have been described as 11-fold helices by me and my colleagues (*Nature* **211**, 227; 1966) provide a particularly compelling example. *A*-RNA duplexes pack in trigonal lattices containing two sets of close-packed triads. Although the triads are the same size, the contacts within them need not be the same. Indeed, for zipper molecules with no helical symmetry, they could never be the same. For 11-fold helical molecules, the contacts within both triads can be the same provided that the value of a particular packing parameter x is 0.368 or 0.458 (Arnott *et al.* *J. molec. Biol.* **27**, 549; 1967). It is a striking fact that there are two crystal forms of *A*-RNA, one with $x=0.365 \pm 0.005$ and one with $x=0.460 \pm 0.005$. Of course our credulity could be stretched by the argument that helical models should be aban-

doned for *B*-DNA alone among the dozen or so polynucleotide duplexes studied by X-ray diffraction. Fortunately, *B*-DNA crystals exhibit the analogous properties which would be expected of 10-fold helices. The unit cell is rectangular and contains two molecules. If these molecules are 10-fold helices and the number of equivalent contacts is maximised, the ratio of the unit cell sides a and b should be $\tan 2\pi/10$. The remarkable fact is that b/a is $\tan(36.15^\circ \pm 0.25^\circ)$. I find it hard to believe that these crystallographic expectations would be realised so accurately if polynucleotide duplexes in general and *B*-DNA in particular were not close to being perfectly regular helices.

For many years attention was concentrated on the base pairing of DNA. It was right that the backbone should be subjected to similar scrutiny. It is unlikely that any major modification will be needed. $\square$

Improved laser test of the isotropy of space

from Bridget Marx

It might be thought that the most sensitive tests of the special theory of relativity would be those performed on systems travelling close to the speed of light. However, a recent experiment by Brillet and Hall (*Phys. Rev. Lett.* **42**, 549; 1979) which relies on a velocity of only 10^{-4} times that of light has produced one of the most precise tests of the Lorentz transformation, and hence special relativity, yet achieved. The basic ideas of the experiment date from 1887 when Michelson and Morley performed their now classic 'ether drift' measurement. That experiment has been improved, modified and repeated several times in the intervening years with little more than an order of magnitude improvement in sensitivity. The new measurement extends the precision of the previous best null result by a factor of 4,000 in the fractional frequency shift.

The basis of Michelson-Morley type experiments is to look for an anisotropy in the velocity of light travelling in a moving frame of reference. The anisotropy for which Michelson and Morley (*Am. J. Sci.* **34**, 333; 1887) were looking was to be attributed to the effect of the Earth moving through the ether, in which it was supposed that light propagated. They measured the round trip times for light to travel in two per-

pendicular directions by looking at the resultant interference produced between the two arms of an interferometer, and then rotated the whole apparatus. They did not see the expected fringe shift and their resolution placed an upper velocity of 5 km s^{-1} on the Earth's motion through absolute space, in which the ether was thought to be at rest. An improved experiment by Joos in 1930 (*Ann. Phys.* **7**, 385; 1930) reduced the limit to 1.5 km s^{-1} . This was far below the expected value of about 30 km s^{-1} , the Earth's velocity about the Sun.

The null result of the Michelson-Morley experiment is considered very

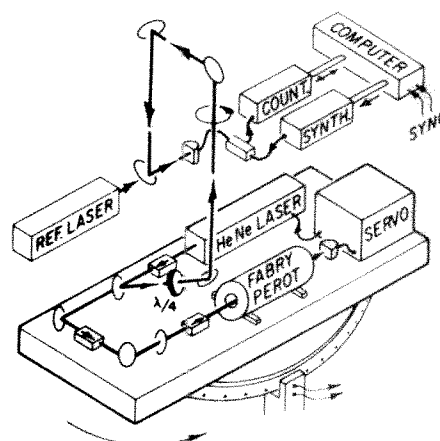


Fig. 1 Schematic of isotropy-of-space experiment. (From Brillet & Hall *op. cit.*).

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important to the foundation of Einstein's theory of special relativity. Indeed Robertson (*Rev. Mod. Phys.* **21**, 378; 1949), in an attempt to replace postulate with observation in special relativity, has derived the Lorentz transformation by assuming the existence of a Euclidean rest-frame in which light propagates isotropically and using the idealised results of the experiments of Michelson and Morley, Kennedy and Thorndike (*Phys. Rev.* **42**, 400; 1932) and Ives and Stilwell (*JOSA* **28**, 215; 1938). In Robertson's rest-frame there is a 'metric', with which one can measure space or time intervals, defined as

$$d\sigma^2 = dt^2 - c^{-2}(dx^2 + dy^2 + dz^2)$$

c being the velocity of light in this frame. In an inertial frame moving with velocity v along x the metric is transformed to

$$d\sigma^2 = g_0^2(v)dt^2 - c^{-2}(g_1^2(v)dx^2 + g_2^2(v)(dy^2 + dz^2))$$

If $g_0 = g_1 = g_2 = 1$ then this metric transform is exactly equivalent to the Lorentz transformation. If $g_1(v) = g_2(v)$ then space in the moving frame is isotropic and the Lorentz contraction follows. The three experiments mentioned above measure g_1/g_2 , g_0/g_1 and g_0 respectively. In fact, the fractional frequency shift observed in the Michelson-Morley type experiments determines $1/2((g_2/g_1) - 1)$, which would approximately equal $\frac{1}{2}v^2/c^2$ in the ether theory. The previous best limit to the value $g_2/g_1 - 1$ was that obtained by Jaseja *et al.* (*Phys. Rev.* **A133**, 1221; 1964) and was $\pm 2 \times 10^{-11}$. This was obtained by measuring the beat frequency between two rotating lasers, the cavities of which were perpendicular to each other. Unfortunately, large spurious noise signals limited their sensitivity to only a factor of three better than Joos had obtained.

The new experiment of Brillet and Hall gives a limit on $(g_2/g_1 - 1)$ of $\pm 5 \times 10^{-13}$, which is a significant improvement over the previous results. Their experimental arrangement is shown schematically in Fig. 1. As the length standard they use a single Fabry-Perot etalon, which is mounted on a table rotating in a horizontal plane. Also on the table is a helium-neon laser at around $3.39 \mu\text{m}$ whose output frequency is locked by a servocontrol system to the length of the etalon. Any change in the round trip time of the laser light in the etalon will result in a compensatory frequency shift of the laser, so that the laser wavelength always satisfies a standing-wave condition in the etalon. The etalon is very stable, made of low expansion glass and temperature controlled to within 0.2°C .

Using this kind of servo-system the laser frequency on a non-rotating table is observed to remain constant to within 20 Hz in a 1 s sampling time. The frequency of this cavity-locked laser is compared with that of a stationary helium-neon laser which is locked to an absorption line in methane, and has a similar frequency stability. Isolation of all parts of the system is important, and to this end the methane-stabilised laser is used to lock a third helium-neon laser at an offset of 120 MHz and this is then used to produce a beat frequency with the rotating, cavity-stabilised laser. Faraday isolators prevent feedback from the Fabry-Perot cavity to its laser. The excellent results achieved in this experiment are attributable partly to the attention paid to stability in all parts of the apparatus and partly to the sophisticated signal averaging techniques applied to the beat frequency data. Any spatial anisotropy will manifest itself in a beat frequency change, due to a change in the frequency of the rotating laser, periodic at twice the table rotation frequency. Each reading, which takes approximately half an hour, is the average of many table revolutions and the time dependence of the beat frequency is analysed to select the changes with the right periodicity. The resulting fractional frequency change is of the order of 2×10^{-13} . However, by taking readings at different times of the day and over 100 days this was reduced. A

genuine anisotropy signal will vary in phase according to our velocity direction through space. Spurious signals will remain largely with a fixed phase, and can thus be partly eliminated by looking for a 24-h phase rotation. By also deciding on what is the fixed, isotropic frame with respect to which the Earth is moving, it is possible also to take into account a yearly phase rotation and thus further reduce the spurious background. This averaging is assumed valid as there is no noticeable hour or day dependence of the data. This brings the observed fractional frequency shift down to 10^{-14} for a 24 h average and $1.3 \pm 2.2 \times 10^{-15}$ for the overall average. This result limits $(g_2/g_1 - 1)$, the spatial anisotropy factor in Robertson's derivation, to $3 \pm 5 \times 10^{-15}$. In terms of the ether theory this corresponds to a limiting velocity of about 20 m s^{-1} .

If we assume that the isotropic rest-frame with which we are comparing our moving frame is that in which the cosmic blackbody radiation is isotropic, then the velocity of the Earth is approximately 400 km s^{-1} . Using this velocity the null result of Brillet and Hall, with a resolution (sensitivity divided by predicted ether shift) of about 3×10^{-9} , becomes the most sensitive test of the Lorentz transformation yet performed. If the sensitivity is improved further it might soon be possible to observe the breakdown of this transformation as the anisotropies introduced by general relativistic effects become important.

Eels: new interest in an old problem

from J. D. McCleave and F. R. Harden-Jones

A RECONNAISSANCE expedition to the Sargasso Sea now underway may help to clear up a long-standing mystery. Where are the spawning grounds of the North Atlantic eels?

The American and European eels (*Anguilla rostrata* and *A. anguilla*) of the North Atlantic are very similar in appearance but individuals can be separated by their mean vertebral counts of $107 \text{ s.d.} \pm 1$ and $115 \text{ s.d.} \pm 1$ respectively. The eels are migratory. According to the classical account of their life history they spawn from

January to May in the Sargasso Sea; their pelagic eggs develop into leaf-like larvae—the leptocephali—which drift for $1\frac{1}{2}$ to $2\frac{1}{2}$ years in the ocean currents towards the continental shelves, and here they metamorphose into elvers which swim up the rivers and so into freshwater where as yellow eels they feed and grow. Several years later the adults leave freshwater and run down to the sea. While yellow eels are caught in coastal fisheries it is as silver eels that both species are believed to return to their parent spawning area, to spawn once, and then die.

This account of the life histories of the two eels follows that put forward by the Danish biologist Johannes Schmidt (*Phil. Trans. R. Soc.* **B 211**, 179; 1922). Schmidt based his hypothesis on the size distributions of eel larvae taken in the North Atlantic by Danish research

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vessels and merchant ships. But spawning eels have never been caught in the Sargasso Sea and only four silver eels have been taken off the continental shelf of Europe. No eel eggs have been identified from plankton samples taken in the Sargasso or from any other area. Schmidt used the distribution of the smallest eel larvae taken by research and merchant vessels to define the spawning area for European eels as being bounded by latitudes 20°N and 30°N and longitudes 48°W and 65°W. Although the collection of American eel *leptocephali* was poor, he suggested that this species spawned in a contiguous area somewhat to the southwest.

Schmidt's hypotheses gained general acceptance and his major conclusions were not questioned until 1959 when the English biologist Denys Tucker challenge the "well-publicised saga" (*Nature* **183**, 495; 1959). Tucker offered a "new and drastically different interpretation of the observed phenomena", namely that European eels do not return to the Sargasso spawning ground but perish in continental waters, that European and American eels are not two distinct species, and that eel populations in Europe are maintained by larvae from American eel parentage. This touched off a lively correspondence (*Nature* **183**, 1405; **184**, 1281; 1959; **185**, 589; 1960) with eel workers critical of his new solution. Perhaps Tucker's major contribution was not so much a solution as a reminder that much of Schmidt's story was based on circumstantial evidence. But while most biologists are reluctant to reject the classical account, Tucker's hypothesis has never been disproved, and little weight has been given to Vladikov's point (*J. Fish. Res. Bd Can.* **21**, 1523; 1964) that the presence of American eels in Trinidad and the Guianas in South America is only consistent with a spawning area much farther to the south of that suggested by Schmidt.

There are a number of outstanding questions to answer in the biology of the North Atlantic eels: are there two distinct species; and if there are, where are the spawning areas and when do they spawn; what are the mechanisms for species separation during the period of larval drift; and what are the migration routes and orientation mechanisms of eels in the open sea?

Renewed interest in the life histories of eels developed from the 'Symposium on eel research and management' sponsored by the International Council for the Exploration of the Sea (ICES) and the European Inland Fisheries and Advisory Council (EIFAC) and held at Helsinki in 1976. In part this is attributable to increasing commercial interests and pressure for management. The first sale value of

adults of the two species is about £10 million annually. They are often under-utilised, being processed for pet food in the United States, whereas when smoked they fetch £20 per kg on the Danish retail market and live unpigmented juveniles—'glass' eels—may fetch £500 per kg on the Japanese retail market. Furthermore there is concern for overfishing in some European rivers and the danger is thought to be real in areas where both elvers and adults are exploited. The Helsinki symposium endorsed a recommendation that ICES and EIFAC should organise an International Expedition to the supposed spawning area of the European eel. International support for the venture was enlisted and the Federal Republic of Germany has made the research vessels 'Friedrich Heincke' and 'Anton Dohrn' available for two cruises to the Sargasso Sea early in 1979 under the leadership of F.-W. Tesch of Hamburg. A reconnaissance cruise is now under way.

The possibility of UK support for the Expedition has been discussed by an *ad hoc* group set up by the Royal Society's British National Committee on Oceanic Research and by the Natural Environment Research Council. Although it was agreed that the 'eel problem' raised scientific problems of great interest and merit which deserved further study, it was thought that existing collections of larval material should be properly examined before launching an International Expedition and this view was sustained at a meeting of the ICES Planning Group held in October 1978. Attempts to secure support for an International Expedition have therefore been postponed until the results of the reconnaissance cruises are to hand and the study of existing collections completed.

Progress is being made with the material. All Schmidt's collections from west of 40° longitude, including much unpublished material, are being systematically examined and analysed by Jan Bøetius of Schmidt's old institute, Danmarks Fiskeri-og Havundersøgelser, Copenhagen, in collaboration with E. F. Harding of the Statistical Laboratory, University of Cambridge. Results from the collections of European eel material will be forthcoming but enough is already known to suggest that Schmidt lacked sufficient material to deduce the spawning area for American eels. Examination and analysis of substantial larval collections pertinent to the American eel is to begin soon at the University of Maine in cooperation with other American universities and the National Marine Fisheries Service. At the University of Maine a computer simulation study of larval drift is being developed for the western North Atlantic. The known freshwater distri-

bution of American eels may, in conjunction with these studies, allow a prediction of a likely spawning area for the American eel.

The return of European eels to the Sargasso could be shown directly by their capture at sea and Tesch might succeed in this aim in 1979. Methods other than direct capture are being considered for the future. Large predators may eat eels and their stomach contents could be examined; if the otoliths of adult American and European eels are distinguishable by chemical analysis, scanning electron microscopy or other means, the screening of bottom sediment cores might provide evidence of their distribution; and specific chemicals from mucus or the gonads of mature eels might be detectable in seawater samples, so allowing the presence of adult eels to be deduced without capture.

Nearly 20 years have passed since Tucker suggested that there was but one species of North Atlantic eel. A search has been made for genetically based biochemical differences in haemoglobins, serum proteins, and various enzyme systems. Chromosome number and types have also been examined. But the results of this work have not yet given conclusive evidence for species distinction.

The mechanism for species separation (or the eco-phenotypic separation of Tucker) during larval migration has been assumed to be simply drift from slightly different spawning grounds, differential growth rate and age-at-metamorphosis. That the separation mechanism is imperfect is demonstrated by the arrival, in small numbers, of American elvers in European waters. New collections of larvae to be made along transects into the southwestern North Atlantic during Tesch's 1979 cruises and the examination of existing larval collections and the larval drift simulation study should help to clarify the problem of larval separation.

As no migrating adult eels have been caught off the continental shelves, nothing is known of their migratory routes and nothing is known of their orientation in the sea. Ultrasonic telemetry tracking studies of the movements of migratory eels and displaced non-migratory eels are now being carried out in European continental waters by Tesch and by McCleave. Greer Walker and Arnold (at the Fisheries Laboratory, Lowestoft), and Tesch plans to follow hormonally-treated adult eels in the Sargasso Sea in 1979. But there is undoubtedly a long way to go before the migration of the adult eels can be described in behavioural terms. Nevertheless, the next decade should see a considerable advance in our understanding of the old 'eel problem'. □

SPRING BOOKS

R. A. FISHER was one of the really great scientists of this century, a man of immense ability, with an independent mind of great penetration combined with wide interests and striking erudition. He is, of course, best known for his contributions to statistics and genetics, but he was interested in any variable phenomenon and indeed made a basic statistical contribution to the study of palaeomagnetism, whose implications for the theory of continental drift he both recognised and expounded. All these he combined with a capacity for eccentric behaviour which, though sometimes exasperating or embarrassing to his friends, is now chiefly remembered for its contribution to the seemingly inexhaustible fund of "Fisheriana"—some of it no doubt apochryphal but most of it firmly based on fact. It has indeed been said that in later years, biometrical meetings not uncommonly developed into gatherings engaged in telling endless stories about the master.

I had the privilege (and it *was* a privilege, even if at times a little wearing) of working closely with Fisher, in the Galton Laboratory, for several years in the mid-1930s. They were unforgettable years, and not just because they saw the publication of that great book, *The Design of Experiments* (which Fisher gave as a lecture course while he was writing it), the introduction of such new statistical techniques as discriminant functions, the analysis

of Nabours' data from wild populations of grouse locusts which revealed selective differentials attaining the hitherto unheard of level of 10%, or even the birth of the serological unit which was to unravel the complexities of the *Rh* gene and do so much for the study of human blood groups. They were unforgettable too for the way in which he gave unstintingly of his time in

Life with Fisher

Kenneth Mather

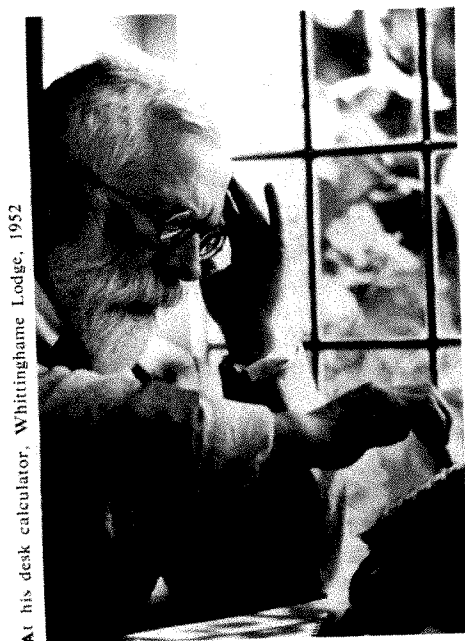
R. A. Fisher: The Life of a Scientist. By J. Fisher Box. Pp.512. (Wiley: New York and Chichester, UK, 1978.) £16.

explaining to a hitherto statistically uneducated young biologist how to approach the analysis of data, including the clarification of the (at any rate, to him) elementary mathematical points at issue, and for his insistence that genetical experiments should be designed with the same care as agronomical experiments, including the calculation of the laboratory resources that would be required. In the case of mouse experiments this was done in terms of mouse-cage-months, and Fisher often based his calculations on optimistic figures for speed and prolificacy of breeding. One soon learned that outright argument about this would serve only to irritate him, but a continuing attitude of concern would lead him to accept a programme more realistic in its expectations, particularly as the numbers of mice failed to mount in the expected way. One learned, also, to look after snails, grouse locusts and dogs as well as mice, and even for one brief period to look after some marsupials which he imported from Australia on the grounds that marsupial genetics had been sadly neglected.

Life with Fisher was indeed a never-ending education. His kindness and patient helpfulness, especially to young people, was something that those of us who benefited from it will never forget. We also saw, however, how coldly and continually hostile he could be to those whom he suspected of being hostile to his views or obstinately misguided in their own. The mid-1930s saw too not only his last polemical

exchange with Karl Pearson but the sad controversy with W. S. Gossett ("Student") with its revelation of a contentiousness so stubborn that it did not even spare an old friend. This contentiousness no doubt sprung from the treatment he had himself received (though not from Gossett) in his earlier struggling years, and it was no doubt also to these early years that we should trace Fisher's deep-seated distrust of accepted authority to whose nature he refers specifically in the introduction to the *Design of Experiments*.

Our personal recollections of this outstanding, and in some ways paradoxical, man can now be filled out and extended by the biography which his daughter, Mrs Joan Fisher Box, has written. It is a lengthy book, but for me at least it made compulsive reading. It supplies us with a full account of his ancestry and early life; and with it we can enter into the life of the Fisher family, its excitements, its happinesses and its sadnesses in a way which is possible only when looking through the eyes of one of the family itself. One can share Mrs Box's nostalgia in looking back at her childhood, as in the brief but touching account of Christmas in the Fisher household, including the story of how on one occasion "the existence of Father Christmas was challenged and one little boy stayed awake and saw for himself the figure who entered and filled the socks; he wore an unfamiliar long gown of

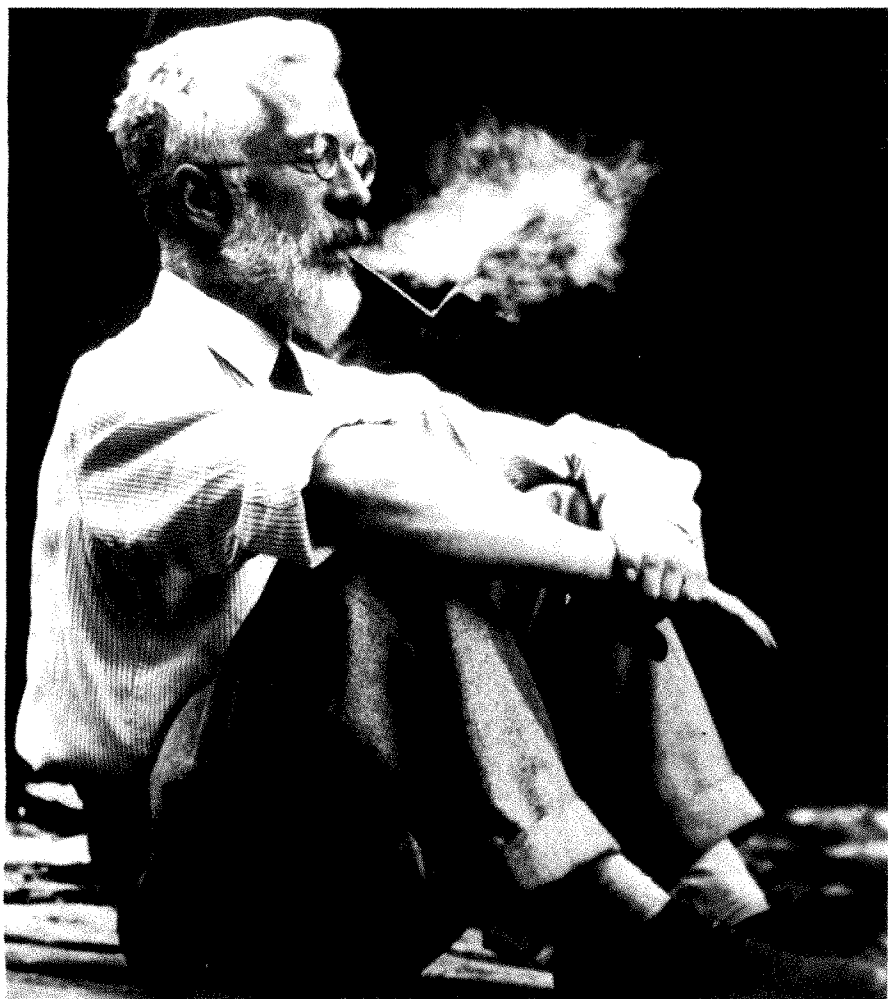


At his desk calculator, Whittinghame Lodge, 1952



At the seaside, 1938

SUPPLEMENT



At Lake Junaluska Assembly, North Carolina, 1956

Courtesy Mrs Horace Norton

scarlet silk, whose pink facings might have revealed to a more sophisticated watcher that Father Christmas was a doctor of science from Cambridge University; a mask with curling silver beard hid his features". This is true Fisher and a worthy addition to our store of Fisheriana. Nor does Mrs Box burke the sad story of the break-up of Fisher's marriage. She paints her father's picture in a way that would have met with Cromwell's approval—"warts and all": blemishes as well as greatness.

It would, however, be wrong to leave any impression that the book is wholly, or even mainly, devoted to Fisher's family life and personal matters. On the contrary Mrs Box presents a full account of his scientific life, his scientific interests and scientific achievements, with which the more personal things are interwoven, as indeed must have been virtually inevitable in writing about Fisher. The early years,

before 1919, not only bring in reference to the paper he gave while still an undergraduate in 1911, in which his interests in biometry and genetics are foreshadowed, but also cover his two early major works on the exact distribution of the correlation coefficient and the correlations between relatives on the supposition of Mendelian inheritance—the latter now a genetical classic.

He went to Rothamsted in 1919, initially for such time as a grant of £200 would cover, but stayed until 1933. During these fourteen years he recast the whole theoretical basis of statistics and developed the modern methods of designing and analysing experiments, while at the same time he was generating his theory of the evolution of dominance and writing his seminal book *The Genetical Theory of Natural Selection*—and this genetical work was all carried out in his spare time.

When he succeeded Karl Pearson at

the Galton Laboratory in 1933, he was able further to develop his genetical interests by undertaking genetical experiments on a sizeable scale and by founding the serological unit. But a wealth of statistical work continued, including the sharp defence of his basic approach against those who chose to criticise it. Much the same balance of interests continued in Cambridge where he succeeded R. C. Punnett as Professor of Genetics in 1943. The Cambridge days saw the publication of the *Theory of Inbreeding*, arising from some of his mouse experiments, and also *Statistical Methods and Scientific Inference* in which he discusses at length the problems and logic of inductive inference. They also saw the flowering of the serological unit and the recognition by many honours from universities and academical bodies in a number of countries, as well as by the knighthood conferred on him in 1952. He travelled widely, especially in the USA and India where his ideas had a great influence, and after retirement in 1958 his Australian friends persuaded him to make his base in Adelaide where he died in 1962.

Each period of his life can be seen as marked by its distinctive scientific achievement, springing from the problems which his various appointments posed and the opportunities which they offered. It is a fascinating story which Mrs Box has to tell, and she tells it well. Her presentation and assessment of his work is comprehensive, balanced and informative, and she points to limitations where she sees them, as for example in Fisher's much smaller interest in the direct study of the hereditary materials than in the numerical relations to be found in breeding experiments.

This is indeed a fascinating book. To those who knew and worked with him it will bring the special pleasure of recalling a past association that was both memorable and eventful. To a wider audience it offers an account, at once informative and human, of a great scientist who gave us statistics as we now know it, while at the same time establishing himself among the great leaders of genetics especially in the study of natural selection and its impact on populations and their properties. □

Kenneth Mather is Honorary Professor of Genetics at the University of Birmingham, UK.

Morgan's crisis

C. D. Darlington

Thomas Hunt Morgan: The Man and His Science. By G. E. Allen. Pp. 447. (Princeton University Press: Princeton, New Jersey, and Guildford, UK, 1979.) \$31.50; £15.80.

DURING his life the work of Thomas Hunt Morgan was the centre of many disputes. The chief of these concerned the validity of his famous discovery: Were the chromosomes the physical basis of heredity? Others concerned secondary questions: What did the discovery mean? How was it made? And who made it? The first question for long divided the scientific world into battling parties. The others divided only the new believers who in the course of 50 years have taken possession of the battlefield. For them the secondary questions have grown in importance. And for their solution the life of this enigmatic man has become of absorbing interest.

We now have two books on Morgan from outside his scientific circle which help us to answer these questions. One is a concise but accurate and convincing account by Shine and Wrobel (Kentucky University Press, 1976). The other is this larger treatise by Allen. Neither comes to grips with the crucial issues, perhaps because no one can who was not himself in the fray. Before it is too late, therefore, we should recall how those issues came to a crisis as they did for Morgan in the spring of 1910.

It was the meeting of two apparently conflicting ideas which created this crisis. Both had come from Europe to the United States in the course of Morgan's teaching life. They had been brought by two unconnected missionaries. One of these was E. B. Wilson who had become the head of Morgan's department at Columbia University. The results of his visits to Europe in 1883 and 1892 were concentrated by Wilson into the three editions of his great book, *The Cell*; a book which cost him the labour, not of one decade (as Dr Allen supposes), but of a lifetime. It was a book which argued the central position of the chromosomes in heredity and development.

The other missionary was Bateson, whose journey from England was different in period, scope and direction. His Silliman lectures in 1907 had argued the central position of Mendelism in the study of heredity. And they had converted to his view several shrewd critics at Yale and at Harvard, notably East on the plant side and Castle on the animal side.

So two schools, sharply divided, came into being in the United States. Neither seriously converted the other. And neither in the least converted Morgan. Indeed it seemed that 20 years of embryological experiment had left Morgan without any theory of his own and ostentatiously sceptical of everybody else's. He doubted Wilson's chromosomes. He ridiculed Bateson's factors. He confused Darwin's origin of species with his natural selection, which he even condemned as teleological. And (to use Allen's word) he "chastised" Weismann for his errors. He paid some respect to the mutation theory of de Vries. But when in 1907 Fernandus Payne, his pupil jointly with

Wilson, asked him for a problem he gave him the Lamarckian task of breeding Castle's *Drosophila* in the dark for 60 generations.

Payne, as he recalled 67 years later, failed to diminish the fly's eyes by two years' life in the dark. But Morgan himself, in the third year, with a hand lens, was able to find something in the light, a mutation, a single male fly with white eyes. The factor responsible proved to be, as it was likely to be, in the unpaired sex chromosome. From this one fly Morgan could therefore with little trouble demonstrate Mendelian segregation, sex-determination by chromosomes, and sex-linkage. Even, if he had thought of it, he could have seen a ratio distorted by natural selection. The progeny of this one fly corrected unmistakably all Morgan's recent mistakes.

In this embarrassing position Morgan quickly wrote a letter to *Science*. Then on 7 July, 1910, writing from Wood's Hole (although Dr Allen does not seem to know) he sent the much corrected typescript to Bateson at Cambridge, offering to share with him his stocks and the theory they might carry with them. Bateson, however, had come to distrust Morgan's judgement and he evidently declined the stocks; he also certainly rejected the theory.

Having failed to enlist Mendelian support, Morgan, back in New York, turned to the chromosome side, to Wilson in the next room to his own. Wilson was able to tell Morgan about the Belgian cytologist, Janssens, who from seeing chiasmata between chromosomes at meiosis recalled, as German cytologists had done earlier, Weismann's theory of meiosis. This theory demanded recombination which would arise by "crossing over" and would explain linkage. Wilson was also able to recommend to Morgan three pupils who had learnt about the chromosome theory from him, and about Mendelism from the books of Bateson's disciples, Punnett and Lock. They would breed his flies.

The results in the fly-room were dramatic. But what exactly happened? The answer I partly discovered for myself when twenty years later I questioned the two leading figures in the drama. Who did it, I asked Wilson; and his reply was quite firm. Morgan's three greatest discoveries, he said, were those three men, Bridges, Sturtevant and Muller. Then I asked Morgan what Janssens' idea had meant for him. Janssens, Morgan replied, being a Jesuit, had seen his problem not scientifically but teleologically. His idea of crossing over must have come from the belief that there could not always be four products of meiosis in all sexual reproduction unless all four were different. Morgan clearly had not read



Nobel Prize was announced, 1935

Courtesy Dr Tove Mohr

Janssens' article. He did not know that in his first sentence Janssens deduced his argument from Weismann, that is, from the belief that only with recombination could natural selection be made to work. I sadly concluded that Morgan had never understood, and perhaps had long made up his mind not to understand, the chromosome side of the chromosome theory.

The gaps in understanding between Wilson, Morgan and Bateson were profound. How profound is shown by their continuance today, and by the failure of Morgan's biographer to notice them. For this reason he does not realise that the process of crossing-over between chromosomes has never been seen in female *Drosophila*. He does not realise that it remains, as Morgan would have said before he inferred it, a pure speculation. Nor does Allen realise the fear Morgan had of discussing the other side of the story, the absence of crossing over in the male *Drosophila*. Was its purpose perhaps to make his experiments possible? We shall never know.

For over 20 years Morgan had demanded facts and denounced speculation. But now, swept forward by the success of his clever young men, he found himself inscribing his name as the head of a grand speculative movement. Dr Allen devotes proper attention to those three young men: Bridges, the quiet easy-going countryman from the north, whose keen eyes discovered hundreds of mutations;

Sturtevant, the observant horse-breeder from the south whose ingenious experiments and interpretations carried him beyond heredity into development; Muller, the intense excitable New York Jew whose imagination went too fast and too far for Morgan's understanding or forbearance.

These men did not create a unified genetic theory. That was too much to expect. But they put together the assembly line of a great *Drosophila* industry. Surely no city but New York at that time could have brought together such a combination of talent. And if Morgan had a "creative genius" as Dr Allen calls it, it was that, when he saw what they could do, he let them do it.

The defect of this *Drosophila* industry lay in the limitations of the fly. It failed to reveal the chromosome processes underlying its heredity and indeed heredity in general. The diosophilists had to avoid meiosis. Making a virtue of necessity, Muller therefore quickly went beyond the chromosome to the gene. There the reluctant Morgan followed him to adopt the gene as his own. The chromosomes could then be taken for granted. Reversing the order of Nature the chromosomes did what the fly required. This was Morgan's "Theory of the Gene".

After the lapse of years Morgan, having lost interest in the experiments of genetics, and never having sustained an interest in its ideas, was awarded a Nobel Prize. Dr Allen's account allows us a glimpse of the embarrassment that once again overtook him. Having delayed his journey to Stockholm, on his way he arrived in Edinburgh still uncertain what, and whom, and how much, he should acknowledge. Such a failure to sort out his intellectual antecedents might be thought to be a merely personal problem and one not uncommon in science. But in this case, its effects have survived far beyond any personal interests. The hiatus in the relations of Morgan, with Bateson on the one hand, and with Weismann, Boveri, Janssens and Wilson on the other, has left its traces in the dichotomy of genetics as it is taught today, a defect liberally illustrated in the present biography.

The Nobel Prize had been heralded by a last general discourse, a book of lectures unhappily entitled *The Scientific Basis of Evolution*. In the one original chapter in this work Morgan finds himself trapped with the too obvious notion that the evolution of man introduces a new biological principle. In his view there are now "two processes in inheritance" in man. The inheritance of culture, he explains, is now independent of the old limitations of genetic inheritance as under-



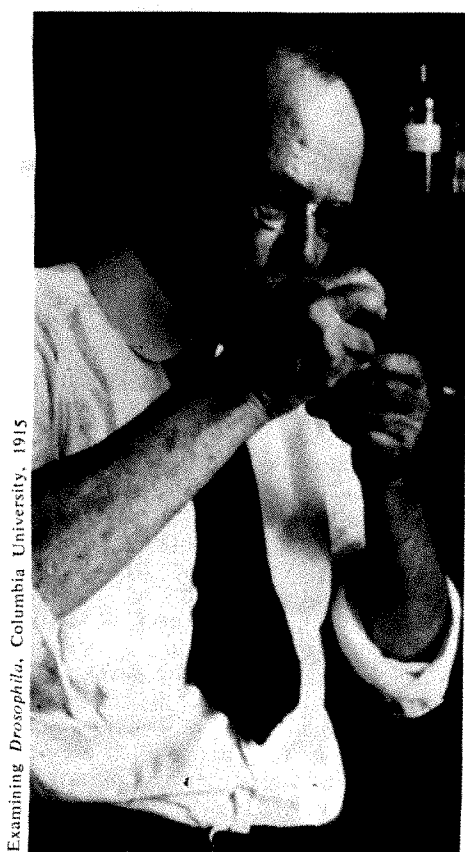
In the Fly Room at Columbia. "Who combed my hair?" Morgan asked when he saw this snapshot

stood, for example, in the fly.

It is surprising that Julian Huxley in a book on evolution which he dedicated to Morgan should have adopted this fallacy of Morgan's 30 years later as his own. Without apparently knowing its source he described it as "psychosocial inheritance". But it is even more surprising that Morgan's biographer, claiming to be a Marxist (who should always know what he is going to discover), has now failed to notice this gem of Marxist dialectic: for here is an idea which opens the way so agreeably to Lamarckism and Lysenkoism. From it the whole froth of our social sciences today, with their specious arguments and calamitous consequences, easily flows and overflows.

Dr Allen must have occupied many years in preparing this work, as his method of assembling it is a recipe for chaos, chaos in writing and chaos in reading. His accommodation of Bateson (and the present reviewer) at the John Innes Horticultural "School" which he places in Merton College, Cambridge, is an extreme example. But what can be expected of a writer who buries his references irrecoverably in 743 footnotes dispersed throughout the text? Clearly his book may help later enquirers into the history of genetics. But not unless they are willing to start at the beginning again, however distressing the results of their enquiries may be. □

C. D. Darlington is Emeritus Professor of Botany at the University of Oxford, UK.



Examining *Drosophila*, Columbia University, 1915

Courtesy Isabel Morgan Mountain

Master of biological research

M. R. Pollock

Selected Papers in Molecular Biology. By Jacques Monod. Edited by A. Lwoff and A. Ullmann. Pp. 753. (Academic: New York, San Francisco and London, 1978.) \$29.50; £19.15.

No doubt there will always be some scientists, overburdened by the appalling mass of literature that has to be sifted through these days, to protest against the publication of reports that have already appeared in print and are generally available in libraries. But there must be many more who will warmly welcome this collection of papers by Jacques Monod, who was unquestionably one of the greatest of the architects and exponents of the new biology. It is, in fact, a selection of less than half his total output of 131 scientific papers and, rather unfortunately, does not include any of his forewords, essays, reviews or recorded interviews on broader themes, which are less easy to get hold of than the others. Indeed, there must be many who would have liked an unexpurgated collection of *The Complete Works*. He was a master of beautiful and clear writing, both in French and English, and his views on almost everything are worth reading.

Presumably the basis of the editors' selection was to avoid redundancy of material and the extravagance of two volumes. The 62 papers reprinted make a fair-sized book which, priced at over £19, seems expensive enough for something in principle already available, but still less than half what would have to be paid for loose xerox copies. The volume is generally well produced and only relatively few articles have had to be reduced to an extent where the size of print is borderline for easy reading. A fair proportion of the papers excluded are abbreviated preliminary reports appearing in *Comptes rendus* . . . , completed versions of which are published elsewhere; but there are some apparent inconsistencies. An introductory explanation of the selection principles used would in any case have been interesting and reassuring.

The book in itself is a great tribute to the work and genius of Jacques Monod. "Occasionally the career of a scientist", write the editors in their preface, "is marked by an important discovery. It is most unusual that it be

illuminated by an uninterrupted series of great discoveries and still more unusual when each discovery gives rise to new concepts and opens new vistas". But it is much more than just a tribute. It is a story, told in the dry, restricted language which research workers are expected to use for recording what they have done and how they think, of one man's contribution to scientific knowledge. And, apart from a very few pieces of work mostly at the beginning of his career, it is the story about a single problem: the specific control of enzyme production in living organisms. This problem originally presented itself, apparently rather narrowly, as the phenomenon of 'enzyme adaptation' in microorganisms and it was tackled by Monod mainly through the



Jacques Monod

Camera Press

study of a single system: the famous β -galactosidase of *E. coli*; but it carried with it tremendous implications for a better understanding of the wider problem of the phenotypic expression of genetic potentiality in general.

But heaven forbid that anyone should read the book like a story from beginning to end! It is, rather, a book of reference and inspiration, to be consulted by research students in order to learn something about how a master of biological research thought and worked, and for historians to understand better thereby the evolution of ideas in one area of biology covering less than 30 years: a period, however, that comprised the profound revolution in our thinking in genetics, biochemistry and ultrastructure which we now call molecular biology.

This period in Monod's work

stretched over only 20 years: from the late 1940s, during which he wrote his first long classic review on 'enzymatic adaptation', published in *Growth*, to his Nobel prize article of 1966. To get a proper perspective, it is illuminating to read these two articles directly in sequence. In the first, the problem was defined, the essential questions posed and some tentative, plausible mechanisms (often incorrect, but that is not the point) suggested. In the last, most, if not all, the questions had been answered.

In between, Monod had master-minded developments crucial to providing the answers. These include (amongst others) the discovery that specific inducers of enzyme formation are not necessarily even ligands of the enzyme, let alone substrates (1950); the illuminating application of the 'allometric plot' to the study of the kinetics of enzyme induction (1952); the demonstration (1954) that enzyme induction involved *de novo* biosynthesis of the specific enzyme protein from amino-acids; specific 'permeases' (1955); and the discovery (1958) that the inducer operated by antagonism of a specific gene-determined repressor. Thereafter followed the 'presentation' of a veritable avalanche of new concepts: 'structural' and 'regulatory' genes, the 'operator', the 'operon', the 'promoter', and finally (1962) the property of allostery which is of fundamental importance to the understanding of the control of enzyme function and enzyme synthesis.

In nearly all these developments Monod played a vital—usually a completely dominant—role, although it is important to realise that outstanding colleagues such as Mel Cohn and François Jacob (and indeed several others whom he guided and stimulated in his inimitable fashion) often also played an important—and sometimes essential—part; not forgetting André Lwoff himself who provided, right from the beginning, the emotional, intellectual and material background support without which almost everything would have been five times more difficult.

Monod's essential talent lay in his ability to combine a precise, logical formulation of a problem (that is, in terms that could be tackled experimentally and had meaning within the current 'paradigm') with an imaginative grasp of its implications in biology as a whole, and the power to initiate plausible interpretations yet move on from a concept, however attractive, once it was beginning to obstruct, rather than promote, the development of a subject. The difficulty, of course, is to recognise the obstruction. It is failure to do this that so often prevents research workers from making original discoveries. And nowhere is this better illustrated than in Monod's final rejection

Royal Society of Medicine International Congress and Symposium series No. 7

New Advances in the Treatment of Candidal Vaginitis

edited by D. Gough

May/June 1979, approx. 55pp., £4.50/\$9.25 (Paper 0.12.791706.3 (Academic Press))

Contents include

S. R. Mayhew and W. E. Suffield: A study of the efficacy of seven-day administration of miconazole in candidal vaginitis. D. Gough: The influence of dosage and duration of administration of miconazole on the cure and relapse of candidal vaginitis. M. Borgers and M. De Brabander: Differential effects of imidazole anti-fungal drugs on the yeast and mycelial phases of *Candida albicans* in vitro. A. Reyntjens: New gynaecological formulations of miconazole. W. Denald: A study on the cure and relapse of candidal vaginitis with a novel formulation of miconazole.

Published jointly by the Royal Society of Medicine, Academic Press and Grune & Stratton

Information Transmission in the Nervous System

M. Uttley

May/June 1979, xiv + 102 pp., £7.50/\$15.50 0.12.709750.3

In this monograph the author puts forward a hypothesis about synaptic variability which offers an explanation of psychological findings in terms of neurophysiological facts, and which is applicable to phenomena at all levels in the nervous system, from single neurones to the whole animal. The hypothesis is described by a single equation from which numerical predictions can be made, and in the first four chapters it is fully discussed together with its consequences in simple situations and its computer simulation in complex situations. The book will undoubtedly be read with great interest by a wide variety of students and researchers in psychology and neurophysiology.

ASA Monographs

18. Social and Ecological Systems

edited by Philip Burnham and Roy Ellen

May/June 1979, approx. 330pp., £8.80/\$18.25 0.12.146050.9

Contents include

James J. Fox: A tale of two states: ecology and the political economy of inequality on the island of Roti. David R. Harris: Foragers and farmers in the Western Torres Strait Islands: an historical analysis of economic, demographic and spatial differentiation. Pierre van Leynseele: Ecological stability and intensive fish production: the case of the Libinza people of the Middle Ngiri (Zaire). Paula Brown: Change and the boundaries of systems in highland New Guinea: the Chimbu. Jonathan Friedman: Hegelian ecology: between Rousseau and the world spirit. Tim Ingold: The social and ecological relations of culture bearing organisms: an essay in evolutionary dynamics.

Society for Applied Bacteriology Technical Series No. 12

Plant Pathogens

edited by D. W. Lovelock

May/June 1979, xii + 122pp., £9.60/\$19.75 0.12.457050.X

Plant pathology is an area in which significant advances are continually being made. This reflects the perennial interest of agricultural and horticultural microbiologists in plant diseases and in the relationship between the causative organisms and the environment. This book records the proceedings of a Demonstration Meeting of The Society for Applied Bacteriology, at which the participants exhibited techniques that they had developed and found useful in the study of plant pathogens. They discussed techniques for use in field study and others that are more suited to the research laboratory. Some of these methods are completely new and can be used in different circumstances and in the study of other species.

Origin, Prevention and Treatment of Affective Disorders

edited by Mogens Schou and Erik Strömgren

April/May 1979, xiv + 308pp., £9.80/\$20.25 0.12.628950.6

In 1978, the University of Aarhus, Denmark, arranged a series of international scientific symposia to celebrate its 50th anniversary. The university wanted to emphasize the internationality of research and to provide opportunities for discussion of the results of past work and the plans for future work. The topics of the symposia were therefore related to the dominant fields of research of the institutes which organized the symposia. The Institute of Psychiatry held a 3-day symposium with Danish and international speakers on the topic of affective disorders, with the emphasis on origin, prevention and treatment, which is the subject of this book. The presentations dealt with lithium therapy and research, rapidity, tryptophan, brain amines, blood and tissue antigens, cerebral localization of memory and depression, neuroendocrinology, genetics, epidemiology and suicide.

Methods in Microbiology Volume 12

edited by T. Bergan and J. R. Norris

April/May 1979, xvi + 268pp., £12.00/\$24.75 0.12.521512.6

Contents of Volume 12

S. D. Henriksen: Serotyping of bacteria. E. Thal: The identification of *Yersinia pseudotuberculosis*. T. Bergan: Bacteriophage typing of *Yersinia enterocolitica*. S. Winblad: *Yersinia enterocolitica* for serotype 0-8. S. Muckerjee: Principles and practice of typing *Vibrio cholerae*. H. Brandis: Vibriocin typing. P. Oeding: Genus *Staphylococcus*. J. Rotta: Group and type (groups A and B) identification of haemolytic streptococci. J. Jelinkova and J. Rotta: Identification and typing of enterococci. H. Brandis: Bacteriocins of streptococci and bacteriocin typing. E. Lund and J. Henriksen: Laboratory diagnosis, serology and epidemiology of *Streptococcus pneumoniae*. Subject index.

Pulmonates Volume 2B: Economic Malacology

with particular reference to *Achatina fulica*

A. R. Mead

April/May 1979, x + 150pp., £12.00/\$25.20 0.12.267541.X

From a Review of Volume 1

'... a stimulant to further research. As a record of what has been achieved in many areas and a pointer to what can be done, this book should take its place on the shelves of research departments.' Joyce E. Rigby in *Nature*.

Contents

Introduction. Dispersal to new areas. Evaluation of dispersal. Economics. Bionomics in control. Biological control. Other methods of control. Disease and decline. Evaluation of control. Future prospects.

Transactions of the Zoological Society of London

Editor: H. Gwynne Vevers

Publication: Volume 35, No. 1

April 1979, approx. 90pp., £12.50/\$26.25

Contents of Volume 35, No. 1 by M. N. Bruton

The breeding biology and early development of *Clarias gariepinus* (Pisces: Clariidae) in Lake Sibaya, South Africa, with a review of breeding in species of the subgenus *Clarias* (Clarias). The food and feeding behaviour of *Clarias gariepinus* (Pisces: Clariidae) in Lake Sibaya, South Africa, with emphasis on its role as a predator of cinchids. The role of diel inshore movements by *Clarias gariepinus* (Pisces: Clariidae) for the capture of fish prey.

Research on Steroids Volume VIII

Proceedings of the Eighth Meeting of the International Study Group for Steroid Hormones

edited by A. Klopffer, L. Lerner, H. J. van der Molen, and F. Sciarra

April/May 1979, xii + 384pp., £15.60/\$32.25 0.12.416050.6

This volume contains the proceedings of the VIII Meeting of the International Study Group for Steroid Hormones, held at the Consiglio Nazionale delle Ricerche, Rome, from 5–7 December 1977. The topics under discussion were 'Control Mechanisms of Reproductive Function' and 'Recent Methods in Steroid Assay'. The reports of recent research covered subjects ranging from blastocyst steroids to mechanisms of action of progesterone on oocytes, hormonal regulation of sertoli cell function and steroid receptors in the testis. The sections on new techniques included details of enzyme immunoassay and affinity chromatography for steroids.

Nonsense Mutations and tRNA Suppressors

edited by J. E. Cells and J. D. Smith

May/June 1979, approx. pp., £16.00/\$33.25 0.12.164550.9

From the preface:

'This book is a record of the proceedings of the EMBO Laboratory Course and Aarhus University 50 Years Anniversary Symposium on "Nonsense mutations and tRNA suppressors" held in Aarhus in July 1978. ... The main aim of the book is to introduce the reader to the field of translational suppression, specifically to nonsense mutations and tRNA suppressors. The book covers classic work on nonsense suppressors in prokaryotes and yeast as well as the latest developments in the search for nonsense mutations and tRNA suppressors in higher eukaryotes. To help the reader the book contains a few general chapters dedicated to tRNA and its role in protein synthesis as well as a compilation of wild type and mutant tRNA sequences.'

Applied Biology Volume 4

edited by T. H. Coaker

May/June 1979, xii + 286pp., £16.40/\$34.44 0.12.040904.6

From a Review of a Previous Volume

'... fills a valuable niche and the high standard of production gives it an assured place on the library shelf.' B. N. K. Davis in *Biological Conservation*.

Contents

N. W. Pirie: Leaf protein as a source of food. C. R. Field: Game ranching in Africa. John H. Steele: Some problems in the management of marine resources. M. S. Johnson and A. D. Bradshaw: Ecological principles for the restoration of disturbed and degraded land. W. Clive James and P. S. Teng: The quantification of production constraints associated with plant diseases. Subject index.

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Kidney Transplantation

Principles and Practice

edited by P. J. Morris

May/June 1979, xviii + 310pp., £17.50/\$36.25 0.12.507450.6 (Academic Press) 0.8089.1142.2 (Grune & Stratton)

This book presents the current position of renal transplantation from the point of view of the practising physician, transplant surgeon, nephrologist, tissue typer, virologist or bacteriologist. The book is comprehensive in the sense that a person entering a transplant unit for the first time could get an overall grasp of kidney transplantation, ranging from the surgical techniques to the diagnosis and pathology of rejection. Thus the book should be of value to all who are involved in the various aspects of kidney transplant programmes, be they doctors, scientists or nurses.

Advances in Microbial Physiology Volume 18

edited by A. H. Rose and J. Gareth Morris

April/May 1979, X + 300pp., £18.60/\$38.50 0.12.027718.2

This series presents critical review articles on all aspects of the physiological and biochemical activities of micro-organisms. *Advances in Microbial Physiology*, spanning the area between microbiology and biochemistry, is able to deal with subjects of interest in both fields which previously have received little attention from reviewers.

Contents of Volume 18

R. H. Garrett and Nancy Klein Amy: Nitrate assimilation in fungi. Paul S. Sypherd, Peter R. Borgia and John L. Paznokas: Biochemistry of dimorphism in the fungus *Mucor*. Michael G. Sargent: Surface extension and the cell cycle in prokaryotes. W. R. Finnetry: Physiology and biochemistry of bacterial phospholipid metabolism. Author index. Subject index.

Food Texture and Rheology

edited by P. Sherman

June/July 1979, approx. ??? pp., £20.00/\$41.50 0.12.639960.3

This book provides an up-to-date appraisal of current thought, developments and practice in the study of food texture. It contains papers which were presented at a recent symposium held in London and organised on behalf of the International Union of Food Science and Technology. The papers offer critical reviews of the present state of knowledge as well as reports on recent original research on food texture. Subjects treated range through the sensory evaluation of textural properties; instrumental test methodology for textural properties and problems encountered in instrumental testing; and the rheology of vegetable proteins, of dough and baked produces and of miscellaneous food products.

Recent Advances in the Biochemistry of Cereals

edited by D. L. Laidman and R. G. Wyn Jones

June/July 1979, approx., 400pp., £21.00/\$43.50 0.12.433950.6

This volume contains the published proceedings of the 16th annual conference of the phytochemical society of Europe. Contributions are mainly concerned with factors regulating processes at a cellular level. They include the biochemistry of mineral ion uptake, grain germination encompassing dormancy and vigour phenomena, and the development of the seed particularly in terms of its carbohydrate, protein and lipid content, and the significance of grain composition in nutrition and food technology. Plant biochemists, plant physiologists, agriculturalists and food scientists should find much to interest them in this book.

Medical Physics series

Non-Invasive Physiological Measurements Volume 1

edited by Peter Rolfe

May/June 1979, xiv + 350pp., £21.00/\$43.50 0.12.693401.7

The aims of this book are to provide a comprehensive description of the techniques for physiological measurement which are presently available, to assess the clinical and research value of each, and to indicate where further developments are needed and likely to occur.

Contents include

An introduction to non-invasive cardiodiagnostic techniques. Quantifying skin disease and *in vivo* using electrical impedance measurements. Electrical impedance pneumography. Ultrasonic Doppler velocimetry. Cerebral blood flow measurement using venous occlusion plethysmography in the newborn human infant.

Analytical Methods for Glycerol

M. R. F. Ashworth

May/June 1979, xii + 220pp., £21.00/\$43.50 0.12.065050.9

As regards its completeness, this book has no rival in its field. It includes sections on oxidation methods, esterification methods, ether formation, dehydration, reactions, physical and non-chromatographic separation methods and chromatographic methods. There are chapters for methods with glycerol compounds and for the enzymic determination of glycerol, each chapter separated into sections, each section comprehensive. Thesis workers in the field of organic chemistry, laboratory staff engaged in organic and biochemical work, and users of university chemical libraries all over the world will know the author as a distinguished specialist in the field of organic analysis.

Electron Spectroscopy

Theory, Techniques and Applications Volume 3

edited by C. R. Bundle and A. D. Baker

April/May 1979, x + 362pp., £21.50/\$45.15 0.12.137803.9

From a review of Volume 1

"A book to be wholeheartedly recommended not only to photoelectron spectroscopists, for whom it will become a standard reference, but to that much wider audience of chemists and physicists who are interested in, or need to know about, the electronic structure of the chemical bond."

D. S. Urch in *Chemistry in Britain*

Volume 3 largely moves away from the theme of the first two volumes, which discussed photoelectron spectroscopy exclusively, by including discussions on X-ray emission spectroscopy, electron impact spectroscopy, and ion fragmentation mechanisms.

Air Pollution Chemistry

John D. Butler

April/May 1979, x + 408pp., £25.00/\$52.50 0.12.147950.1

The presence of chemicals in the air poses questions about their concentration, toxicity, distribution, stability and influence on climate. The aim of this book is to introduce the methods used to answer these questions and assess the current knowledge and ideas about air pollution. Although written from the viewpoint of a physical chemist and dealing with the applications of chemistry to air pollution, little chemical knowledge has been assumed. Wherever possible concepts have been explained and defined before their application, thus making the text readily comprehensible to specialists in other disciplines.

The Electrochemistry of Lead

edited by A. T. Kuhn

May/June, xviii + 468pp., £28.00/\$59.00 0.12.428350.0

The studies in this book, now revised and expanded from their original form, are derived from a Symposium at the University of Salford on the same subject. Subjects covered include the electrodeposition of lead and its alloys; the electrochemistry of lead in molten salts; the use of lead-dissolving electrolytes whether in refining of the metal, in reclamation of scrap lead, or as the basis of a new battery; hydrogen evolution on lead; and lead dioxide anodes. Although not intended to be a book primarily about the lead-acid battery, the work considers in detail the electro-chemistry of the lead-water-sulphuric acid system, much of it 'battery-orientated'.

Food Science and Technology: A series of Monographs

Food Microscopy

edited by J. G. Vaughan

April/May 1979, xvi + 652pp., £29.60/\$61.25 0.12.715350.0

Modifications to the light microscope and the development of the electron microscope have greatly increased the importance of microscopy in the practice of food science. However, no comprehensive account of these advances has yet appeared. This book breaks new ground in showing how light and electron microscopy can be used to investigate problems of food processing, acceptability and deterioration. It also covers the problem of the identification of food constituents, which has been intensified by recent legislation, particularly with regard to animal feeds. The book will be of interest and forensic scientists, biologists and microscopists, and for undergraduates in departments of Food Science, Biology and Nutrition at universities.

Proceedings of the 11th International Power Sources Symposium Power Sources 7

Research and Development in Non-mechanical Electrical Power Sources

edited by J. Thompson

May/June 1979, xii + 774pp., £65.00/\$134.50 0.12.689150.8

The importance of the biennial International Power Sources Symposia, and the series of books that records their proceedings, continues to increase. This volume contains the 49 papers presented at the 11th Symposium, held in September 1978 and attended by some 450 delegates from 30 countries. The contributors are drawn, principally, from Government research establishments, industry and universities, and the Symposium provided them with a forum within which they were able to present and discuss their own progress in the study, development and use of electrical power sources. Their major concern was with the many types of primary, secondary, high-temperature and reserve batteries, fuel cells and other types of non-mechanical power sources.

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tion of the cloying, but once attractive, hypothesis that the close similarity between the specificities of enzyme induction and enzyme function must mean that the mechanism of induction involves combination between the inducer and a receptor molecule consisting at least of a modified version of the enzyme, if not the enzyme itself. But the essential point to be grasped was that the relationship is functional, not chemical. Once Monod had discarded the false implications of the specificity similarities, he was free to

postulate the true nature of the receptor, characterise and define the *lac* repressor and discover the phenomenon of allostery.

Thus, almost more than from the actual discoveries themselves, we can benefit from studying the way he used his mind. And that is one of the chief reasons why this collection of masterpieces is so valuable. □

M. R. Pollock is Emeritus Professor of Biology at the University of Edinburgh, UK.

Strategies of dissent

Vera Rich

To Build a Castle: My Life as a Dissenter. By Vladimir Bukovsky. Pp.352. (André Deutsch: London, 1978.) £7.50.

IN 1961, Vladimir Bukovsky was a biology student in Moscow. Today he is a biology student in Cambridge. *To Build a Castle* relates the history of the intervening years, which, with a few brief intervals of relative freedom, were spent in the prisons and psychiatric hospitals of the USSR.

This period spans the rise of dissent and opposition in the USSR. One curious feature of the dissent movement is that, at least in Russia proper, scientists played a major role, and at the beginning, the leading role. (In the non-Russian republics, such as Lithuania and Ukraine, other factors such as national aspirations make the picture more complex.) At one end of the dissent spectrum, leading scientists could defy the régime from a position of strength, knowing that the state needed their expertise; at the other, it was among the students of the natural sciences that latent doubts began to take a more concrete form—total ideological conformity and commitment being ensured in the humanities and social sciences by the preliminary screening of University entrants.

Bukovsky's book outlines how such doubts could grow and crystallise in a student living in the immediate post-Stalin era. After his expulsion from the University in 1961, we follow him through the long routine of arrests and incarcerations. This book is not, however, just another set of labour-camp memoirs. The *Castle* of the title is an imaginary one, which Bukovsky patiently constructed in his mind through long years in prison and psychiatric ward, as a means of

therapy and self-protection against the grim reality which surrounded him.

Accordingly, the main interest in the book is to be found in what may be called the strategies of dissent. Nowadays, it is taken for granted that any civil rights protest is essentially a demand that what is guaranteed by the constitution should be implemented in fact. Zhores Medvedev, the geneticist, for example, waged a major campaign against Soviet censorship on the grounds that "Secrecy of correspondence is guaranteed by law". Bukovsky relates how this procedure was first developed in the late 1960s and how, when Alexander Yesenin-Volpin, the mathematician, first urged it, many budding dissidents were sceptical of its potential value—a scepticism which, as Bukovsky describes, has been refuted over and over again in the past decade.

Most fascinating, however, are the chapters dealing with Bukovsky's experiences of mental hospitals, for here we have the first-hand background to the reports which Bukovsky, in a brief spell of freedom, managed to transmit to the West. These reports for the first time drew attention to the new Soviet theory that political discontent equals mental disorder. Here too is the practical experience of psychiatric examinations and medical "commitments", which led Bukovsky, in collaboration with psychiatrist Semeon Gluzman, who is still serving a seven-year sentence in a labour camp, to write their famous *samizdat*, *Manual of Psychiatry for Dissidents*. This is a slim but detailed handbook of how to behave under interrogation, with some pungent analyses of the main psychological types of interrogator likely to be encountered, and the best strategies for circumventing and frustrating them. In a book of the stature of *To Build a Castle*, it is regrettable that space could not have been found to include the 'Manual of Psychiatry' as an appendix—this is, however, one of the few faults one can find with an otherwise excellent work. □

Vera Rich is Nature's regular correspondent on Soviet and East European affairs.

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MEDICAL MICROBIOLOGY VOLUME 1: MICROBIAL INFECTIONS

Edited by J. P. Duguid, B. P. Marmion and R. H. A. Swain

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Eye-opener on life

A. J. Cain

Life on Earth. By D. Attenborough. Pp. 319. (BBC/Collins: London, 1979.) £7.95.

THIS pleasant book is not, and was not intended to be, anything like a scientific text. Although based on the highly successful television series, it is also far from a mere coffee-table selection of excellent pictures. In character, it probably approaches most closely Kenneth Clark's *Civilisation*. To quote from the Introduction, the purpose in making the films was "to try and survey the whole animal kingdom and consider each great group of animals in the light of the part it has played in the long drama of life" and in order to do so on a practicable scale "to try and perceive the single most significant thread in the history of a group and then concentrate on tracing that, resolutely ignoring other issues . . .". To do so, one must know something about the anatomy and physiology of the basic design in each group—"The quintessence of, for example, a lizard is only fully understandable in the light of the particular possibilities and limitations dictated by its reptilian nature, and that, in turn, only becomes comprehensible in the light of its past."

David Attenborough has never recovered from his first experience of the tropics, and he is quite right not to; no one, including professionals, is really a biologist until he has experienced personally the incredible richness, the diversity, the multitudinosity of life where it really burgeons. And when he has, it is an experience he knows will be valuable to everyone, not only professionals, if it can be communicated. This book will be for very many people, especially young ones, literally an eye-opener on life; but it will be much more, because there is a definite theme, or rather several, running through it. It is the welcome antithesis to those woolly-mammal-pretty-girl books that bored everyone some years ago in the name of conservation.

It is based, therefore, as far as possible on living forms—indeed, one of the surprises of the television series is that while taking full account of time, and sufficient outstandingly well-preserved or interesting fossils, and of plate tectonics, it goes extremely lightly over our old friends the dinosaurs and other extinct reptiles, and most of the extinct mammals. For the purposes of

the book, this is probably right—far more can be said accurately of the anatomy, physiology and ecology of living things, and there is a rich array of 'living fossils' to draw on. Occasionally, this approach seems to bring a weakness of treatment; if one wishes to show the extraordinary continuity of groups, even among the vertebrates (which after all are a fairly compact group), then the beautiful sequence from early crossopterygians to early reptiles should surely not be omitted. To jump from lungfish and coelacanth to modern amphibians with only a passing mention of *Eryops* leaves one gasping in a very rarefied atmosphere. But a rigid exclusion was obviously necessary, and it is ungracious to pick out omissions.

One should be grateful, in fact, for how much there is in the book. Hardly any 'living fossil' of importance is omitted, and most get considerable discussion. (The only one I wished had not been left out is that tropical plant that so closely parallels the fossil land-plants (psilophytes) of the Rhynie Chert.) In each case, the present mode of life is discussed with expert knowledge and assistance. Every time, each group is related to the state of the Earth when it arose, the nature of the fauna and flora it had to live with, and what it did or does that others did not or could not do.

Inevitably there are many gaps in our knowledge, and the author is rightly insistent on them. Why do so many groups of invertebrates acquire fossilisable hard parts at the beginning of the Cambrian? Was it really a sudden cooling of climate that extinguished the large land-reptiles (and many small ones) at the end of the Mesozoic? What exactly is the evolutionary relationship between molluscs, arthropods and annelids? As against that, the author gives an excellent picture of life before the oxygen-shield developed, and living things could safely get out on to land. He points out the enormous advantages of being a reptile (you can live on one-fifth of the food necessary to keep an equivalent mammal stoked up), as well as the inevitable disadvantages.

All through, he avoids the fallacy of believing that a primitive form is necessarily inefficient; normally it is efficient enough for a particular mode of life. He is especially interesting in this connection on the marsupials, where, in fact, he seems to miss a point. The radiation of marsupials in South America in the Palaeocene seem to have taken place in the presence of eutherian mammals. So, far from being extinguished as more primitive and inefficient, they seized the ground insectivore, arboreal nocturnal insecti-

vore and carnivore modes of life so successfully that they held them to the exclusion of the South American eutherians. These took up all other possible modes, and the combined fauna did not become largely extinct until 50×10^6 yr later, when very different eutherians came in from the north and both marsupials and local eutherians suffered badly. Equally, there are numerous endemic eutherians in Australia today, coexisting with the marsupials and making up a complete range of fauna. With the ruin of their habitats by cultivation, both sorts become extinct.

Inevitably, some specialist will quarrel with almost any sentence of the book, but what is so useful is indeed the breadth of treatment and the insistence on living things as living things, solving particular problems of existence. Many of the points raised will stimulate naturalists to much further thought. It has been known for centuries that cormorants and darters get their plumage soaked under water, and have to sit out in the sun, drying themselves out. A survival of a primitive character? Possibly, but why should it survive? If every other sort of aquatic bird has evolved waterproof clothing, why can't they? But if, as suggested, the air trapped in this waterproof clothing makes the bird buoyant and it has to struggle to get down under water, does this mean that although cormorants and other pelecaniforms can stay under for only a short time, they are more adept in catching fast fish? If so, then the lack of air in the plumage may be a secondary adaptation, specially selected for. After a while, one begins to wonder if any character is merely primitive, merely there because one's ancestor had it. Is it not kept because it still has a use? The author comments on the retention by modern ratites (ostrich, emu, rhea, etc.) of adaptations for flight, such as air-sacs in the bones. Possibly when their ecology and physiology are better understood we shall see why they didn't convert their legs to horse-like ones. Whereas in the nineteenth century so much had to be explained away as the retention of ancestral characters, much now makes sense when we know how exactly an animal lives.

Coming to the book after the television programmes engenders mixed feelings. One is glad to have the book as a permanent record, and still more because it is extremely well written and enables one to grasp the themes of the whole series. The text is clear (I noted only four misprints—and one transposed caption); the price is low. The illustrations are superb, and only a very few are spectacular without much biological content (I noted the Goliath



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edited by **S.A. Rice**, *University of Chicago*.

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Patterns in Crystals

by **N.F. Kennon**, *Dept. of Metallurgy, University of Wollongong, Australia*.

The book has been prepared for students of any age embarking on a study of crystals for the first time. It is appropriate for courses in crystallography, physics, chemistry, geology, mineralogy, ceramics, metallurgy, materials science and engineering at university, college, polytechnic or high school level. It is also particularly relevant to gemmology and lapidary hobby groups. The presentation is absolutely basic and directed to the reader seeking some understanding of the fundamental characteristics of a crystal and so learn the language that is used in the more advanced works.

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208 pages
(cloth) \$23.00/£10.00
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Scattering of Light by Crystals

by **W. Hayes**, *University of Oxford*
and **R. Loudon**, *University of Essex*.

A description of phenomenon of inelastic light scattering by crystals and its use in obtaining information about the various kinds of solid-state excitation. The experimental techniques are largely common to the study of all types of excitation and the main components of light-scattering apparatus are described in detail. The properties of the excitations are derived in a general way, and experimental examples and simple theoretical interpretations are clearly presented.

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Interpretation and Processing of Vibrational Spectra

by **M. Horak** and **A. Vitek**, both of the *Czechoslovak Academy of Sciences, Prague*.

This handbook contains a description of and methodological hints concerning basic procedures for the handling and interpretation of vibrational spectra. All commonly-used definitions, mathematical relationships, tables of constants, conversion factors and computation schemes for numerical treatment of experimental values have been collected. The book is supplemented with FORTRAN programs for the computer solution of many problems in molecular spectroscopy.

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414 pages
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Organic Liquids: Structure, Dynamics and Chemical Properties

edited by **A.D. Buckingham**, *Cambridge University*,
E. Lippert, *Technische Universität, Berlin*
and **S. Bratos**, *Université Pierre et Marie Curie, Paris*.

This book is the first to be devoted to the theme of organic liquids. New physical techniques for studying the structure and properties of liquids are fully described and numerous applications of particular organic liquids presented. Emphasis is placed on the energetics and the dynamics of molecular interactions in organic liquids.

December 1978
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362 pages
\$45.00/£18.00

Organic Reaction Mechanisms 1977

edited by **A.C. Kripe** and **W.E. Watts**, both of the *School of Physical Sciences, New University of Ulster, Coleraine, Northern Ireland*.

The thirteenth volume, continuing the pattern now established for the series, surveys work on organic reaction mechanisms appearing in the literature dated December 1976 to November 1977. The aim, as before, has been to provide a concise and comprehensive coverage of work published in the period under review; where, at the time of writing, any particular results seemed of outstanding significance, these are described and discussed. The remainder are listed.

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750 pages
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Acousto-Optics

by **J. Sapriel**, *Centre National d'Etudes des Telecommunications*,
translated by **S. Francis** and **Barbara Kelly**.

Discusses the interaction of two types of waves; acoustic waves of mechanical origin, caused by the elastic properties of the material, and light waves which are electromagnetic. For the purpose of this monograph, some physical properties, such as elasticity and piezo-electricity, have been given a microscopic and a phenomenological treatment. For the most part, however, *Acousto-Optics* provides a molecular and theoretical treatment of the physical properties of solids, the interaction of light and acoustic waves, and acousto-optic devices and methods for the investigation of the properties of materials.

February 1979
0471 99700 5

142 pages
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Charge Coupled Devices and Systems

edited by **M.J. Howes** and **D.V. Morgan**, both of *Dept. of Electrical & Electronic Engineering, University of Leeds*.

This book deals with the physics, technology and applications of charge coupled devices. It is the third in a series of books which develops the concept of integrating the device physics with the circuit problems. Charge coupled devices are novel in that they have as their basic parameter electric charge rather than currents and voltages. (*Wiley Series in Solid State Devices and Circuits*)

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Section B

CHEMISTRY REVIEWS—Volume 1 (1979)

edited by M.E. Vol'pin Institute of Organometallic Elements, Moscow

In this inaugural volume, two reviews deal with modern aspects of catalysis, one presents new achievements of a well-known Soviet school on reaction mechanisms involving organometallics, while the fourth paper is devoted to a rather new field of research—the effect of magnetic fields on chemical reactions.

The intention in this and subsequent volumes is to reflect the most important developments in chemistry in the U.S.S.R.

Contents: New Developments in the Reaction Mechanisms of Organometallic Compounds (I. P. Beletskiy); Molecular Bases for Heterogeneous Catalysts by Acids through the Participation of Brønsted Centers (V. B. Kuzinsky); The Effect of the Magnetic Field on Radical Reactions in Solutions (Yu. N. Molin, R. Z. Sagdeev and K. M. Salikhov); General Principles of Fermentation Catalysts (I. V. Berezin, A. A. Klyosov and K. Martinek)

June 1979

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Molecular Motion in Polymers by ESR

edited by S.E. Kelneth and R.F. Boyer (Volume 1 of MMI Press Symposium Series)

Contributors: Aleksandrova, T.A., Berliner, L.J., Bobst, A.M., Buchachenko, A.L., Bullock, A.T., Cameron, G.G., Clarkson, R.B., Coon, D.L., Hyde, J.S., Kovarski, A.L., Kurnier, P.L., Kusumoto, N., Liang, T.M., Lindberg, J.J., Miller, W.G., Ranby, B., Rudolph, W.T., Smith, P.M., Tormela, P., Vekasi, Z., Weissman, A.M., Weber, G., Wu, C.C.

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Molecular Dynamics of Nucleic Acids as Studied by Spin Labels

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Published for the Commission of the European Communities, Directorate-General for Scientific and Technical Information and Information Management and EURATOM

A large amount of important fundamental research in radiation protection and allied subjects is sponsored by the Commission of the European Communities/EURATOM. This invaluable compendium is essentially a "who is doing what" compilation. Each research team head is asked annually to report on the work and progress of his team; this large publication is the result. Anyone engaged in research in the field of radiation protection will need this progress report to keep abreast of the considerable and varied amount of research work being done in Europe.

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Fission Gas Behaviour In Nuclear Fuels

edited by C. Ronchi, H.J. Metzke, J.v.d. Laar and H. Blank

Proceedings of the Workshop held at the Joint Research Centre, Karlsruhe Establishment (European Institute for Transuranium Elements), Federal Republic of Germany, October 28–27, 1978

(Volume 1, Number 1 of EUROPEAN APPLIED RESEARCH REPORTS—Nuclear Science and Technology Section)

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June 1979

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147 Dfl (\$73.50)

Sixth Symposium on Microdosimetry

Brussels, Belgium, May 1978

edited by J. Boaz and H.G. Ebert

Commission of the European Communities, Directorate-General for Research, Science and Education, Biology, Radiation Protection and Medical Research

The increasing use of nuclear energy and use of ionizing radiations and radioactive nuclides in medicine, industry, and research has resulted in an ever-growing potential hazard to man from radiation contamination. The determination of such hazards call for substantial efforts in microdosimetric and radiobiological research.

Contents include papers on: Biological and Chemical Effects of Radiation from External Sources and Incorporated Radionuclides (as interpreted with the aid of microdosimetry); Physical Principles of Radiation Interaction and Energy Deposition; Spatial and Temporal Distribution of Radiation Interaction Processes; Experimental and Mathematical Determination of Energy Deposition Spectra and Their Mean Values; Definition of Radiation Quality; Differentiation of Radiolytic and Nonradiolytic Radiation Mechanisms in Incorporated Radioactive Nuclides; Analysis and Interpretation of Dose-Effect Relationships; Models of Radiation Effect Mechanisms.

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times the number of plates, and a nationally assisted edition with ten times the number? Its value for schools alone would justify the expenditure of government money, and help to assuage the feelings of those who now have to teach spelling in the Universities. □

A. J. Cain is Derby Professor of Zoology at the University of Liverpool, UK.

Darwin's legacy

C. M. Yonge

The Beagle Record: Selections from the Original Pictorial Records and Written Accounts of the Voyage of HMS Beagle. Edited by R. D. Keynes. Pp. 409. (Cambridge University Press: Cambridge, 1978.) £30.

HARDLY to be realised by that monarch or indeed by any of his subjects, yet—viewed in the light of what was to come from it—the voyage of *HMS Beagle* could well be considered the most momentous event during the short reign of William IV. That vessel departed on her circumnavigation of the globe 18 months after he came to the throne and returned less than a year before his death and the accession of Queen Victoria. The numbers of those aware of the course and significance of this voyage have recently been vastly increased following the broadcast of the imaginatively impressive television series by BBC-TV.

Putting aside for the moment the eventual consequence in *The Origin of Species*, there was early appreciation of the immediate achievements of the voyage. Darwin's original account of what he saw and did was initially somewhat hidden because it formed volume 3 of the *Narrative of the Surveying Voyages of HMS 'Adventure' and 'Beagle' between 1826 and 1836* published in 1839. This appeared before a wider public six years later as the *Journal of Researches into the Natural History and Geology of the Countries visited during the Voyage round the World of HMS 'Beagle' under the Command of Captain FitzRoy RN*, which a reviewer in the *Quarterly Review* was to describe as "One of the most interesting narratives of voyaging that it has fallen to our lot to take up, and one which will always occupy a distinguished place in the history of scientific navigation".

How fortunate it was, one may well reflect, that the prime concern of the

voyage was a survey of the southern shores of South America. As a naturalist attached to a naval vessel, Darwin might have gone to the Antarctic as Joseph Hooker did, to Australia and New Guinea as Huxley was to do, or perhaps not further than the eastern Mediterranean, the limit for Edward Forbes. But South America, not long freed from restrictions on scientific enquiry only penetrated by Humboldt, was a different proposition. To the prematurely judicious eye of Darwin, only 22 when the voyage began, this continent presented so very

however, with products of later years at Down House, would have been no more than the work of an outstanding naturalist had it not been for the publication of *The Origin of Species*. Darwin then changed the direction of human thought and based his demonstration of the fact of evolution on "certain facts in the distribution of organic beings inhabiting South America, and in the geological relations of the present to the past inhabitants of that continent". From that date all that Darwin had done and thought becomes of profound interest—above all his activities during the voyage of *HMS Beagle* which ended on November 7, 1836, "having completed the unusually long period of five years and one hundred and thirty six days" in commission.

Apart from the *Journal of Researches*, his granddaughter, Lady Barlow, edited *Charles Darwin's Diary of the voyage of HMS Beagle* in 1933 and twelve years later unpublished letters and notebooks in *Charles Darwin and the Voyage of the Beagle*, with



Two Fuegians fishing from a canoe

Drawing by Conrad Martens

many matters for contemplation—recent earth movements, a wealth of unusual animals and plants, above all of fossil remains—during the long process, with constant re-checking, of FitzRoy's surveys. There was the final bonus of the parting visit to the Galapagos Islands before the long journey home.

After return the first scientific outcome was *The Structure and Distribution of Coral Reefs*, the subsidence theory a result more of his previous observations of earth movements in South America than of those on the few coral formations encountered in the Pacific and Indian Oceans. All,

his letters to Henslow (1831–1860) in *Darwin and Henslow: The Growth of an Idea* in 1967.

The present, and most impressive, volume came initially into being—Professor Richard Darwin Keynes, a great grandson, informs us—after a chance encounter with Dr Armando Braun Menendez at Buenos Aires in 1968. His rich collection of books, charts and pictures dealing with the exploration of the southern hemisphere was found to include two of the sketch books of Conrad Martens. Darwin may introduce him; writing to his sister Caroline from Monte Video on November 13, 1833, with initial

reference to the first official artist, he states: "Poor Earl has never been well since leaving England and now his health is so entirely broken that he leaves us—& Mr Marten, a pupil of C. Fielding & excellent landscape drawer has joined us".

Such indeed were his qualifications as revealed by the great majority of the illustrations, many in colour, that adorn this book. They mainly depict scenes around the southern coasts because, after nine months, Martens left at Valparaiso, FitzRoy without money to employ him longer. But in these beautifully reproduced sketches of Port Desire, in the Beagle Channel, at the Santa Cruz River, at Chiloe and elsewhere, we view scenes that Darwin and all aboard HMS *Beagle* must have gazed upon. The few pictures not by Martens include John Gould's painting of *Rhea Darwinii* and FitzRoy's sketch of Coquimbo in Chile, with the simple drawing of Napoleon's tomb at St Helena by Darwin's servant Syms Covington.

The skilfully edited text, aiming at a general narration of events, is a judicious blend of extracts from the *Diary*, with all of Darwin's letters to his father and sisters, together with those, largely on scientific matters, to Henslow. FitzRoy is represented by extracts from the second volume of the *Narrative* with letters, both official and personal, to Captain Francis Beaufort, Hydrographer to the Navy and under whose instructions the voyage was carried out. There are concluding passages from the *Narrative* in which he defends the biblical story with the record of the rocks—potent evidence for evolution in Darwin's hand—all explained as a result of the flood!

Charles Darwin has been well served by his family—first by his sons, then by his granddaughter; and now a great grandson has produced the culminating tribute. □

Sir Maurice Yonge is Honorary Fellow in Zoology at the University of Edinburgh, UK.

Saga of a research vessel

George Deacon

On Almost Any Wind: The Saga of the Oceanographic Research Vessel "Atlantis". By S. Schlee. Pp301. (Cornell University Press: Ithaca and London, 1978.) £10.50.

THE *Atlantis* was built for the newly founded Woods Hole Oceanographic Institution in 1930–31, where, to begin with, most of the work was done in summer by scientists who held teaching appointments. Nevertheless the Trustees recognised that progress required a moderate sized ship, with reasonable living quarters, able to work in the deep oceans. H. B. Bigelow, Director of the Institution, and Columbus Iselin, general assistant and future master of the ship had much to do with her specification. She was a steel auxiliary, rigged as a ketch, with fore and aft sails, engined by a 280 hp diesel engine. Sail was chosen for economy, long range and steadiness, though it has often been suggested that yachtman's preference for sail had something to do with it. It was, perhaps, something of an anachronism from the start. UK oceanographers had proved Captain Scott's square-rigged, coal-fired *Discovery* unsuitable for making widespread observations in

reasonable time, and had replaced her by a fully-powered vessel built for the same price as the *Atlantis*, though costing twice as much to run. But the *Atlantis* proved ideally suited for pioneering work with new techniques and ideas, and it was not until post-War expansion, involving multi-ship operations, and widening international activities, extended and tightened the work load that "slogging across the ocean at a walking pace" proved unacceptable. As time went on she used her engine more and sailed less, till in 1960, when her tail-shaft broke—after post-war fitting of a more powerful engine—she had to call for a tug.

By 1935 she had greatly extended

our knowledge of the water circulation in the north-western Atlantic Ocean and Caribbean Sea, of daily plankton migrations, nutrient cycles, bacterial populations in sediments, the Gulf Stream, Sargasso weed, and CO₂ in atmosphere and ocean, among other basic problems. She went on to make extensive biological and physical studies of the Georges Bank area. She had also taken part in the development of a large quantitative plankton net with a triangular opening, with 50 ft sides, and the Leavitt release gear for opening as well as closing. She began seismic exploration at sea with Maurice Ewing "who was constantly banging on the Institution's door" and was used for development of more efficient core-samplers and the bathythermograph.

She was laid up when America joined the war, and afterwards used extensively in studies of underwater acoustics and underwater explosions. Her coverage was extended to the mid-Atlantic ridge, the Mediterranean Sea, the South Atlantic, the deep trench west of Chile and Peru, and to the Red Sea which proved a severe test of her suitability. There were fairly hectic times when she cooperated with the *Vema*, Ewing's own converted yacht which is still at work though without her rigging. Progress was most rapid when specialists of different kinds worked together. "Hersey's gang . . . charged across the frontiers of acoustic technique and instrumentation . . . like an institution within the Institution". Cooperative studies of the Gulf Stream were also very effective.

Schlee's book gives a lively and realistic account of life at sea in a small ship, based on the journals of her scientists and officers as well as the Institution's archives. She was often uncomfortable, "most of one's existence is passed exchanging one small discomfort for another". Shortage of funds was an obvious as well as concealed cause of trouble. She had a deep-sea winch from the start and a precision echo sounder in 1953, but no powered windlass or freshwater showers till 1954. She is not the only small ship in which continued discomfort has led to bother at sea and trouble in foreign ports, but she had her share.

In 1957 most of the trans-Atlantic sections were worked by a larger fully-powered ship the *Crawford*, soon joined by the *Chain*, and in 1963 by the *Atlantis II*. The old 'A-boat' was going down hill, but still used for tasks that she could do as well as and sometimes better than larger ships. In 1966 she was sold at a nominal charge to the National Council for Scientific Investigation in Argentina. She was re-



Courtesy Woods Hole Oceanographic Institution

fitted in Boston "such care had not been lavished on the vessel since the end of World War II".

When she entered Woods Hole for the last time all the town turned out to greet her. She could not have continued so long or done such good work if long-serving captains and crew had not taken continuing pride in her, their unusual spirit and enthusiasm more

than making up for her old-fashioned design. She is still working from Buenos Aires, mainly for training cruises.

Her fine story is well told. ☐

Sir George Deacon was Director of the former National Institute of Oceanography. He joined the RRS Discovery on 28 January, 1979, for further study of the Antarctic Ocean.

Varnished tale of Scripps

Edward Bullard

Scripps Institution of Oceanography: Probing the Oceans, 1936 to 1976. By E Noble Shor. Pp. 502. (Tofua Press: San Diego, California, 1978). Paperback \$5.95.

THE Scripps Institution of Oceanography was founded in 1903; in 1967 Helen Raitt and Beatrice Moulton published a history of its first fifty years. The present book starts in 1936, when Harald Sverdrup became Director, and brings the story up to 1976. It is a truly remarkable story. How did a small marine biological station grow to be the biggest oceanographic institute in the United States and, perhaps, in the world and then, in the 1960s, spawn a new campus of the University of California? The story is largely that of the three great Directors—Harald Sverdrup, Roger Revelle and Bill Nierenberg.

Sverdrup saw that the future of Scripps lay beyond the coastal waters of California. He obtained a ship, the beautiful schooner *E. W. Scripps*, built for racing and pleasure cruising, and converted her to a research vessel. It was Sverdrup who made Scripps a real oceanographic institute.

At the end of World War II he returned to Norway and, after a period of transition under Carl Eckart, Revelle became Director. Revelle had learnt during the War to 'think big'. He started a frantic race to raise money, hire people and build buildings. It was he who made Scripps larger and better and more fun than any other oceanographic institute. After a few years he grafted on to it a graduate school of science and engineering and turned this and Scripps into the University of California at San Diego, with himself as acting Chancellor. His strategy was original and brilliant. Most universities start with under-

graduate teaching and then, years later, develop a graduate school. This ensures a start with a rather pedestrian staff. Revelle's scheme enabled him to engage a prestigious staff from the start: among his early captures were Harold Urey and Maria and Joe Meyer. When the time came to choose a Chancellor for the new campus the Regents did not appoint Revelle. In 1964 he left California to become Director of the Population Studies Institute at Harvard.

It is a dramatic story and Betty Shor has told the part that concerns Scripps in meticulous and well documented detail; but somehow the realities of what happened have slipped away. Everything is so smooth, everyone behaves so well, everything succeeds, there are no rows and only the merest hint of the struggles that raged before Revelle was made Director and during his unsuccessful attempt to become Chancellor. We are told the names and deeds of all kinds of rather minor figures—for example, of the lady who presides so charmingly and efficiently over the aquarium bookstore—but the name of Edward Pauley who, as a Regent of the University, nearly wrecked the whole enterprise does not occur at all and, to come nearer home, no clue is given as to who at Scripps opposed Revelle's appointment as Director.

To be fair it must be said that Mrs Shor could hardly have told an unvarnished tale. She was asked by the Director to write the book and most of those mentioned in it are her friends. It would have been instructive to have reproduced some of the letters sent at that time to the President of the University and to see who said what and when; but it is clearly too much to expect.

After the turbulent reign and abdication of Revelle there was a short period during which Fred Spess was Director. He picked up the pieces, smoothed the ruffled tempers and continued the building programme; he then handed over to Bill Nierenberg. Nierenberg had the difficult and unenviable task of maintaining and expanding Scripps in a time when the country had lost its belief in the worthwhileness of anything. For a

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while it looked as if Scripps would become 'Scripps rent-a-ship', an organisation that provided ships for anyone who could raise the money to run them. This has not happened; Scripps has remained what it was, an organisation practicing and encouraging all branches of oceanography; indeed it has taken on new responsibilities, such as the Deep-Sea Drilling Programme, and has built fine new buildings. Again the drama and the struggle are largely missing from the book. Again it all happens so smoothly and easily with no sense that but for Nierenberg, it might have come out very differently.

In reading the book one does learn a lot about what happened and about who did what and went where. There are accounts of projects and expeditions with well chosen extracts from letters, ships' logs and radio signals. The facts are set out with great care and detail. It is clearly a book that everyone interested in oceanography and in Scripps should read. It is not the ultimate book on the personalities and the period, but it is an essential contribution to the writing of such a book in 50 years time. ☐

Sir Edward Bullard is Emeritus Professor at the Scripps Institution of Oceanography, La Jolla, California.

Predator-prey interaction in arthropods

A. F. G. Dixon

The Dynamics of Arthropod Predator-Prey Systems. By M. P. Hassell. Pp. 237. (Princeton University Press: Princeton, New Jersey, and Guildford, UK, 1978.) Hardback \$20; £10 70; paperback \$8 75; £4.40.

DR HASSELL has given us a lucid, clearly illustrated and eminently readable account of the development of analytical models of parasite-host interactions. This book is a most welcome exposition and summary of the current position in a rapidly developing aspect of ecology to which the author himself has been a major contributor.

Starting with the Nicholson and Bailey model the author develops step by step elegant analytical models framed in difference equations. This is achieved by the incorporation of more biologically significant parameters into the model. First, it is shown that stability can be predicted if an expression for density dependence is incorporated. A consideration of the functional responses of predators to prey density reveals the difficulties of extending relationships derived from studying mainly parasitoids. In true predators the search rate and handling time vary with stage of development of the predator and size of the prey, and this complexity poses difficulties in modelling this set of interactions. Consideration is given to the consequences of non-random search by a predator as opposed to the random search assumed by Nicholson and Bailey. More realistic prey and predator distributions tend to make the interaction very complex and difficult to explore analytically. However, the author shows that descriptive models have been useful in this context.

Next is revealed the powerful stabilising effect of mutual interference among searching parasites on parasite-host interactions, the constant area of discovery assumed by Nicholson and Bailey being a special case. However, although a widespread phenomenon in laboratory studies of parasite searching, it should be stressed that the extent to which mutual interference occurs in the field and the significance thereof still needs to be resolved. Many of the laboratory studies on mutual interference have been done at unrealistic densities. In considering the predator

rate of increase the author reveals another difference between predators and parasitoids. Prey density influences development, survival and fecundity in predators. If these factors are to be incorporated then the author suggests that we may have to abandon simple time dependent difference equations in favour of mathematically less tractable models.

Switching by predators from eating one prey species, to eating predominantly another species, as the proportion of the alternative prey increases, is shown to stabilise prey populations providing the predator population remains constant. Likewise, if switching is shown by the top predator in a community it can enable competing prey species to coexist. This is followed by a consideration of how it is possible for competing predators and hyperparasitoids to coexist.

The book ends with a most useful chapter on the theoretical basis for biological control which builds on the previous chapters. This reveals that an ideal parasitoid for use in biological control programme, all other things being equal, is one that has a high search rate and a marked ability to aggregate in areas of high host density; and it is argued that these are more likely to be attributes of specific rather than generalist parasitoids. However,

the case of the biological control of winter moth in Canada is used to show that when 'things are not equal' then predicting the outcome is difficult. Multiple introductions of parasitoids are shown now to be justified, as additional species will either coexist with the first species or replace it, and, whatever, the outcome, the equilibrium density of the host will fall.

The author justifies the book's title by regarding parasitoids as simple predators. He stresses that "true predator-prey models need to recover from the neglect at the hands of essentially parasitoid models". Only further study will reveal just how much of a special case the parasitoid-host interaction is and whether it can be usefully applied to the arthropod predator-prey system.

This book will undoubtedly be the standard text for both students and research workers in this field for some time to come. It is also a tribute to Nicholson and Bailey who, although unable to model the stability they observed in insect populations in the field, nevertheless produced a model which has been developed by the author and others to further our understanding greatly. At £4.40 the paperback version is excellent value. □

A. F. G. Dixon is Professor of Biology at the University of East Anglia, Norwich, UK.

Creative personality

Macdonald Critchley

Chase, Chance and Creativity: The Lucky Art of Novelty. By J. H. Austin. Pp. 237. (Columbia University Press: New York, and Guildford, UK, 1978.) \$16 20.

NOT for a long time have I enjoyed a technical apologia so much. Disjointed it may be, but the book is none the worse for that. Obviously the author is not only a biochemist of conspicuous ability, but he is something much greater, a thinker who writes well, and who is versed in the humanities. The book opens in a naive, autobiographical but intensely interesting fashion. We learn how as a junior hospital officer his interest was captured by a fascinating and argumentative case-conference between two of America's outstanding neurologists. His stint in the pathological laboratory was highlighted by the opportunity to study that rare and deadly disorder, metachromatic leuco-

dystrophy. He became intrigued by the presence of highly coloured urinary deposits which in an odd fashion changed in hue from reddish-purple to a vivid golden brown. What was their nature and what was the meaning of their metamorphosis? Patiently over the ensuing years Dr Austin pursued his quest, following one clue after another, communicating with other workers far and wide. Eventually he narrowed down the responsible factor to a deficiency in the enzyme sulphatase. This accomplishment led the author to investigate the nature of the Lafora deposits within the neurons in cases of myoclonus epilepsy. They proved to be comprised essentially of a glucose polymer. Important findings such as these inspired Dr Austin to extend his investigations into the chemistry of Alzheimer's disease and then the ageing of the brain.

This introduction leads on to sections 2 and 3, in which we are introduced to such fascinating topics as the nature of scientific discovery; the role played by mere lucky chance; the factor of intellectual preparedness; and the place occupied in research by serendipity. Concerning the latter, Dr Austin submits an allied but deviant conception which he calls "altamirage". This term was named after the "discovery"

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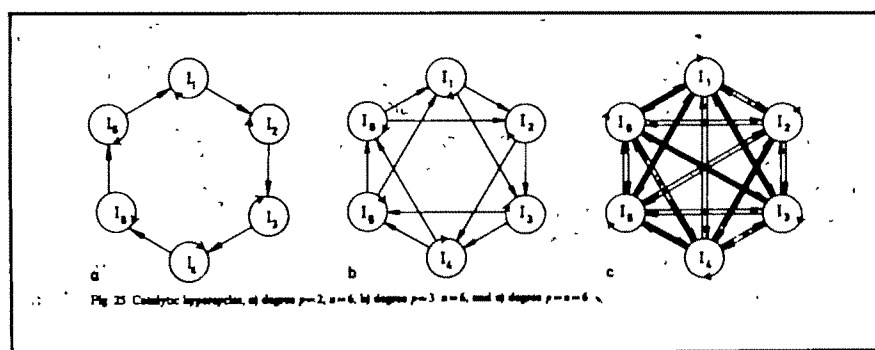


Fig. 25 Catalytic hypercycles. a) degree $p=2$, $n=6$; b) degree $p=3$, $n=6$; and c) degree $p=n=6$.

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of the prehistoric art within the caves at Altamira by Don Marcelino—more particularly by his dog, and especially by Maria, his 9-year-old daughter. It was this little girl who was the first to direct her gaze upwards, and thus become the discoverer of the glories of the "Sistine Chapel of prehistory". Altamirage is defined as "the facility for encountering unexpected good luck as the result of highly individualised action". It transcends the boundaries of serendipity in its role of personal action in chance.

The erudite and stimulating chapters which make up the final sections are psychological and philosophical in nature, and deal with such subjects as, for example, the creative personality. In an interesting fashion the author reviews the considerable literature concerning the anatomy of creativity. He emphasises, in a manner which merits the attention of all senior academics who dispense power and patronage, Dragstedt's essay "Who Killed Cock Robin" in which is described the gradual death of creativity in a researcher who becomes enmeshed in too many committees. In Dragstedt's words, "the early days in the laboratory are the golden years for many scientists. After he becomes known, the volume of mail, telephone calls, number of visitors, organisational activities, including committees by the dozen, and demands for lectures, reviews and community activities grow insidiously and will destroy the creativity of the scientist if unopposed".

Throughout his book Dr Austin recurrently mentions the part which the arts may have contributed to his scientific career. Music is there, and more particularly an almost obsessional, lifelong preoccupation with colour. It is tempting to believe that the author is, or was, a synaesthetic thinker, though he nowhere explicitly says so. Likewise he speaks of a reality familiar to some who have closely studied the phenomena of the body-image, and which the reviewer has never before seen expressed in print. According to Austin, "Even now, writing these lines, I retain a clear visual image of the scene at the desk where these ideas burst forth. This time, in a kind of mental double vision, I am both a participant in the process and a spectator hovering over a point about five feet up and ten feet directly to the rear." (My italics.)

The reviewer has nothing but admiration for *Chase, Chance and Creativity*, and confesses that Dr Austin is a man he would very much like to congratulate in person. □

Macdonald Critchley is Honorary Consulting Physician at the National Hospital, London, UK.

Major flowering plant families

Peter D. Moore

Flowering Plants of the World. Edited by V. H. Heywood. Pp. 335. (Oxford University Press: Oxford and London, 1978.) £7.95.

It is an unusual experience to come across a book which demands unreserved praise; this one does. In both concept and execution this work is eminently laudable. It aims at providing a simple introduction to the major flowering plant families of the world; over 300 are described, so it approaches a comprehensive coverage.

The book commences with a brief introduction to the structure and biology of the angiosperms together with an outline of their evolutionary history and the criteria which are used in their classification. As the book is likely to attract a wide market outside the circles of academic botany, this is a very necessary section. Even within such circles it will prove a useful summary for students. It includes Stebbins' diagrammatic portrayal of evolutionary relationships within the flowering plants.

There follows a synopsis of classification in which families are arranged into their ten Orders. On the whole the Stebbins system is used, except where some small families are fused with closely related ones.

Technical terminology in plant morphology is, according to Heywood, one of the major obstacles for the amateur, which keeps him from the appreciation of much taxonomic botany. Undoubtedly he is right in this, and the provision of an illustrated glossary should go a long way towards demolishing this obstacle, especially as the attractiveness and clarity of the illustrations make it quite the most explicit glossary of its kind currently available.

The bulk of the text (300 pages) consists of a systematic treatment of the plant families. The description of each family includes an assessment of its distribution, both verbally and cartographically. The distribution maps are a unique feature of the book, which will prove particularly interesting to those interested in phytogeography. The verbal descriptions are necessary supplements; for example, although the family Magnoliaceae is

found in southeast Asia and in North, Central and South America, 80% of the genera come from the former region. A map on its own could be misleading.

The diagnostic features of each family are then described, and here the use of technical terms for the sake of brevity will necessitate frequent use of the glossary. Major subdivisions within the family are then described and finally its economic value is considered. The latter is a source of much interesting information and includes medicinal, timber and horticultural uses.

Quite the most striking features of the book are the illustrations. Each family has a few characteristic members illustrated both by habit drawings and flower sections. Many of the illustrations are reproduced in a sepia monotone with certain parts, often the flower or fruit, picked out in full colour. It is impossible to convey in words how aesthetically pleasing and technically informative this form of representation has proved. It combines the clarity of a line diagram with the naturalness of a well-composed painting. Victoria Goaman, Judith Dunkley and Christabel King, together with the publishers, must be congratulated on a set of illustrations which are unsurpassed in current popular botanical literature.

It would be petty to dwell upon errors in the text; they are present, but fortunately scarce. For example, the primrose is referred to as *Primula veris* rather than *P. vulgaris*.

There are no comparable books which could compete with this one, either in quality of production or in price (at under £8 it must be rated a bargain). Older books, such as G. H. W. Lawrence's *Taxonomy of Vascular Plants* (Macmillan, New York, 1951), which have served generations of students, lack the fine illustrations and the distribution maps of Heywood's book. These features will also appeal to the very considerable amateur market in both Europe and North America.

This book is more than a textbook of taxonomy, it is a publication which will stimulate an interest in plants within any who open its covers. It is an item which all biologists should have on their bookshelves. Oxford University Press are to be congratulated on the production of a book which will be of great service to botany and which is a credit to British publishing. □

Peter D. Moore is Senior Lecturer in Plant Sciences at King's College, University of London, UK.

Occasions of experience

Eric Ashby

The Star Thrower. By L. Eiseley. Introduction by W. H. Auden. Pp. 319. (Wildwood House: London, 1979.) £5.95.

THIS book is about Nature but it is an unusual book to be reviewed in *Nature*. Its author was a professor of anthropology but the book would score zero in an examination paper in anthropology. It is about Man in the Darwinian scheme of evolution but it is concerned with "the phase beyond the evolutionist's meager concentration upon survival".

Loren Eiseley was a poet who became a professional scientist. The essays and poems in this volume were collected for publication shortly before his death in 1977. They are autobiographical musings written by a deeply sensitive man who never reconciled himself with what he calls the "crystalline and icy objectivity" of the scientific attitude towards Nature. The imagery is vivid and personal; the style ornate, and in places opaque. His naturalist's eye observes something—a playful fox cub too young to know fear, a spider keeping its web under surveillance, a fossil bone, a flint arrow head—these ignite his imagination and illuminate Man's relationship with the other living things around him.

The most interesting essays are those on science and humanism. Eiseley is distressed by reductionism. He does not dispute the need to think about living things at the level of atoms and molecules but he asserts that the things worth understanding about Nature cannot be discovered at this level; which of course raises the question: What, then, does he think is worth understanding? He answers this question by drawing the reader away from Darwin to Emerson and Thoreau. It is the numinous in Nature which matters to Eiseley, the attitude which the Germans call *Ehrfurcht*, the eye that sees the whole. "The finest intellect", he writes, "is that which employs an invisible web of gossamer running into the past as well as across the minds of living men and which constantly responds to the vibrations transmitted through these tenuous lines of sympathy".

Eiseley is not the only scientist who recognises the limits of reductionism and the dilemma of a theory of evolution from which the idea of purpose has been excluded. But, for my part, I do not think an appeal to the heady

prose of Emerson or the (sometimes querulous) protestations of Thoreau is likely to clarify these issues. Two essays on Thoreau—by far the best in the book—raise an interesting question, namely: What is the difference between imagination as it is used in science and imagination as it is used in poetry? Unfortunately Eiseley misses the opportunity to discuss this question. Imagination is as important in science as it is in poetry. The difference in the use made of imagination is this: the scientist disciplines imagination to make it comply with observation or experiment; the poet gives imagination free rein to range over whatever imagery his mind can design. Both these uses of imagination are legitimate. But it is confusing to mix the usage, and that—so it seems to me—is what Eiseley frequently does.

The Star Thrower has earned eulogies from other reviewers. I confess that I find the book disappointing. I think the reason for my disappointment is the use Eiseley makes of imagination. He uses it as a poet does, displacing (as he says of Emerson) "the sedate white doorstone into nature by something wild and moon-haunted, whether in science or art." In art, yes; Wordsworth, Emerson, Walt Whitman have

put on record vividly the human response to Nature. But in science, moon-haunted descriptions are not satisfactory records of Nature. Eiseley has tried to do something very important: to describe the intricacies of the biosphere as compellingly as Watson and Crick describe the intricacies of the double helix. It is an immensely difficult task and to have tried and failed is in itself creditable. Some poets—Robert Bridges in the *Testament of Beauty*, for instance—have got closer to it. So have some essayists—Lewis Thomas, for instance, in his charmingly allusive *Lives of a Cell*. I think the prize goes to A. N. Whitehead (and Eiseley generously acknowledges this), for he set out, with impeccable logic, the concept of process in evolution in a way which does enable one to think about the biosphere with the clarity Eiseley was seeking. It was Whitehead who linked together what he called "occasions of experience" like bars of music in a sonata. Eiseley's book, too, is a record of occasions of experience. It is not a clear record, but it is the testimony of a sincere and sensitive poet. □

Eric Ashby is a Fellow of Clare College, Cambridge, and Vice-Chancellor of Queen's University, Belfast

Belief in a created Universe

P. E. Hodgson

The Road of Science and the Ways to God. By S. L. Jaki. Pp. 478. (Chicago University Press: Chicago; Scottish Academic Press: Edinburgh, 1978.) £9.50.

PROFESSOR JAKI is already well known as an historian of astronomy, with major works on the Milky Way, Olbers' Paradox and the Planetary System. In his *Relevance of Physics* he reviewed the history of physics and its relationship to other disciplines, and in *Science and Creation* (for review, see *Nature* 251, 747, 1974) he showed why science had its only viable birth in seventeenth-century Europe and not in the civilisations of antiquity. In the present volume, containing his Gifford Lectures for 1975 and 1976, he extends this line of thought by showing the deep connection between scientific creativity and natural theology. His thesis is that the belief in a created Universe implies the

epistemology that underlies scientific creativity, so that the road of scientific development and the ways leading the mind from the created world to a knowledge of the Creator are mutually sustaining and inextricably intertwined.

Science first came to maturity when Newton found the middle way between the empiricism of Bacon and the rationalism of Descartes. Newton's mind was dominated by a vision of truth incarnate in nature, a truth that can be revealed by a dynamic interaction between bold speculation and meticulous experimentation. His theory of gravitation was a creation of the mind—a leap beyond sensory data—but because it was a vision rooted in the data provided by nature it could become a vigorous science. Newton's awareness of the created nature of the world implied a recognition of its contingency, and hence the necessity of painstaking experimental work to uncover its secrets. The middle way found by Newton thus transcended empiricism without becoming trapped in a *priorism*.

The reverse side of this thesis is illustrated by detailed discussions of subsequent philosophers including Hume, Locke, Kant, Fichte, Hegel, Schelling, Mill, Comte and Mach. Their admiration of Newton's achievement and their ambitions to extend his

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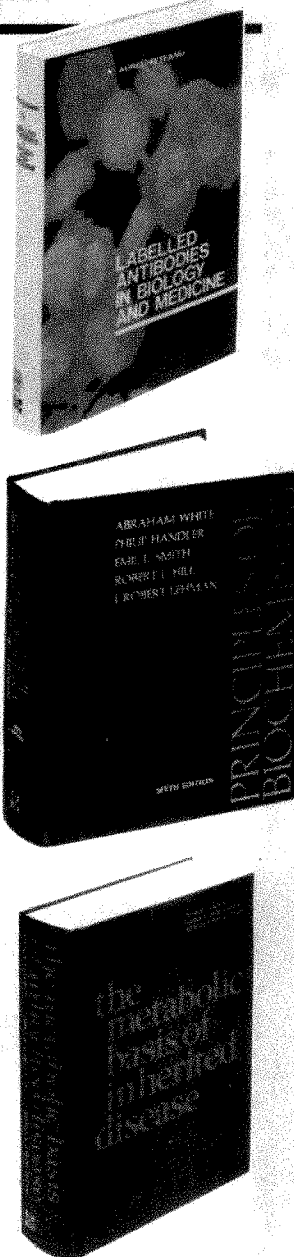
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method far beyond physics was seldom matched by any comparable understanding of his scientific work, still less of its epistemological roots. Insofar as they departed from the epistemology inherent in the ways to God, they developed theories of science that would have been lethal if scientists had taken any notice of them. Fortunately they did not, so no great harm was done, at least to science itself.

The second great period of scientific creativity was initiated by Einstein and Planck, and here again the wellsprings may be traced to the same theistic source. Although initially Einstein was influenced by Mach, his scientific creativity gradually forced him to repudiate Mach's sensationalism. Its ravages are still evident in the philosophy of quantum mechanics and in Bohr's theory of complementarity.

Fragmentation of culture

W. H. Brock

Nature and the Victorian Imagination. Edited by U. C. Knoepfelmacher and G. B. Tennyson Pp. 519. (University of California Press: Berkeley, Los Angeles and London, 1978.) £15.

"WHAT a glorious title, *Nature*, a veritable stroke of genius to have hit upon", wrote the mathematician J. J. Sylvester enthusiastically to *Nature's* editor, the astronomer J. N. Lockyer, on the first appearance of the journal in November, 1869. Yet, although the title was copied by French, Italian, Dutch and Norwegian commercial science publications, it is significant that when American scientists founded a similar journal in 1883 they chose the title *Science*. Again, T. H. Huxley, in translating a German nature poem for the first issue of *Nature*, could think of no more fitting a preface for a periodical "which aims to mirror the progress of that fashioning by nature of a picture of herself, in the mind of man, which we call the progress of science". Yet, as the editors of this elegant book point out, by 1918 any serious writer, painter or scientist would have thought it "impossible, quaint, archaic, Victorian . . . to rise from a bird's plumage to a consideration of man's position in the Universe".

That the scientific community pioneered this "denaturing" process few would deny. Put briefly, the collective theme of this interdisciplinary feast of a book is what S. F. Cannon has called "the fragmentation of culture", whereby the common

At the present time, increasing evidence of the singularity of the Universe is again pointing to its radical contingency.

Professor Jaki develops his theme with a masterly command of the original sources, and the text is supported by over a hundred pages of notes and references. It is so densely packed with ideas that it is not easy to read, but scientists, philosophers and theologians will find it a mine of stimulating analysis. Inevitably his thesis raises many other questions such as the relationships between the Christian churches and science, which have not always been harmonious, and the acceptance of science by radically different cultures; and these are apt future subjects for Jaki's pen. □

P. E. Hodgson is Lecturer in Nuclear Physics at the University of Oxford, UK.

religious, moral, poetic and scientific perspective of the early Victorians was gradually fragmented into, on the one hand, a literary consciousness which continued for a long time to seek symbols in Nature; and on the other, the various scientific forms of imagination which pursued separate, and sometimes, unrelated, disciplinary goals by measurement, experiment and abstraction. That "Ruskin . . . in looking at botanical specimens . . . saw nothing of what Darwin saw in them", is, for example, brought out by F. Kirchoff in a fascinating analysis of Ruskin's floral mythology in *Proserpina*.

Apart from R. Smith's fine essay on the human significance of biology, the question whether value systems were completely abandoned by scientists is, unfortunately, little explored by the 26 contributors to this volume, which is primarily addressed to the literary reader. Concentrating on the changing artistic interpretation of Nature, there are only three essays by historians of science who actually explore the directions taken by chemists, physicists and biologists during the century. These, together with a fine essay on science education from the Arnold scholar, R. H. Super, stand for the systems of knowledge that challenged traditional "inscape". Ignored, except for the editors' allusion to the nature writer, W. H. Hudson, is the powerful tradition of natural history (whose social history has been so ably analysed by D. E. Allen in *The Naturalist in Britain* (Allen Lane: London, 1976) which may well be seen as continuing a moralistic science long after Darwin. By the same token, the essays are exclusively concerned with the intellectuals' imagination; whereas cursory reading of "working-class" natural history journals suggests that their readers continued, like the literary

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and artistic intelligentsia (but unlike laboratory-bound biologists) to find mystery and feeling in Nature.

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W. H. Brock is Director of the Victorian Studies Centre and Reader in the History of Science at the University of Leicester, UK.

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Empirical, useful, predictive enterprise

J. Z. Young

Reliable Knowledge: An Exploration of the Grounds for Belief in Science.

By J. Ziman. Pp. 197 (Cambridge University Press: Cambridge, 1978) £7 95

PROFESSOR ZIMAN is a mathematical physicist but in this book he has devoted himself to the task of trying to define the aims and methods of all science. Wisely he begins with his own subject and bravely he goes on to extend his thoughts to biology and even sociology. Probably each scientist will like best the parts of the book that deal with subjects other than his own. But this is not to say that any of it is superficial or inaccurate, at least so far as my limited knowledge goes. Quite the contrary, he has succeeded in distilling the essence both of the results and limitations of very wide areas of science.

His general thesis is that "*the goal of science is a consensus of rational opinion over the widest possible field*" (p3) (italics throughout are his own). This is achieved, because of the very specific "internal sociology" of the scientific communities, which allows for the communication of what he calls "consensual knowledge". The definition of this is that it is unambiguous and agreed to be true by all participants. The ideal form of it is mathematics and Professor Ziman takes a lot of trouble to explain how this approaches an ideal language. It is refreshing to find that he is under no illusions that it is perfect: "Unfortunately deficiencies of consensibility can be found even in the most sophisticated mathematical systems". In particular he emphasises that "the hope of finding a unique and perfectly logical language of this kind is vain". In order for scientists to agree, they have to share certain fundamental beliefs, for example, in the Kantian categories of space and substance. He is unwilling at first to define and delimit the range of these "supreme principles". As his exposition proceeds it becomes clear that he believes that they depend upon certain innate human capacities, especially "the mysterious skill that we call pattern recognition". Moreover,

this is not amenable to complete logical analysis and is not even completely uniform amongst all men.

Having thus warned us that mathematics is not a perfect tool he nevertheless believes it to be the best available and proceeds to give an excellent account of how it functions. His beautiful description of the diverse powers of synthetic and analytical methods is presumably elementary for the mathematician but simple and direct for the rest of us; and done almost without abstract symbols. He follows it through to discussion of the place of experiment and of models and hypotheses. Popper's criterion of falsifiability he holds to be "strategically sound but tactically indefensible", a polite way of saying that it is no use. Here, as very often, he speaks of the scientist actually at work, a rare virtue in a theoretician. Every theory can be falsified by some of the observations, the question is whether it is the best available; and he illustrates this by the controversy over the missing solar neutrinos, which are much less numerous than theory predicts. He rightly contrasts the "calm order and perfection of well established theories" with the "controversy, conjecture, contradiction and confusion" of the frontline of research.

His determination to show science as it really is leads him next to discuss the principles of observation and particularly pattern recognition. "The assumption that *all observers are equivalent*" is not merely a basic principle of Einstein's theory of special relativity; it is the foundation stone of all science. However, obvious though this seems, it in fact implies that "scientific knowledge cannot be justified or validated by logic alone". As observers differ, "statements about the real world are always subject to uncertainty. They cannot all be given precise status—'true' or 'false'—their logic is *three-valued*, falling into the categories 'true', 'false' and 'undecided'." Ziman gives much discussion of the implications of this thesis which, as he says, may not be of great importance in physical science but imposes limits in biology and especially in sociology. In particular he emphasises the great influence of the sociology of belief in determining the observations that are made. Every scientist must use the paradigms of his age and Ziman gives examples of how they determine the observations he makes and the interpretations that he puts upon them.

So much cautious scepticism might well lead to scientific pessimism. But Ziman is much too practical to be a defeatist. Whatever the fundamental

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logical difficulties of validating science, "there is nearly universal human consensus concerning certain aspects of of the material domain". He leaves open the question "whether the categorial framework of everyday realism is the only possible scheme for describing and understanding nature in its physical aspects". He is content to leave it like this, but is not optimistic that "psychology and neurophysiology can arrive at a better understanding than, say, the ancient art of introspection"

Here I should disagree with him. Although he has taken the trouble to study these biological subjects seriously, I do not think he appreciates the speed with which they are approaching the capacity to achieve consensual communication that is useful even if not completely unambiguous. It is not too wild a statement to say that we are within sight of a 'complete' science of biology with principles that cover the origin, evolution and present operations of living things. It is true that the extension of this to include Man raises special difficulties. He rightly dwells in

his concluding chapters on the difficulties of precise definition of such concepts as "intelligence", "caste", "peasant" or "psychosis". In fact "we do not have a taxonomic framework for human behaviour, individual or social, in which the categories are both meaningful and distinct". True enough, and we all deplore the weakness of sociology. The difficulties introduced by hidden variables will of course always make the study of complex systems more difficult than that of simple ones. If the aim was to find some logically perfect science of man then indeed I should be pessimistic. But Ziman has taken pains to show us that the whole of science is simply an empirical, useful, predictive enterprise, characteristic of modern *Homo sapiens*. The life sciences are a part of this enterprise that is at present developing very rapidly, and I believe that his careful attention to them has already been very helpful and will be even more so in the future. □

J. Z. Young is Emeritus Professor of Anatomy at University College, London, UK.

Systematic theory of meaning

George Steiner

Truth and Other Enigmas By M. Dummett. Pp. 470. (Duckworth: London, 1978.) £18.

For Michael Dummett, Frege's *Grundgesetze der Arithmetik* is not only "unquestionably the most brilliant sustained performance of its length in the entire history of philosophy," but the whole of logic "came of age only with the work of Frege." It follows that the "most urgent task that philosophers are now called upon to carry out is to devise what I have called a 'systematic theory of meaning'." This systematic theory will proceed from the cardinal distinction between 'sense' and 'reference' in Frege and from Frege's concepts of the relationships between logic and mathematics on the one hand and logic and language on the other. Concomitantly, the philosopher, though fully aware of the attractive 'nihilism' in Wittgenstein's belief that philosophy is not "in the least concerned with establishing true propositions", will turn to Frege's systematic programme to establish a logical foundation for the determination of such propositions. And though, again, aware of the teasing finesse of Quine's thesis on the

indeterminacy of natural language propositions, he will reject this thesis. Only in this way, argues Professor Dummett, can philosophy aspire to a 'scientific' status and emerge from a 'scandalous' historical situation in which interminable effort and ingenuity "appears to engender nothing but unending disagreements."

Dummett's own papers represent this programme. They are closely-argued, rigorous and subtle steps towards a 'theory of meaning' capable of generating and testing a better understanding of 'truth', of the conditions under which a sentence may be correctly asserted, about whether the reality that renders key classes of empirical statements true or false can be shown to be fully determinate. These concerns are closely linked to a series of highly technical considerations (of special interest to the mathematician, to the mathematical logician but also, at several points, to the scientist in general) on the conflict between realists and intuitionists in mathematics, on the philosophical reach of Gödel's theorem and on the justification of deduction. Physicists, familiar with notions of time-reversal, will find much to instruct and delight them in two essays on "Can an Effect Precede Its Cause?" and "Bringing About the Past". Professor Dummett is a master of logic and of analytical exposition, and where one fails to agree—has he allowed for the characteristic and shrewd pragmatism which underlies Quine's thesis on the indeterminacy of translations between languages, a thesis which cannot be

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simply reduced to an intralinguistic model?—one does so at one's peril.

Professor Dummett's conviction that "we have now reached a situation" where what he postulates to be *the* fundamental philosophic problem, the theory of meaning, "can be resolved to the satisfaction of everyone" and, above all, be successfully resolved "within a finite time", is one of radical innocence. It may have at its core a certain personal element—even the most abstract, analytical thought has a living presence and agent behind it. There are tantalising hints that even in respect of mathematics, Dummett's provisional realism has a theistic basis or that 'metaphysics' is for him far from being nonsense if a systematic theory of meaning underlies it. Indeed, says, Dummett, "If I have made any worthwhile contribution to philosophy, I think it must lie in having raised this issue in these terms." The implications are very wide and of the greatest importance.

It is the more regrettable that the two papers in which Dummett deals with the history of 'linguistic philosophies' in this country, and with the

attacks (however technically inept) made on the narrowness, on the purely technical scholasticism of these philosophies, should decline to engage the real issue. This issue is not whether Strawson has been misinterpreted or whether Ayer has seen the importance of Frege's logic. It is whether or not the concerns which Professor Dummett so brilliantly pursues are central, are adequate to the concept of philosophy in society and the lives of individuals. The charge, and Dummett does not answer it, is simply this: Oxbridge linguistic logic has trivialised the enterprise of the philosopher. It has sought to eliminate ethics, aesthetics, metaphysics in the very sense in which Dummett invokes it. It has chosen to ignore, in Owen Chadwick's remarkable phrase, the extent to which, since the 1930s, "the agonies and passions of mankind started to enter and complicate the philosophies." The challenge is a real one. No-one would meet it better than Professor Dummett himself. □

George Steiner is Professor of English and Comparative Literature at the University of Geneva, Switzerland

Panorama of modern neurobiology

Leslie Iversen

Molecular Neurobiology of the Mammalian Brain. By P. L. McGeer. Sur John C. Eccles and E. G. McGeer. Pp. 644. (Plenum: New York and London, 1978)

THIS substantial volume represents the results of an ambitious undertaking by two biochemists who have pioneered the application of biochemical approaches to studies of chemical neurotransmitters in the brain, and one of the most eminent neurophysiologists of our time. An ambitious undertaking because they seek to bring together in one volume virtually all of the achievements of modern cellular neurobiology and to show how far this basic information can be used to explain the integrative functions of the mammalian brain. This is promising stuff, and the reader is further encouraged by the authors' principle that they should provide not merely a bare factual account of present knowledge, but attempt to give some outline of the key events in the historical development of such knowledge from the interplay of hypothesis and experiment. We may even hope that the basic cellular data will

throw some light on the workings of the human brain—"This organ, weighing about 1.5 kg, is the most highly organised matter known in the universe".

The *Molecular* title indicates a leaning to the 'wet' approach to neuroscience, which emphasises the explanation of brain function in chemical terms; but this is in fact misleading, as virtually all aspects of cellular neuroscience are dealt with in the first and largest two sections of the book. These, in some 360 pages, review basic information on the architecture, organisation and signalling properties of neurones, and concepts of chemical synaptic transmission in the periphery

and in the central nervous system (CNS). A series of fairly detailed chapters summarise current knowledge of the specialised biochemical properties associated with neurones using acetylcholine, amino acids, catecholamines, or 5-hydroxytryptamine as transmitter, and others cover the new field of 'promising peptides' and various other putative CNS transmitters, accompanied in each case by a brief outline of the actions of drugs on neurotransmitter mechanisms.

The coverage of neurotransmitters is good and up to date, although more explanation and examples could have been given of ligand-binding techniques for the study of neurotransmitter receptors in CNS, a development which has had a major impact on neurochemical studies of such mechanisms in recent years. The undue emphasis given to acetylcholine and catecholamines (almost 100 pages) continues to reflect the inadequacy of our current knowledge of other CNS transmitters, rather than the true importance of the monoamines in CNS function.

A third section of some 200 pages gives a wide-ranging review of integrative aspects of brain function, from development and plasticity to motor control mechanisms, learning and memory, and perception, speech and consciousness. The aim is to refer back in this section to the basic properties of neurones described previously. This aim is sometimes successfully achieved: for example, in the description of the extrapyramidal motor control systems in basal ganglia, in which anatomical, physiological and neurotransmitter aspects are well integrated, with discussion of human basal ganglia disease, such as Parkinsonism and Huntington's Chorea and their treatment by drugs, neatly interspersed. On the other hand, the description of cerebellar function completely omits any mention of the neurotransmitters involved, despite reference in earlier sections to the important role of the putative amino acid transmitters, γ -aminobutyric acid and glutamate, in this region of the brain. An ambitious chapter on basic aspects of behaviour includes a description of central endocrine influences and the CNS actions of psychoactive drugs, together with a review of current neuropharmacological theories of mental illness. As complexity increases with each succeeding chapter it is inevitable that the book ends with a discussion of perception and consciousness, in which the dualist-interactionist hypothesis—described in more detail in the recent book by K. R. Popper and J. C. Eccles *The Self and Its Brain* (Springer: Berlin, 1977; for review, see *Nature*, 272, 770, 1978)—is restated. The reader is given plenty of advance warning of this conclusion: in discussing the ques-

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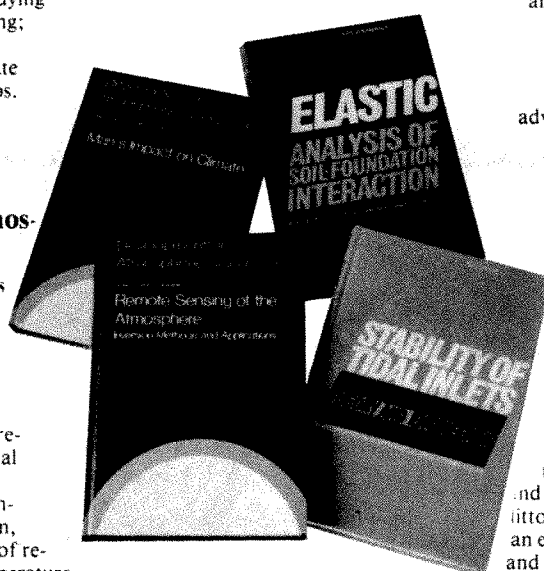
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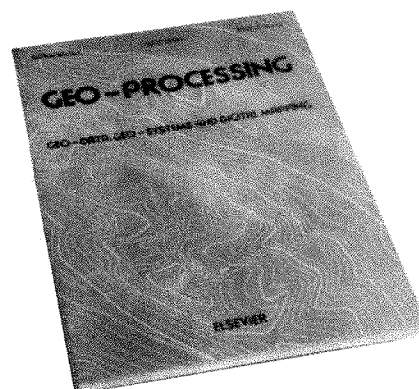
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tion of consciousness (p3) "We shall see that it is beyond our comprehension how these subtle properties of the conscious self came to be associated with a material structure, the human brain, that owes its origin to the biological process of evolution." However, whether one agrees or not with this strong dualist stance, we could all agree that "We believe it to be important for the molecular neurobiologist to be confronted with the problems arising in higher brain functions."

In any volume with as wide a scope as this one, each of us will no doubt find what seem to be important omissions or failures of emphasis. For example, I found it hard to understand why so little attention was given to a clear description of modern anatomical tracing techniques, using anterograde and retrograde flow of marker materials, in view of the enormous and continuing impact such techniques are having on our understanding of neuronal circuits in mammalian CNS. (Pick up any current issue of a brain research journal and count the number of papers involving peroxidase tracing methods!)

The overall impression left by this book is that of a courageous enterprise which did not quite succeed in its objectives. The variation in styles between the authors is often very obvious, and this together with the attempt to cover almost *all* aspects of mammalian neurobiology inevitably makes the book somewhat disjointed, with the individual chapters often divided and sub-divided over again into a multitude of smaller subsections. The illustrations, however, are copious and of excellent quality, and there is a substantial bibliography, with more than 1000 references (though with few more recent than 1976). One is left with admiration for the energies of the authors who have succeeded in putting together an attractive volume, but also in some doubt as to its likely readership. The book is probably too large and expensive to become widely used as a neurobiology student text, where other excellent volumes in any case already exist—for example, S. W. Kuffler and J. G. Nicholls' *From Neuron to Brain* (Sinauer Associates: Sunderland, Massachusetts; UK Publisher, Place, 1976). Nevertheless, it deserves to find a wide readership, and will certainly provide an accurate and sometimes inspiring panorama of modern neurobiology for those who want to know more about the field as a whole, or who feel the need to bring up to date what they thought they once knew. □

Leslie Iversen is Director of the MRC Neurochemical Pharmacology Unit, Department of Pharmacology, University of Cambridge, UK.

Boundaries of palaeo-anthropology

G. E. Kennedy

Early Hominids of Africa. Edited by C. Jolly. Pp. 598. (Duckworth: London, 1978.) £28.

HISTORICALLY ill-defined, the boundaries of palaeoanthropology have recently been delineated by several excellent volumes. Although all of these volumes have emphasised the interdisciplinary character of palaeoanthropology, none has so effectively designated the scope and nature of the contributions from geology, anatomy, biology and archaeology better than this one.

Early Hominids of Africa is comprised of papers given at a conference held in New York more than five years ago. That the conference was organised (largely by Cliff Jolly, who also edited the volume) with care and some foresight is attested to by the overall currency and freshness of the papers. A number of the papers are, in fact, of considerable value in designating the current structure of the discipline.

Glynn Isaac, in his contribution, for example, discusses the scope of palaeolithic archaeology within the context of palaeoanthropological investigations. He points out that such studies focus less on traditional questions of tool typology and more on broader areas of tool function and the behaviour, selection and origin of raw materials. His suggestion that tools represent "self-recording behaviours" typifies the approach where more detailed and subtle questions are now asked of empirical data than was usual in earlier years.

So, too, with the palaeoecological papers contributed by Butzer, Behrensmeyer and Cooke. These papers, each from a different research perspective, focus on the reconstruction of the environmental context of the early hominids. Butzer's paper is perhaps the most successful of these and indicates the new directions taken by the various contributory disciplines within the context of palaeoecological reconstructions. Butzer argues that many of the environmentally deterministic models of anthropoid and hominid evolution were erroneously based on unidimensional reconstructions and both the

environment and the sources of selective pressures were more complex than the earlier, more simplistic models would allow.

The section on anatomical evidence, the largest in the book, reflects a strong trend in research interests away from the cranium; all but two of the papers deal with analyses of postcranial anatomy and locomotor capabilities. Papers by Day, Robinson and Lovejoy are informative in these areas, although they provide little that has not been published elsewhere. The two papers on cranial morphology are innovative and highly specialised. Wallace's study on the ramifications of early closure of the premaxillary suture in hominids is both insightful and soundly based on the fossil evidence. His discussion on the ontogenetic, phylogenetic and adaptive aspects of this process is an excellent example of the subtlety of the questions now being asked of the fossil data. So, too, with Holloway's discussion of endocranial casts which continues his series of stimulating, frank and challenging papers.

A section devoted to the interpretation of diversity in early hominid samples continues the interdisciplinary theme. Delson's paper on cladistic analysis, which emphasises trait congruity as a taxonomic tool, contrasts strongly with Campbell's, which seems to emphasise temporal congruity for the same purposes. Simons contributed an analysis of hominid evolution from a vertebrate palaeontologist's viewpoint. Although valuable for methodological as well as interpretative reasons, this paper is of particular interest for its discussion of *Ramapithecus freybergi*, a later Miocene hominid from a site near Athens.

Papers on the most productive of the east and south African localities were submitted by the sites' principal investigators. Although much of this information has been published elsewhere, these concise summaries are useful.

The long delay in publication has resulted in very few shifts in the interpretation of data. The most noticeable of these shifts involves Johanson's evaluation of hominid material recovered at Hadar, Ethiopia. In 1972, he suggested that two taxa were present at Hadar, a view recently formally rejected in favour of a single taxon. The only significant criticism which can be levelled at this volume is the cost. The high price will put it beyond the reach of most advanced undergraduate and graduate students, groups which would benefit most from it. □

G. E. Kennedy is Assistant Professor of Anthropology at the University of California, Los Angeles, California.

Technological medicine

Maurice Lessof

Beyond the Magic Bullet. By B. Dixon. Pp. 249. (George Allen and Unwin; London, 1978.) £5.50.

THE essential thesis of this readable and well written book is that expensive, technological medicine has failed. It has distorted our approach to health problems and has led to the neglect of the epidemiological, social and psychological components of illness. Worse than this, it is not even effective. Waterborne infections, which kill an estimated 10 million people annually throughout the world, could be contained more readily by measures that are concerned with nutrition, hygiene and water supplies than ever they could by antibiotics.

There is a second theme which is a little more difficult to sustain. The author believes that, in a world setting, the approach to disease through individual causes and specific cures has largely been a fiasco. 'Specific aetiology' is an idea that has had its day; for even infections with an identified cause are very complex in their origins. A technological approach to treatment is inappropriate in the Third World, where the death rate for measles is increased 400-fold by malnutrition.

The fallacy of this proposition is perhaps worth considering, for the argument is very seductive. The use of vaccines, much applauded by the author, is the fruit, *par excellence*, of research into 'specific aetiology'. It is also, incidentally, a product of the much-criticised pharmaceutical companies, with their 'magic bullet' mentality. In making the case for better nutrition and hygiene, it is tempting to deride the scientific approach. Yet vaccination has eradicated entire diseases from many countries throughout the world. The evidence of this book, so impressive in other ways, cannot therefore be accepted for this curiously science-bashing purpose.

The author's thesis is a powerful one and may be re-stated in another way. The Western approach to medicine is characterised by a search for antidotes and glamorous technology. It has led to overspecialisation, overprescribing, and the development of incongruously elaborate techniques for curative medicine and surgery. It fails to take into account the fact that the illnesses of mankind are determined by a combination of many factors, which require social remedies rather than pill-pre-

scribing and high-technology doctoring. In the West, cigarette-smoking and obesity, in the Third World nutrition and hygiene—these should receive our attention and our emphasis.

In its writing, this book is delightful, and the historical section includes an account of Pasteur's experiments which is strangely exciting. (Poor Jenner gets no credit, but never mind!) The author is not merely an anti-medical Illich, following a fashionable witch-hunt against doctors. He notes that some at least of the medical profession share his concern with the relationship between social problems and health care. The medical establishment is, however, unable to cope.

The problems are indeed formidable—how to assert, in a liberal democracy, an effective anti-smoking, anti-alcohol, anti-obesity policy; how to persuade a Third World country to modify, however slightly, the priority given to prestige hospitals and universities, in order to emphasise the importance of hygiene, health education and good water supplies; and how to encourage solutions which, even when they cost nothing, require a firm stand by politicians, who themselves depend on populist support.

Much of the quoted evidence is beyond dispute. There are some massively important problems, which we neglect at our peril and which require largely political solutions. The medical or scientific reader may find little comfort in the author's message. The implication, almost throughout, is that the purveyors of modern, conventional medicine and research are oblivious to the real world, shackled to an obsolete view of current health needs, obsessed by high technology and magic bullets, and unable or unwilling to adapt either their thinking or their teaching of the next generation of doctors. This is simply not true. The problems are as formidable as the author says they are. In seeking to combat them, it needs to be more clearly stated that doctors, and even scientists, are quite frequently on the side of the angels.

Switching from the Third World to the sophisticated West, there is not only a need to change our social priorities but also a need for more humanity in our provision of health care. This is illustrated by a vivid account of a patient with ankylosing spondylitis, who had an unquenchable determination to get better, though "one of the experts put the chances of recovery as one in 500; another said he had never witnessed recovery from this comprehensive state". (On the story given, this was probably not ankylosing spondylitis at all but post-infective reactive arthritis, from which a rapid recovery is usual. Even in true anky-

losing spondylitis, it should be stated that some 80–90% of patients are earning their own living 20 years later.) The story is a good one, recording a patient's determination and also a doctor's unstinting support for an unconventional treatment which had nothing to commend it except for the patient's faith. The point is well made. A practising doctor looks after sick people, not disembodied disease. Hope and encouragement may do more than medicine—even more so in diseases such as cancer, in which definitive medical treatment may not be available.

Having reviewed all the evidence, this book suggests that we must deploy both our doctors and our financial resources to better effect. The message could, however, be dangerously misread. On the advice of our politicians, we in Britain have already suffered an expensive and ineffective reorganisation of the Health Service; a wasteful but well-meaning allocation of money to ineffective types of social work; a crazy emphasis in our Community Health Councils, which give priorities to 'watchdog' functions rather than to a responsible collaboration with professional health workers; and an opportunistic encouragement of specialised kidney units which are now, for political reasons, deprived of funds.

What about the needs of the Third World? Who can doubt that great benefits could follow from better nutrition, hygiene, good water supplies, and the Chinese barefoot doctor's approach to health education and simple health care. But even here, the problem is not a simple one. In many parts of the world the mere fact of a reduced infant mortality rate results in the exposure of even more children to the ravages of malnutrition. In this context, social priorities must therefore include population control. This, the most important problem of our time, is merely touched upon in this book. Alone among the Third World countries, the Chinese have shown that population expansion can be controlled by means of public education and persuasion. In the West, however, this social and political approach has not succeeded. Here the contraceptive pill and the intra-uterine device have, indeed, provided another victory for high technology, and their future potential blessings have yet to be realised.

In summary, the siren song of the anti-science lobby needs to be heard but then resisted. The moral to be drawn from this book is absolutely clear. We need both social policies and high technology to solve our problems. It is a partnership, not a conflict. □

Maurice Lessof is Professor of Medicine at Guy's Hospital, London, UK

Complex mental functions

Kevin Connolly

Mind in Society: The Development of Higher Psychological Processes. By L. S. Vygotsky. Edited by M. Cole, V. John-Steiner, S. Scribner and E. Souberman. Pp. 159. (Harvard University Press: Cambridge, Massachusetts, and London, 1978.) £8.75.

L. S. VYGOTSKY is known largely to the English reading community through a little book, *Thought and Language*, which has received very wide acclaim. It was published posthumously in 1934, suppressed in 1936, reissued in 1956 and translated into English only in 1962. The book has had a great impact on Western psychology and revealed Vygotsky as a truly original thinker and insightful observer of children. He was born in Orsha in Byelorussia in 1896 and graduated from the University of Moscow in 1917 having specialised in literature. From 1917 to 1923 he taught literature and psychology in a school and began his systematic work in psychology only in 1924. In 1934 at the age of 38 he died of tuberculosis. During this all too brief period of research in psychology he was immensely productive. He worked first at the Institute of Psychology in Moscow and then in the Institute of Defectology, of which he was a founder. Over this 10 year period he taught courses in Moscow and Leningrad, undertook a medical training, and wrote more than 160 books, papers, reviews and lectures. Much of this work remains unpublished and very little has been translated into English. It is now over 40 years since Vygotsky died and yet much of what he had to say is fresh and deeply significant for contemporary psychology. This newly-translated material is of historical interest but not primarily so; he was writing clearly about fundamental issues which Western psychology has recently been congratulating itself on having articulated.

Vygotsky was interested in complex mental functions, or as they were more usually called 'higher psychological processes'. He rejected the notion that an understanding of these processes could be gained from an extension of the principles derived from animal psychology, and like the Gestalt psychologists did not believe that an adequate analysis was to be found in

reductive stimulus-response theories. He sought a comprehensive approach which would provide both description and explanation in terms acceptable to natural science. His goals for such a programme were immensely ambitious including as they did the identification of relevant brain mechanisms, the developmental history of behaviour, and most important specifying the societal context in which the behaviour developed. That he did not fully achieve these goals is hardly surprising but he did show himself to be an astute and remarkable thinker.

For Vygotsky the developmental method is a central feature of psychological science. He saw the scientist's task as one of reconstructing the origin and course of the development of behaviour and consciousness. A distinctive theme of his writing is the emphasis which he placed upon the unique qualities of man. As a species we actively realise and change ourselves in the varied contexts of history and culture; in the course of his history man affects and changes nature to create for himself new conditions of existence. To the Western tradition Vygotsky seems less an experimenter and more a thinker. He was a brilliant observer of children and an ingenious experimenter, but his work is not characterised by elaborate experimental designs and statistical analyses. Rather the experiment is a means of gaining insights which illuminate the ideas and carry them along. The experimental 'leg work' will no doubt be done by others, who perhaps find thinking harder.

The book contains eight of Vygotsky's essays, the first five of which are taken largely from a hitherto unpublished manuscript of 1930 entitled, *Tool and Symbol in Child Development*. Here Vygotsky attempts to relate Marx's Theory of Society to concrete psychological questions. The concept of tool implies a specific human activity, the transforming effect man has upon nature in gaining mastery over it to serve his ends. Like tool systems, sign systems (speech, writing, number) are created by societies and change with the level of cultural development. In this way the mechanism of individual developmental change is rooted in society and culture. The last three essays explore educational implications of his ideas. Again the penetrating quality of his thought is striking. He explores the relationship between learning and development, and in his concept of the "zone of proximal development" can be seen the antecedents of the competence/performance distinction which psychologists have made much of over the



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past decade. He proposes too that intelligence could be better assessed by an examination of the child's capacity to benefit from teaching rather than the normative comparisons embodied in psychometric tests. In an essay on play he concludes that it is not a predominant feature of childhood but that it is a leading factor in development. The last essay on writing is a masterpiece which has profound practical implications in education.

This little book is an intellectual excitement; it abounds with all manner of ideas, insights and novel formulations. For anyone feeling jaded with contemporary cognitive or developmental psychology the book should lift the spirit; for the graduate student in search of ideas it cannot fail to raise and widen the horizons. The editors provide, two by two, a useful and sharply focused introduction and concluding piece.

Vygotsky comes high on my list of people I regret not having met and I am grateful to the editors for having helped me know him in the next best way. I recommend the book warmly and without reservation. □

Kevin Connolly is Professor of Psychology at the University of Sheffield, UK.

Invitations to enquiry

Mary Waring

Science, Curriculum, and Liberal Education: Selected Essays. By J. J. Schwab. Edited by I. Westbury and N. J. Wilkof. Pp. 394. (University of Chicago Press: Chicago and London, 1978.) \$24; £16 80.

For some twenty-five of his fifty years at the University of Chicago, Joseph Schwab participated in an experimental undergraduate programme in general education, and it was this experience that gave impulse and direction to all his writing. This book contains a selection of twelve of the fifty or so essays that make up the bulk of his published work, an Introduction written by the Editors, and a bibliography.

The Introduction outlines the context in which Schwab's ideas developed. It stresses the continual interplay between, on the one hand, reflection upon the immediate practical concerns of his day-to-day teaching and, on the other, extensive explorations in psychology, psychiatry, literature, philosophy, science and the philosophy of science, an interplay that was to become the hallmark of his approach to education. His mastery and use of many perspectives and this interrelating of ideas and action gave Schwab both an intellectual framework and an integral and distinctive approach to problems. Both were greatly enriched by continuing dialogue with colleagues like Louis Thurstone, Irving Lorge, Ralph Tyler, Robert M. Hutchins and, above all, Richard McKeon, who introduced Schwab to hermeneutically-inspired interpretative schemata and, as a former student and colleague of John Dewey, gave him direct access to a philosophy that was to be a major influence on his thought.

Although many of the essays are well known and highly regarded, the fact that most have been written for special occasions and purposes and have hitherto been published individually in a variety of journals, has meant that they have proved difficult for many readers who have come to them in isolation or at relatively long intervals of time. Collecting them into one volume and providing the orientation afforded by context creates a whole which is much more than just the sum of its parts; it reveals so vividly the comprehensiveness and coherence of Schwab's understanding and it encourages and facilitates greater fluidity

of thought on the part of the reader. Grouped under three headings ("On Liberal Education and Science", "On the Foundations of the Curriculum" and "On Curriculum Building"), the essays are concerned primarily with a carefully reasoned enquiry into the practical problems of giving new meaning and vitality to the conception of a liberal and liberalising education and, within it, to the teaching of science. Schwab leads the reader through the complex web of considerations which enmeshes each of the issues explored—enquiry and interpretation, personhood and experience, scientific enquiry and the nature of science and liberal education, educational testing, teacher education and curriculum development and research—providing a series of deep insights and provoking reflection upon the articulation of ideas and action in the search for means and ends. There is no attempt, however, to argue for

outdated, elitist conceptions of liberal education: the concern is with the search for essentially practical ways of helping the young to become 'actively intelligent' people who enjoy and thus continue to seek out knowledge and understanding, and who possess the critical and organising powers that make possible informed choice and action.

This is a challenging and important book, offering none of the slogans and easy solutions of those whom Schwab describes as "honing a problem down until it looks simple" or basing their ideas on "unexamined notions of reality". Emphasising, always, that matters of the curriculum are matters for doubt and critical enquiry, Schwab is concerned to "move men to action", rather than to prove or to prescribe.

Mary Waring is Lecturer in Education at the Centre for Science Education, Chelsea College, University of London, UK.

A forbidden transition

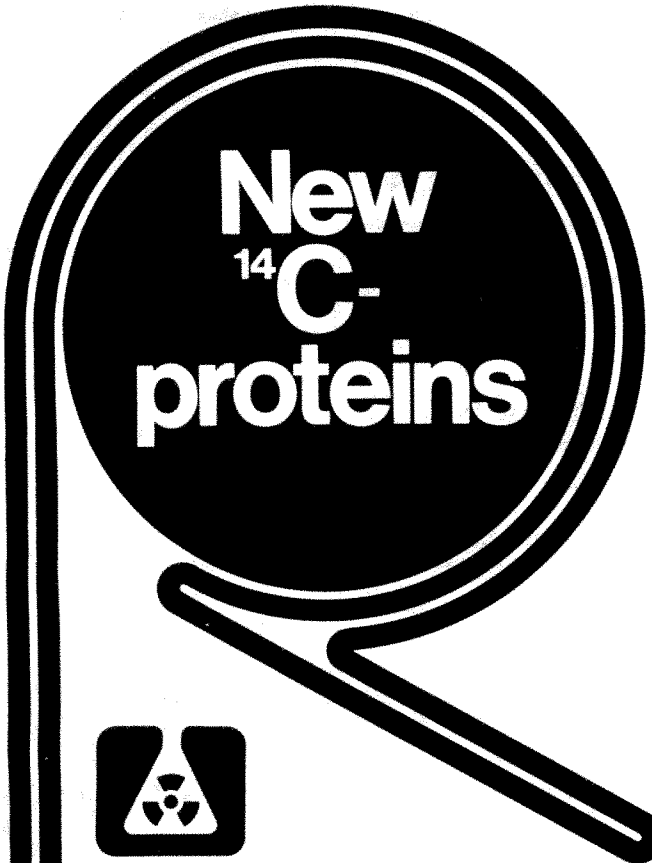
Fred Dainton

Higher Education Developments: The Technological Universities, 1956-1976. By P. Venables. Pp. 407. (Faber and Faber: London, 1978.) £25.

This book has a simple structure. In the introductory chapter the reader is taken at a brisk trot through the history of post-school technical education by way of the Percy Report; the establishment in 1956 of the Colleges of Advanced Technology (CATs), and of the National Council for Technological Awards; the Robbins Committee's recommendations of 1963, most of which were accepted almost immediately by the Government and which led to the CATs being transformed into technological universities with a consequential transition from ownership and government by Local Authorities (or Government in the case of Loughborough); to self-government and financial support from the University Grants Committee (UGC). The next eight chapters are a description of all that happened in and to these institutions in the decade 1966-1976. This section is abundantly provided with graphs and tables and is a gold-mine of factual information which is made very easy to quarry by the use of a very well constructed index. The tenth chapter is entitled "The Polytechnics" and is in fact a fascinating comparison of these institutions with the technological universities, in which the author seems to

invite—almost quizzically—the reader to look at the facts assembled in four tables and draw his own conclusions. In the last two chapters the author looks at higher education in a broader perspective, as it is and as it is likely to be, and he also lifts the veil ever so discreetly on his own credo and ideals. The book ends with four appendices; a glossary of acronyms, potted biographies of the eleven institutional actors; a few more statistics which I feel would have been better placed in the text; and the questionnaires which evoked the responses on which much of the information purveyed by the book is based. The wealth of data assembled in the book contrasts somewhat strangely with a curious omission. Nowhere did I find a really clear definition of technology and how it should be distinguished from natural science and engineering—if it is.

The non-British reader may be surprised that a whole book should be devoted to the detailed examination of such a small subject—namely, the emergence, infancy and early childhood of eleven institutions containing only about one-tenth of the total university population and therefore only about half that proportion of the total in higher education. The significance of the subject does, however, go far beyond what this mere numerical comparison might suggest, for the process by which the technological universities were created can never be repeated for as long as the policy of the "binary system" holds sway. That transformation would now be a "forbidden transition". The book is therefore important as a case study of special value to two groups of people. On the one hand it will be important to those who will determine future tertiary education policy in Britain and on the other hand to the historians



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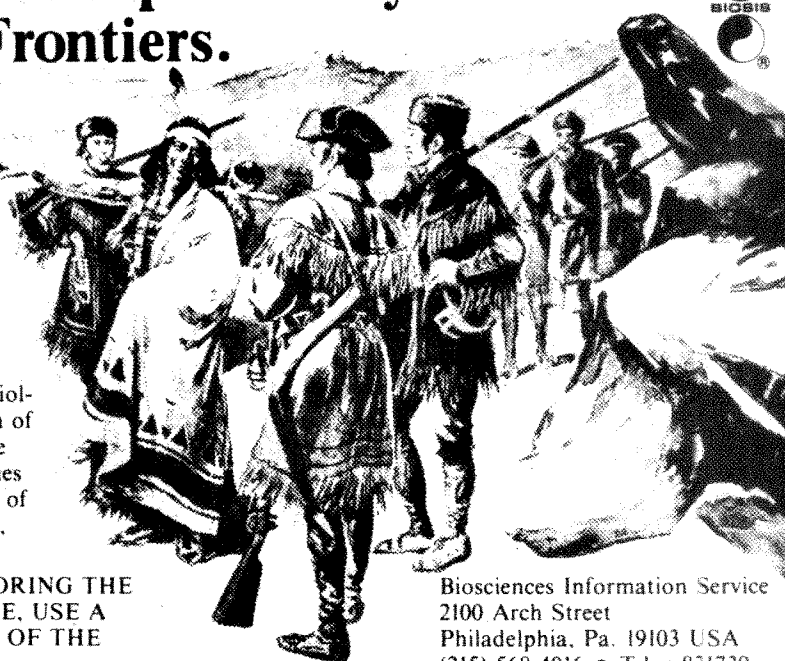
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of the politics of education. It can be strongly recommended to both because it is soundly based on a careful scrutiny and scholarly evaluation of documents and an exhaustive enquiry; above all, it is written by an "insider" (the author's own term). This last point is of special importance for although there are many pieces, published and unpublished, which are written about higher education in Britain few of the authors have Sir Peter Venables' very wide experience. These writers fall into a very wide range. Thus at one extreme the authors are lonely scholars, either MA or PhD thesis writers or their own senior colleagues, more often than not embedded in a School of Education, itself on the fringe rather than in the mainstream of the activities of its parent institution. Such authors' sources are restricted to public documents and their experience is unlikely to embrace that of treading the corridors of a local education authority, the Department of Education and Science, the Scottish Education Department, the Welsh Office or even the UGC. The fruits of their labours are, however, likely to be published perhaps in book form or as articles in journals for all to see, dissect and evaluate. At the other extreme there are the civil servants whose duties are to prepare influential position (and policy) papers which rarely ever find their way into the open literature—at least legitimately. Their own direct experience of higher education is likely to have been limited to a few years at a university ten, twenty or even thirty years earlier. So the scholar suffers from ignorance of the "real", as distinct from the "openly acknowledged", political and intellectual reasons for decisions; and the civil servant who is well placed to influence those decisions has no living, working contact with the "coal-face" of higher education.

This gap is deplorable, as it is likely to militate against the formulation of the most beneficial policies. How is it to be bridged? The Royal Commission is one device. It is certainly open and thorough but also often cumbersome and slow; and it is not a permanent bridge. There are a few helpful signs in, for example, what seems to be a greater willingness and/or freedom of the public servant to discuss issues with scholars and researchers and the welcome tendency to issue "Discussion Documents" and "Green Papers" before decisions are taken. Then there is the occasional forum or symposium which is, however, rarely productive of meaningful interaction between the civil servant who, restrained by the rules and conventions of his employment, may elect to stay silent at a public gathering on just those topics which, being of greatest contemporary interest and importance, are therefore also the most sensitive issue.

The situation would be much improved if there were some exchange of people between posts in Academe and the Public Service. But mobility, though repeatedly

advocated as desirable, remains and is likely to remain far too infrequent. In our British system the best we can hope for is the lucky chance either that academics and ex-civil servants are able to collaborate (as in the case of Becher, Embling and Kogan's brief study of higher education) or that some able person who has been deeply involved as a teacher and administrator in higher education institutions for most of his life, helping to shape them during a period of rapid development, who has also served on many national bodies in ways which have brought him into close contact with civil servants, local authority officials and politicians, will make time to take stock of the past and present and point out the options for the future.

Such a man is Sir Peter Venables. His career in higher education, especially in technological universities and their precursors, will be known to many as

qualifying him well for this task, to which he has also brought the scientist's insatiable appetite for facts and a method of enquiry borrowed from the woman who is both his sociology instructor and wife. The result is predictable and well worth the effort. I have only two regrets. The style is almost too 'dead pan', too detached and at a number of places I would have liked Sir Peter to be a little more 'unbuttoned' and to have given us the benefit of his own opinions even when they could not be completely buttressed by evidence. His own long and devoted service to the cause of technological education have earned him that right. My second regret is that the price should be so exorbitant and therefore the book may not get the circulation it deserves. □

Sir Frederick Dainton was, until 30 September 1978, Chairman of the University Grants Committee, London, UK.

Energy melting pot

Peter Rost

Energy and the Environment: Democratic Decision-Making. Edited by C. Lenzer, C. Phipps, J. Valleix and J. Surrey. Pp. 141. (Macmillan: London, 1978) £5.95.

It was not until the oil crisis of 1973-74 that politicians woke up to realise that they now had to face yet another challenge. Until then the provision of energy at an acceptable price was taken for granted. Energy policy was regarded as the preserve of technical experts and the balance between supply and demand was satisfied by the market. Now energy has become a dominant world political problem.

The energy debate has not lacked documentation. Students anxious to be alarmed, or reassured about the prospects for mankind's future energy resources have been deluged with reading material. But each such contribution to the argument has been a personal interpretation, or a consensus by an expert group. What has been less readily presented to the informed but enquiring reader, is an actual cross-section of the debate itself.

This is why this book is an important contribution to the evolving energy debate. It records a two-day colloquy organised in November 1977 by the Council of Europe, which brought together 70 politicians from Europe and North America, with an equal number of independent experts. The Parliamentarians represented a formid-

able array of spokesmen many of whom have established their national and international political reputations for their specialist contributions to energy studies, whereas the technical and industrial experts included many reputable figures from international organisations concerned with energy problems, of high standing in scientific, academic and international circles.

Such a rich mixture into a two-day melting pot could easily have created an indigestible meal. But the themes were well defined and the debate was disciplined. Four principle aspects were tackled—energy supply and demand to the year 2000; the contribution of fossil fuels and renewable sources; the nuclear debate; and options for the future.

Perhaps surprisingly, some consensus emerged. No one energy source will solve the future, real costs will rise and conservation must move up the league table of priority options.

Revealing too, is the admission by decision takers, that they (the decision takers) will have to face up to unpopular measures! Long term policies to provide enough energy, yet protect the environment, maintain living standards and preserve democracy, may require electorally unpopular choices.

This book should help to reassure the informed public that, although the experts will never agree any more than politicians, at least the debate is being carried forward on solid intelligent foundations of scientific knowledge and political maturity. We must hope that Parliamentarians will follow up with the correct policy decisions. □

Peter Rost is Member of Parliament for South-East Derbyshire, a Member of the Select Committee on Science and Technology Sub-Committee on Energy Resources, and Member of the Conservative Parliamentary Energy Group.

Civil nuclear policy

Walt Patterson

The Politics of Nuclear Power. By Dave Elliott, with Pat Coyne, Mike George and Roy Lewis. (Pluto Press: London, 1979.) Paperback £1.95.

THERE cannot be many issues which would bring John Biffen, MP, and Robin Cook, MP, into the same voting lobby at the House of Commons. Each is generally considered to be well toward the outer flank of his party—Biffen toward the right of the Conservatives and Cook toward the left of Labour. Yet on 15 May 1978 Biffen and Cook were together among the 80 Members who challenged the joint wisdom of both Front Benches and voted against the proposed expansion of nuclear fuel reprocessing at Windscale. Articulate Parliamentary critics of British civil nuclear policy, whose numbers have increased impressively in the past two years, speak from virtually every corner of the House—as do nuclear advocates.

It is an interesting phenomenon, worthy of much closer scrutiny. But it is not the subject of *The Politics of Nuclear Power*. Perhaps it should be, for the issues which crystallise around civil nuclear policy do not lend themselves easily to the conventional left-right of traditional party politics in Britain. However, Dave Elliott and his colleagues have here set themselves a more immediate task: that of persuading trade union members, and others on the left of the political spectrum, that it is in their interest to re-appraise official union policies which presently support civil nuclear expansion. Elliott and his colleagues can point to France, where the Confédération Française Démocratique du Travail (CFDT), the country's largest union, has played a leading role in attacking French government plans for headlong nuclear programmes; and to Australia, where the Australian Council of Trade Unions has been confronting the government for some four years, opposing official plans to mine and export uranium. However, it is also true that trade unions elsewhere—notably in the US and the Federal Republic of Germany—have been among the most enthusiastic and vehement proponents of civil nuclear activities. Even on a broader party-political basis the case is

by no means clear-cut. French and Japanese socialists, among others, are critical of nuclear power; but West German and Swedish Social Democratic governments have pressed ahead with nuclear expansion—only to find, in the Swedish case, a non-socialist party ousting the Social Democrats from office on a promise to phase out the nuclear programme. In the Soviet Union, of course, the civil nuclear programme has long had top priority, leading to the ironic spectacle late in 1978 of a party of American journalists, touring Soviet nuclear facilities under the auspices of the US Atomic Industrial Forum, champions of nuclear free enterprise. In short, the picture is confused, and confusing, and the time-honoured soapbox rhetoric of two-legs-good, four-legs-bad—or indeed *vice versa*—is unilluminating and unhelpful.

Fortunately, Elliott and his colleagues try to avoid sloganeering, concentrating on substantive factual information and analysis of particulars. An introduction by Elliott opens by declaring that "This book focuses on a number of problems, questions and issues relating to nuclear power likely to be of concern to workers in the nuclear industry and elsewhere". Elliott then offers a brief description of nuclear technology and its status in Britain and elsewhere. The main body of the book then falls into three sections: "The political economy of nuclear power", "Nuclear power and employment" and "Political strategies". Pat Coyne recounts the chequered history of British civil nuclear development, identifying what he calls a trend to "state corporatism" and concluding that "nuclear power in the UK has been developed and kept alive by a combination of political will and institutional inertia. Economically it has always been seen in terms of future needs rather than present benefits, and the arguments for its necessity are open to serious doubts . . . the consensus of political interest which previously sustained it shows signs of cracking". Mike George delineates the details of ownership and control of nuclear industry; and Elliott asks whether—given the industry's sorry economic record to date—central planners find nuclear power appealing because it lends itself well to centralisation. Elliott then discusses past attitudes to nuclear matters on the part of trade unions in Britain, through the TUC Fuel and Power Committee, who "simply ask for more of everything—coal, oil, gas and nuclear", and sees this attitude gradually changing, as "wider economic and employment issues are gradually beginning to be raised".

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cusses health and safety and trade unions rights in the nuclear industry, and points to the need for more information. Consideration is then given to jobs, energy and industrial strategy, and alternative policy options of the kind recently detailed by Gerald Leach *et al.* in their report *A Low Energy Strategy for the UK* (Science Reviews Ltd and IIED: London, £7.50; for review, see *Nature* 277, 162; 1979). According to Elliott, "Because the alternative technologies are mainly, by their nature, decentralised they can support a decentralised society, based on community and workers' control. But their introduction into society as it is clearly doesn't necessarily guarantee that such a state of affairs will come about". However, Elliott concludes, "The fight for alternative technologies is not an alternative to the fight for socialism. It can and should be part of it"—a sentiment making up in vigour what it lacks in grace. Part three of the book discusses how such aims can be pursued, by trade unionists and environmentalists working for common objectives.

All four authors write clear, readable prose, in language which is accessible

and blessedly low in jargon. However, it is a pity that more attention was not paid to fine detail; too many trifling but irritating errors of fact have survived into print. (There are eleven British Magnox stations in all, not twelve, including a total of 26 reactors; there is no power reactor in Denmark, only three small research reactors; Belgium, far from being a nuclear "poor relation", has three substantial power reactors already in operation and four more under construction, and generates a larger percentage of its electricity by nuclear power than any other country; the proposed commercial fast reactor would be 1,000 MW, not 100 MW; and so on.) It would be easy to correct virtually all these fluffs in a second printing. To do so would remove the most irksome blemish on what is otherwise a very useful book, one which deserves to be widely read and discussed, by trade unionists, environmentalists, and all those other 'ists who make up our disputatious society. □

Walt Patterson is Energy Consultant to Friends of the Earth (London) Ltd.

Peer review in the NSF

S. S. Blume

Peer Review in the National Science Foundation: Phase One of a Study. By the National Research Council. Pp. 193. (National Academy of Sciences: Washington, DC, 1978.) \$10.75.

Most readers of *Nature* will be well aware that the peer review system (the system by which research grant applications are evaluated by experts in the disciplines concerned) has recently been subjected to considerable criticism in the USA. Members of the general public as well as congressmen have attacked an 'old boy network' said to have control of research funds. The National Science Foundation has steadfastly refused to make confidential referees' reports (and their names) available to members of congress, and this has been held to be a concealment of the Foundation's biased procedures. Politicians have also accused the Foundation of giving inadequate weight to the needs of society (as distinct from those of science) in the selection of projects for funding, and

of discriminating against scientists in less prestigious institutions. The fault, some claim, lies in the peer review system. Ripples of these criticisms—the view that justice should not only be done but be seen to be done, that criteria of equity applicable in other areas of public resource-allocation should apply also in science—have reached these shores. For example, not long ago the UK Research Councils had to consider the claim that they dealt unfairly with scientists working in polytechnics.

This volume reports on the first stage of a study of the operation of the peer review system in the NSF, commissioned by the National Academy of Sciences. The researchers were given full access to reports and files on all applications to the Foundation. They interviewed programme directors (who unlike British committee secretaries have final responsibility for deciding which applications in their field get funded). In the study the authors focus particularly upon three things: How are reviewers (referees) chosen? How do the referees respond to various characteristics of the applicant other than their assessment of the quality of his (very rarely her!) application? And how does the final decision (that is, of the programme director) relate to the referees' assessments?

The study provides no ammunition to the critics of science. An effective combination of statistical and qualitative analysis is used to show, most

simply, that the best research gets funded. No evidence for an old boy network is uncovered, and findings suggest that referees are not much influenced by the status of the applicant's institutional affiliation, or by his seniority, or by whether he has or has not recently had a Foundation grant. They simply judge the application on its merits. Moreover, it seems to be these evaluations which do largely determine what gets funded. To be sure, if one tabulates 'successful' compared with 'unsuccessful' applications by extraneous variables such as those suggested above, one finds clear differences. But all of these vanish when reviewer's evaluation is introduced. Of course there are curiosities. A not infrequent one seems to be the eminent scientist whose application is rejected because it consists of little more than "I'm me—give me the money"! So the system seems to work as intended, and this study shows this to be so.

Nevertheless, as I am sure both authors and sponsors appreciate, the study will not silence the critics. It is not simply that the 'enemies' of science are unsusceptible to reason (though one wouldn't really expect them to succumb to a piece of applied social research). It is also because the study does not—perhaps could not—tackle all the criticisms made. For example, referees are required by the NSF to consider an application in the light of criteria reflecting the competence of the investigator, the scientific merit of the project, its relevance and utility, and its broad implications for American science and science education. The brief section of the report devoted to the problem of weighting these considerations against each other is all too sketchy, and will hardly persuade the cynic that much attention is paid to questions of practical applicability. One gets no feel for this at all. The critic can still claim that if the scientist from a poor southern college is 'fairly' treated, that is not enough. After all, positive discrimination in favour of the disadvantaged is an accepted feature of many areas of policy—why not in science? And even the favourably disposed but budget-conscious politician may feel uneasy in defending a government agency which makes "no systematic attempt to monitor the outcomes of research grants" (p. 158)—that is, to see how its money was spent.

But perhaps for the reviewer to attack a methodical piece of research for failing to resolve an essentially political dispute is not only naive, but inappropriately partisan. □

Stuart Blume is in the Department of Social Science and Administration, London School of Economics, University of London, UK.

Tribology textbook

Robert Schnurmann

History of Tribology. By D. Dowson. Pp. 677. (Longman: London and New York, 1978.) £35.

IN 1964, the Rt Hon. the Lord Bowden of Chesterfield as Minister of State for Education and Science had asked Mr H. Peter Jost to set up a working party to report on lubrication research and teaching requirements. The academic world was represented on this working party by Professor Dowson. Before its findings were published in February, 1966, the "Jost Committee" had asked the editor of the Oxford Dictionary for a "pithy name to summarise in one word the closely related subjects of Friction between Solid Bodies, Wear, and Lubrication, and he suggested 'Tribology'. Thus, HMSO published the report of the working party under the title *Lubrication (Tribology), Education and Research—A Report on the Present Position and Industry's Needs* which specifically mentioned "a conservative estimate" of the potential annual savings to British industry from improvements in education and research of "about £515 million, the biggest single item being savings in maintenance and replacement costs of £230 million".

This triggered off a spate of activity. Three centres of tribology were established in this country, two at Universities (Leeds and Swansea) and one in a government establishment (Risley). Tribology committees were formed, both national and international. Later, the Institution of Mechanical Engineers, which had already sponsored in 1937 a "General Discussion on Lubrication and Lubricants" and had followed this up twenty years later by another conference on "Lubrication and Wear" (organised jointly with the American Society of Mechanical Engineers), set up a Tribology Trust which has made annual awards (gold, silver and bronze medals) since 1972. Also in 1966, a news sheet called *Tribology News* was launched. A flood of publications and conferences was unleashed. 'Tribology' had arrived.

In 1969, Professor Dowson, who is the founder and director of the Institute of Tribology in the University of Leeds, decided to write a textbook on the subject with an opening chapter on its history. This 'chapter' grew to become the present book of 677 pages.

Looked at in a down to earth man-

ner, friction between solid bodies can either be unlubricated or lubricated, and in the latter case it depends on the operating conditions whether the film of lubricant is thick enough to sustain hydrodynamic lubrication—also called 'thick-film' lubrication—or whether one has to consider 'thin-film' lubrication—where the film of lubricant is not thick enough to allow of hydrodynamic flow. The word 'dry', in connection with unlubricated friction should only be used within inverted commas, because the surfaces of all solid bodies carry layers of one contaminant or another, either oxides or sulphides, or adsorbed or condensed matter. Even if these were removed by volatilisation in an ultra-high vacuum, recontamination of the surfaces, which were made 'naked' by volatilisation, would commence instantaneously.

The case is not analogous to the experiments of Agnes Pockels and Irving Langmuir, who scraped and thereby created a 'naked' water surface simultaneously with the application of an organic liquid. This is not the way in which Professor Dowson has set the scene. He would probably agree, however, that friction between solid bodies is a very complex phenomenon. The 'Jost Committee' rightly said that it is "multi-disciplinary". This has been very little heeded in the literature to which Professor Dowson refers. Even when he approaches the end of his journey through the ages and turns to his own subject of Bio-tribology, he uses the misleading word "cold-welding" for what is in fact the adhesive action of films so extremely thin that internal shear in these films is no longer possible (Lord Rayleigh's celebrated 'cup and saucer' experiment).

Throughout the book there is a great deal of confusion between adhesion and cohesion. The wide use of Johansson thickness gauges does certainly not support Professor Dowson's claim of the "cold-welding" of like metals, within the true meaning of the term 'welding' as an interpenetration of crystal grains. True, each gauge block is coated with lanolin before they are wrung together, but the process of wringing them together is necessary to make the residual film thin enough for strong adhesion. In this context, Amontons (p154) had coated his blocks of either copper, or iron, or lead, or wood with cart grease, not necessarily with "old pork fat". Amontons himself said "... enduits de vieux-oint". The other blocks "of similar materials and different sizes" which were loaded against the greased ones were ungreaed. Nowhere is there any mention of the significance of Amontons' Law as a wear criterion and its importance for the safe operation of machinery.

Neither is it Professor Dowson's thesis that it now seems that Sir William Hardy's introduction of the term 'boundary lubrication' was perhaps unfortunate, particularly if it is used to differentiate qualitatively between materials which function by the same mechanism though with marked quantitative differences, such as additives for so-called 'boundary lubricants' and additives for 'extreme pressure lubricants'. Similarly, referring to the chapter on wear, the important qualitative difference is between "mild wear" and "catastrophic wear" and not between "adhesive" and "abrasive wear".

Professor Dowson has included in his account 16 pages of history of the petroleum industry and of the origins of the major oil companies. John D. Rockefeller was indeed a dominant figure for the greater part of his life. He became a philanthropist at the end of his business career, and for many years, the Rockefeller Foundation did a great deal for the promotion of some outstanding scientific research by enabling promising young scientists to spend one year's leave of absence working with people, such as Lord Rutherford, Niels Bohr, Enrico Fermi, Otto Stern, to name but a few.

The petroleum industry discontinued some years ago the use of 'a-hole-in-a-pot' viscometers, such as the Redwood, or Saybolt, or Engler, and nowadays uses glass capillary viscometers. Incidentally, the c-g-s unit of kinematic viscosity is "one Stokes" and not "one stoke". The SAE classification of lubricating oils concerns their viscosity levels and has nothing to do with the dependence of their viscosity on temperature. Even Dean and Davis's 'viscosity index' (VI) only compares the viscosity-temperature dependence of a lubricating oil with that of two other lubricating oils, one of which is a Pennsylvanian oil which has a comparatively very flat viscosity-temperature curve, whereas the other one is a Gulf Coast oil which has a relatively very steep viscosity-temperature curve. Therefore, the VI-numbers assigned to these two types of comparison oils were arbitrarily taken as respectively 100 and 0. This was an important marketing asset in the days when only one of the American oil companies had a monopoly on Pennsylvanian Brightstock, which was added to petroleum distillation cuts and raffinates as a VI-improver. With the advent of mineral oil-soluble polymers, values of VI greater than 100 could be attained, and the Dean and Davis scale cannot be used here. For these values of VI above 100, W. A. Wright constructed a different VI-scale. The *Standard Methods of Testing Petroleum and its Products* of the Institute of Petroleum, the American Society for Testing Ma-

terials, and ISO have therefore given since 1966 "two procedures" for calculating VI, one for values between 0 and 100 and, if calculation should indicate a higher value, another one for values above 100.

Professor Dowson explains in his introductory chapter the chronological "Structure of the book", which is divided into 11 chapters, and his belief in "the wisdom of the 50-year rule", in connection with chapter 11 which has the heading: "Towards tribology: 1925—the present". The "50-year rule" is Professor Dowson's own variation on someone else's comment that "... a wise historian usually stops 20-30 years before his own time, because he cannot see the wood for the trees; the so-called thirty-year rule". The book has an appendix of 25 biographical sketches which were modelled on a book by one F. R. Archibald.

The book is remarkably free from misprints. There are the odd ones, but they are few and far between, although it is a little startling to find on p443, for instance, -11.8°C as the conversion of 0°F , when the right figure of -17.8°C is given 5 lines further down; that 121.1°F is given on p30 instead of 119.1°F as the conversion of 49.5°C is less easily explained; nor that 0.001 oz is said to be equal to 0.038 g, whichever kind of ounce it may be. But these are details. What is disturbing are the many mistakes both in the body of the text and in the "chronological tables".

Bourgeois chemist

Colin A. Russell

Gay-Lussac: Scientist and Bourgeois. By Maurice P. Crosland. Pp. 333. (Cambridge University Press: Cambridge, 1978.) £15.

DECEMBER, 1978, was a busy one for chemical historians. The bicentenary celebrations of Humphry Davy's birth were followed by similar festivities in honour of the French chemist, Joseph Louis Gay-Lussac. Both men were born in December, 1778, though in the country of his birth Davy is much better known. Although equally distinguished for his chemical work, Gay-Lussac has had to wait until his bicentenary for a full length biographical treatment in English. The urgent need for such a book has now been met by the timely appearance of this study by Professor Maurice Crosland.

To most modern chemists, Gay-Lussac is merely a name associated

This is not the time and place to list them all. A few randomly chosen examples must suffice here. Lord Rutherford discovered the atomic nucleus in 1911. In 1919 has great achievement was the 'splitting of the atom'. He coined the name 'proton' for the positive hydrogen ion because, before the discovery of the neutron in 1932, he considered protons and electrons to be the elementary particles of the atomic nucleus. C. T. R. Wilson's cloud chamber dates from 1897; and 1926, not 1924, is the date for Schrödinger's wave mechanics. The name of the discoverer of X-rays was Wilhelm Conrad Röntgen and not "Wilhelm von Röntgen". Sir William Hardy and Philip Bowden were members of the Senior Common Room of the same Cambridge College, Gonville and Caius. The chronological tables are not without their own mysteries. In the juxtaposition of "Political and social events", "General, technical and scientific developments", and "Tribology", one wonders why the "Tribology" column was chosen to record such events as Henry Deterding assuming "control of Royal Dutch Company", the "merger between Royal Dutch and Shell", and the move of the Timken Company to Canton, Ohio. □

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with two or at the most three notable achievements, the best known of which is his Law of Combining Volumes (1808), asserting a simple ratio between volumes of combining gases and their products (if gaseous). He has also been associated with the law connecting gaseous volume with temperature, though more frequently this is referred to as "Charles' Law"; in fact Gay-Lussac was the first to publish (1802), but his experiments were based on earlier work by J. A. C. Charles. Thirdly, chemists will recall the "Gay-Lussac tower" as part of the lead chamber process for making sulphuric acid, being a device for recovering nitrogen oxides from the effluent gas. The last Gay-Lussac tower that I know of in this country disappeared when the final lead chamber plant was dismantled about four years ago, so a further association with Gay-Lussac will be lost to future chemists.

However, as Crosland has been at pains to show, Gay-Lussac is worthy of commemoration for much more than this. His Law of Gaseous Volumes not only provided the foundation on which Avogadro and Cannizzaro were later to build their own theoretical structures; it seems that it confirmed to its

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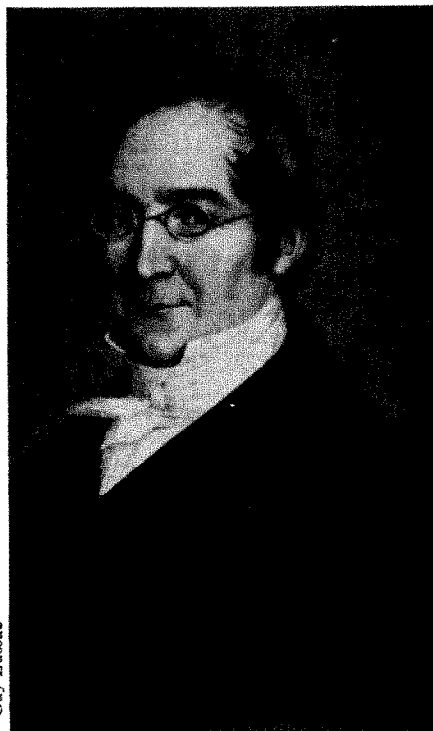
discoverer the fundamental importance of volume measurements—even greater than that of weights. This conviction was nurtured by Gay-Lussac's respect for his mentors Berthollet and Laplace, whose concern for establishing general laws in science proved more attractive to their young disciple than the mere multiplication of new compounds, rather in the old natural history tradition. Oddly enough, recognition of the Law seems to have been unexpected and came (as did many other generalisations in nineteenth-century chemistry) from studies of relatively obscure and complex phenomena, in this case the reaction between ammonia and boron trifluoride.

Associated with Gay-Lussac's work on gaseous volumes was his recognition of vapour densities as accessible and significant parameters for a compound. They were prominent in his researches on the new substance iodine. Gay-Lussac studied it extensively, concluding it to be an element similar to chlorine. He described and analysed hydrogen iodide, and discovered iodic acid, two chlorides of iodine, iodoethane, and so on. Closely related to this work was his research on cyanides. He obtained "prussic acid" in a pure state and prepared cyanogen for the first time. Wavering in his patriotic devotion to the memory of Lavoisier he recognised that oxygen need not always be present in acids, and to cover hydrogen halides and cyanide introduced the term "hydracid".

Nor was Gay-Lussac's concern with volumes confined to gases. In a largely industrial context he pioneered analytical methods for solutions. To have "introduced scientific rigour and established volumetric analysis as a branch of chemistry" was no mean achievement. By fitting a vertical side-tube to a graduated cylinder he devised a primitive burette that may still be occasionally encountered (though it soon turned out to be too fragile for the industrial purposes for which it was designed). He developed the pipette to something like its present form, introduced arsenic (III) as a standard for chlorimetry and made many further innovations. Other scientific work ranged from a study of fermentation to the development of lightning conductors. He studied meteorology and heat, and became one of the few Frenchmen to follow up the discovery of the Voltaic pile.

Gay-Lussac's chemical research is described in this book with verve and skill. The historian will delight in the clarity with which arguments are marshalled and the degree of historical analysis that enables his work to be seen within the context of contemporary science. Chemists will welcome a historical account that actually takes

the trouble to describe the chemistry being done—an increasing rarity these days. They may cavil at a nomenclature that sometimes is neither ancient nor modern and at the use of "react" as a transitive verb, but these are small blemishes in an account that takes seriously the scientific content of Gay-Lussac's lifework.



Gay-Lussac

For all his obvious sympathy and respect for Gay-Lussac, the author does not portray him as either saint or hero. Rather charmingly he includes in the index an entry "Gay-Lussac, Joseph Louis, mistakes of"! It has five references. Rightly he does not classify the rejection of atomism as a "mistake", though with hindsight it might be said to have been a pity. In Gay-Lussac's thinking there was a streak of pre-Comtean positivism later to find fuller expression in the uncompromising anti-atomism of Berthelot. This reluctance to go beyond experimental data taken in conjunction with Gay-Lussac's predilection for general laws suggests a tension which is never completely resolved.

Gay-Lussac is presented as a product of the middle classes determined at very least to maintain his social position. This resolve seems to have been a mainspring for his lifework, leading him to accept a plurality of academic posts, to develop his consultancy practice and to do all he could to improve his financial position. Two bar-charts illustrate that as his productivity (in terms of scientific papers) declined, so his salaried income rose to a peak. It is interesting to contemplate this recipient of a 50,000 Fr. salary insisting on patent rights for his

"Gay-Lussac tower" (and receiving 150,000 Fr. as his reward), while a self-taught plumber on Tyneside declined to patent the very device that made the Frenchman's invention a practical proposition—the "Glover tower". However some may wonder whether financial gain was all that being a "bourgeois" meant for Gay-Lussac's science. Did "bourgeois" attitudes colour the solution as well as the selection of scientific problems? Was his commitment to laws of nature (for instance) a reflection of middle-class convictions about the world? On these matters the author says little, probably for the good reason that there is little that can be said with any confidence, although he does allude to Gay-Lussac's ambivalent attitude to metrication, when "bourgeois" and "scientific" values were in conflict. He also gives a brief account of the chemist's activities as a Member of the Chamber of Deputies and his typically protectionist attitudes to industry and (especially) agriculture.

Gay-Lussac's institutional affiliations give his biography special interest for historians. He held posts in the Ecole Polytechnique, the Paris Faculty of Science, the Muséum d'Histoire Naturelle and the Ecole d'Application des Tabacs; he was a member of the National Institute and of innumerable official committees; he was joint editor of *Annales de chimie et de physique*; he became assay master of the Mint and was closely associated with the chemical plant of the great Saint-Gobain Company; above all, his early years were spent in the unofficial scientific society that met at Berthollet's home at Arceuil. Such a wealth of association gives the author an opportunity to place his subject in an institutional context of extraordinary breadth, and this he does remarkably well. Quite apart from the importance of Gay-Lussac himself, this biography gives a valuable insight into French scientific institutions of the early nineteenth century. Because of Gay-Lussac's specialist training in science, and because he subsequently earned his living through its practice, Crosland regards him as "one of the first generation of professional scientists". One wonders how useful a category of analysis this may be for an individual in early nineteenth century France, in view of the ambiguities lurking within the concept of 'professionalisation'. It may suggest false analogies between different countries or different periods. Only when such a term is rigorously defined does it become meaningful, and precisely at that moment it becomes redundant. If professionalisation is seen at the time to be a crucial issue then that of course is a different

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matter, but this does not seem to have been the case with Gay-Lussac.

The author declares "this biography will help give flesh and bones to one of the names found in science textbooks but, in so doing, it may also make a small contribution to a fuller understanding of the development of science". I believe that he is both over-sanguine and too modest. For me, at least, Gay-Lussac never emerges as a rounded human personality. Here and there we catch glimpses of a somewhat unbending autocrat tending to inspire respect rather than affection and himself under pressure from a financially ambitious wife. But in fact we learn little of his family life, his hobbies ("no great interest in the arts") or his religion (Enlightenment humanism). We do not even know the circumstances of his death. On the

other hand, for science itself the book makes more than the "small contribution" suggested by the author. It is a solid piece of research meticulously executed with tables, footnotes, bibliographies, appendix and indexes, and is a substantial addition to our understanding of the development of chemistry at an important stage of its history. At £15 one might have expected the publishers to allow the author more illustrations than the solitary portrait that appears as the frontispiece. But we must be grateful to Gay-Lussac for having contributed so much to chemical science and to Maurice Crosland for an illuminating and soundly-constructed essay in scientific biography. □

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Salt in perspective

Frank Greenaway

Neptune's Gift: A History of Common Salt. By R. P. Multhau. Pp. 325. (Johns Hopkins University Press: Baltimore and London, 1978.) £15.75.

"SALT was known to the Ancients". So began for fifty years the lectures on the halogens given by an imperishable Oxford don. Robert Multhau might well have begun his excellent book with the same words, conveying as they do the antiquity and continuity of his subject. A book which is primarily historical in intent, but concludes with a reference dated 1976 is, to say the least, unusual, but Multhau is an unusual man.

He has been a man of action and saw the world before entering on a career at the Museum of History and Technology at the Smithsonian Institution which made him so well known as a leading historian of chemistry. His reach is long and his grasp firm. This work on salt has all the fluency and density of significant detail which make his earlier work *The Origins of Chemistry* so valuable.

The book falls into two main sections: "The Age of Culinary Salt" and "The Era of Chemical Salt". The transition is set later than would seem obvious—that is, in the nineteenth rather than the seventeenth century. Multhau sees the food preservative role of salt as its more important feature well into the nineteenth century. Only with the expansion of the

chemical industry as a whole and the concomitant expansion of large-scale manufacturing industry and long distance bulk transport does he depict salt as a key chemical raw material.

Salt is a universal necessity. Without an adequate intake, direct or indirect, normal health cannot be maintained. Yet salt is not so widespread a natural substance as might seem from its invariable association with animal life. It has to be sought by human and animal alike; so since ancient times, it has had the scarcity value which makes for trading and taxation. A history of administration could be written around salt, "an ideal article for fiscal management". There was a universal demand for it, its supply could not be left to chance and it was a fairly easy commodity to control, as was found not only in Europe but also in China and elsewhere. The salt tax, the *gabelle*, was hated in France and seems to have been one of the precipitating causes of the French Revolution. However, one must not take too simple a view of the historic role of salt taxes, which came and went in a very irregular way in the United States, and were much confused in Indian history by the close relationship of salt with the important saltpetre industry and trade.

The extraction of salt is an exceptional example of the stability and continuous survival of techniques which, though crude and simple, were efficient enough to render modification unnecessary for centuries. There were several methods, some of which might be found in use within a short distance of each other: evaporation of seawater by fire or solar heat, surface mining, deep mining, leaching, and so on. Simple pottery pans for boiling down brine were so customary that their fragmentary remains are good evidence of the

existence of a salt industry. The largest salt industries, however, were eventually those based on deep-mined rock-salt, the demand being that of the new chemical industries. (One of several sections contributing to the wider history of technology is that dealing with drilling.)

Before salt entered its modern phase of being the raw material for sodium compounds and for chlorine, it was one of the most important means of sheer survival, being an invaluable food preservative. Lynn White has drawn attention to the importance of improved second-class protein supplies to the development of mediaeval technical vigour, but the argument can be continued: new technical improvements in salt manufacture improved the availability of meat and fish protein and so progressively helped to bring about the energising of Western Europe which ushered in what we can still usefully call Modern Times. But even greater expansion was to come as salt-mining benefited from the steam-engine and the development of iron machinery. Multhau uses an enlightening analogy: comparing early salt-mining to an agricultural pursuit because of its often seasonal character, and showing how it was transformed into a non-seasonal industry. By 1850 the alkali industry took up about a third of British salt production. In the United States, the new meat-packing industry called for a great deal, but industrialised food preservation was much less important elsewhere. Direct culinary use levelled off about 1850, using about half of the British total at that time. The industry expanded, however, and the second half of the book explains why. The nineteenth-century chemical industry had soda as one of its pillars with salt as the base, and the vast increase in the use of salt-derived chlorine in solvents, plastics, pesticides and the like. Multhau points out how in the case of salt, as with so many other substances, the dominant industry is the automobile industry, using the plastics and solvents, which need chlorine, in direct manufacture, but also using very large amounts of salt as such for road treatment in winter.

The book is well produced, particularly well documented, and equipped with valuable maps and statistical tables.

Salt was known to the ancients, but, in spite of its importance, is little considered today in the perennial discussion of the effects of technology. *Neptune's Gift* should do much to equip us to see it in its correct perspective. □

Frank Greenaway is Keeper of the Department of Chemistry at the Science Museum, London, UK.

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Shifting paradigms in chemistry

Maurice Crosland

Chemistry Transformed: The Paradigmatic Shift from Phlogiston to Oxygen. By H. Gilman McCann. Pp. 179. (Ablex: Norwood, New Jersey, 1978.) \$14.95.

THIS book is in many ways a pioneering study. Using T. S. Kuhn's idea of 'scientific revolutions' Dr McCann has spent more than ten years analysing one particular case. It is comparatively easy for the non-specialist to understand the main features of the so-called 'Chemical Revolution' of the eighteenth century, and a succession of philosophers of science have used it to illustrate their views about theories, falsification, and so on. This is the first time that a sociologist of science has tackled this historical question and we must respect the author's study of the relevant chemical journals and a wide range of secondary literature. This is no superficial survey but an attempt to achieve a better understanding of what really happened. The author also touches on the beginnings of the professionalisation of chemistry in France. With his great industry in statistical analysis of his data McCann deserves to succeed in saying something new and important. Certainly his approach opens up several interesting perspectives.

It is difficult to say exactly when Lavoisier's oxygen theory came to take the place of phlogiston. McCann marshals his data into periods: 1772-77 being the period during which Lavoisier was developing his theory and 1778-84 the years when Lavoisier was trying to justify his theory to others and meet their objections. 1785 was the year of Lavoisier's experiment on the composition of water which helped win over Berthollet, with other major chemists soon to follow. In Britain conversion to the new views was slower, and much ingenuity is exercised by the author to explain this. Rejecting nationalism as a possible ground, McCann believes that the British "chemical community" (if there was one) was more elderly than the French and therefore more conservative. Indeed the importance of age is claimed to be one of the great 'discoveries' of the quantitative sociological analysis used. But the author passes far too

easily from a plausible hypothesis about receptivity to one about innovation. It is claimed that what made Lavoisier unique was not (as has been assumed) his genius but his comparative youth, being only 29 at the time of his vital work which recognised the gain in weight of phosphorus on combustion. But what about other French chemists who were about the same age? One might mention the provincial chemists Cornette (28) or Opoix (27). Of course these did not have the advantage of living in Paris, of being members of the Academy, or of having a special interest in gases. But if age was so important in being open to change why is Lavoisier's exact contemporary Opoix remembered (if at all) as a late champion of the phlogiston theory?

Perhaps after all one needs to examine various other factors including



Antoine Lavoisier

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the arguments used on both sides, an aspect glossed over in the sociological treatment. The superiority of the oxygen theory is assumed and it is never mentioned that this 'correct' theory whose acceptance we are analysing included the (incorrect) 'caloric'. This is particularly serious in so far as it affects the very concept of a 'paradigm shift' mentioned in the subtitle, as it has been suggested that in some ways 'caloric' was phlogiston under another name. On p96 there is a remark about "the period of crisis as the French chemists solved problems that stymied the phlogistonists of Britain" but there is no hint that the phlogistonists provided problems such as that of 'heavy inflammable air' which were insoluble in terms of the oxygen theory until Cruickshank in 1801 showed that this was carbon monoxide.

The attempt to gain new knowledge

by a quantitative approach is worth serious examination and it is a pity that this brave beginning is open to a wide range of objections, of which one is the small size of the sample used. Thus in the period 1778–80 one new author could write 20% of the articles cited. The problem of the disproportionate number of articles written by Lavoisier himself is fully appreciated by the author, who presents data in alternative forms to meet this objection. There is also the problem of biographical detail for some of the lesser well known chemists which might affect the picture of the 'average chemist'. Finally it is

not clear to the reviewer how we can count "oxygen articles" in journals before the discovery of oxygen.

I am sure that a careful use of a quantitative approach may provide valuable new insights in understanding the development of science but it will be all the more useful if it is brought in as an additional tool rather than as an alternative. In counting heads let us not ignore what was in those heads.

Maurice Crosland is Director of the Unit for the History, Philosophy and Social Studies of Science at the University of Kent at Canterbury, UK.

Respecting aberrant figures

J. R. Ravetz

Man and Nature in the Renaissance. By A. G. Debus. Pp. 159. (Cambridge University Press: Cambridge and London, 1978.) Hardback £7.95; paperback £2.50.

THE history of the science of the period of the Renaissance is directly relevant to the practice of science today, for our present ideas of what constitutes genuine science, and what demarcates it from false imitations, were forged then. For a very long time it had been common for historians to take the evaluations of the seventeenth-century innovators as simple fact: those who adhered to older methodologies of world-views were simply reactionaries, fools or charlatans. Scientists who achieved great science (by our standards) in spite of such defects (as Kepler or Gilbert) were explained as being "strange mixtures of the old and the new".

In recent decades, there has been a thin but growing stream of research

that treats some of the aberrant figures with respect, both for their stature in their own times and for such 'positive' achievements as can be gleaned from their work. The most promising candidates for such re-evaluation are in the tradition of alchemically tinged practical chemistry, relating directly to metallurgy, medicine, and having implications for philosophy, religion and politics. The leading figures in that 'chemical philosophy' were Paracelsus and van Helmont; its leading interpreters Page, Rattansi and Debus.

Now this school has produced an introductory textbook by which students are shown how much more there was to the scientific revolution than the developments centring on the work of Galileo. In it we find brief and illuminating accounts of the 'chemical philosophy'. Otherwise, it is a competent survey of developments in science and in methods in the sixteenth and early seventeenth centuries. As it is read and used, we may expect that the 'chemical philosophy' will achieve a sort of respectability among those who learn or teach the history of science.

This is a very valuable achievement. A most urgent educational task is to show that the Galilean style of reductionist, abstracted research is not the uniquely true approach to a proper knowledge and control of nature. A modest and uncontroversial introduction to a broader conception of science

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will have a greater diffusion in teaching establishments than a strident polemical tract. But a price is paid for this very Fabian approach to intellectual reform; there is a loss, not so much of ideological militancy as of historical drama. Our sort of science was conceived and propagated by men with a prophetic mission. The issues in their debates ranged over all human values and experience. Different conceptions of reality were in open collision then, and ideological commitments (based on a fusion of political and theological concerns) could be as important as experimental facts in determining positions in the still immature natural sciences of the time. Of all this, we get only the most mild of hints. The received historical doctrine of the rise of modern science as an automatic consequence of the adoption of correct attitudes and methods, is here not so much challenged as modified at the fringe. But perhaps that is appropriate for a pioneering venture in textbook writing; and as a simple, lucid and accessible introduction to its subject, the book succeeds well. □

J. R. Ravetz is Reader in the History and Philosophy of Science at the University of Leeds, UK.



Van Helmont (left) and Paracelsus



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Wheelwrights of the heavens

A. W. Sleeswyk

Geared to the Stars: The Evolution of Planetariums, Orreries and Astronomical Clocks. By H. C. King, in collaboration with J. R. Milburn. Pp. 442. (Adam Hilger: Bristol, 1978.) £25.

IN twenty-one chapters and over 366 pages, illustrated with 333 diagrams, reproductions of engravings and photographs, sprinkled with 2,236 footnotes, this book gives an account of planetariums, telluriums, orreries, globe clocks, astrolabes, lunariums, astronomical clocks, equatoriums, satellitians, cometariums; all of them, to the modern eye mechanical analogue computers of the Solar System, but differing in the way the data are displayed. These models—often of great beauty—are now, as are all analogue computers, being superseded. At the end of the book the most modern planetarium is described, with the heavenly bodies represented by spots of light projected on the interior of a dome vaulting over the audience, and controlled by a digital mini-computer.

Astronomers, who often earned their keep as astrologers, have observed from the earliest times onward the recurrent motions of the heavens, and the bodies moving in them—Sun, Moon and planets. Whether or not awed by “the infinity of those spaces”, they determined with ever-increasing accuracy the different periods of these motions, whose ratios are never exactly expressible as finite quotients. Hence the challenge to clever craftsmen to produce close approximations by ingenious gearing.

The material is arranged in a roughly chronological order, starting with the little we know about Hipparchos and Archimedes in connection with astronomical models—the protohistory of the subject. Under-water archaeology brought to light the next item, the astonishing Antikythera mechanism, probably made by a Rhodian craftsman in 87 BC, lost in a shipwreck north-west of Crete in about 80 BC, and salvaged by sponge-fishers in the year 1900. Its 30-odd gears were conserved in lumps of calcareous accretion, which only in recent years became amenable to analysis, by X-rays and gamma-rays, on which D. J. Price in 1974 could base the definitive interpretation. The mechanism computed the synodic month and the lunar year and

the positions of Sun and Moon on the basis of Meton's (about 430 BC) approximation, in which 19 solar years correspond with 254 sidereal revolutions of the moon, or 235 lunations. As many later geared models, it made use of differential gearing.

Fourteen centuries passed before anything nearly as elaborate was made. The interim is filled by the Arabs with their geared astrolabes, and the Chinese with the waterwheel clock made by Chang Ssu-hsün exactly a millennium ago. Later, this clock was perfected by Su Sung, and lost as a link in the development of technology when the Chinese capital K'ai-feng fell to the Chin Tartars in 1126.

The first mechanical clocks date from the end of the thirteenth century, and the equal hour system gained universal acceptance only a century later. Not surprisingly, the first clocks about which we possess detailed information, those of the fourteenth century of Richard of Wallingford and Giovanni de' Dondi, were astronomical clocks. The positions of the Sun, the Moon and the five planets were displayed on separate dials in the latter clock, which is adequately discussed. Regrettably, the same cannot be said for the discussion of Richard of Wallingford's clock, reconstituted by J. D. North and published in final form in 1976: it is unnecessarily brief and marred by errors of detail. Astronomical clocks have been with us ever since those early examples were made.

Astronomical modelmaking received some powerful impulses from developments in cosmography. In 1543 Copernicus published his *De Revolutionibus*, replacing Ptolemy's geocentric Universe by the much more orderly heliocentric one. In 1609 appeared Kepler's *Astronomia Nova* on the properties of the elliptical planetary orbits, providing a new basis for the “equation of time”, that is, the difference between sundial time and mean solar time, indicated in many astronomical clocks. In the course of time the production of these display clocks diverged increasingly from scientific clockmaking, which aimed at greater accuracy, culminating in Harrison's marine chronometer of 1735.

The trail of astronomical clock making brings us back to the late sixteenth century, to the court of the landgrave William IV of Hesse-Cassel, who employed some masters of the craft, Eberhard Baldewein and Jost Bürgi; then to England in the seventeenth and eighteenth centuries, when Tompion, Mudge and Pinchbeck made clocks catering to the tastes of educated gentlemen of the time. Astronomical clocks of heroic complexity were made in Central Europe: for instance, by

Aurelius a San Daniele and David a San Cajetano at the royal court monastery at Vienna during the second half of the eighteenth century; above all, perhaps, the ‘World Machine’ finished by Ph. M. Hahn in 1791. Schwilgué made the astronomical clock in Strasbourg in 1842, more or less in this tradition. His workshop was the germ of the firm of Ungerer, makers of a most elaborate clock for the cathedral of Messina in 1933.

The line to be drawn between astronomical clocks and other astronomical displays is, of course, vanishingly thin. In France a development occurred of clock-driven armillary spheres, of which Antide Janvier finished an outstanding example in 1801. In England, orreries were mainly developed as aids in adult education by instructors such as James Ferguson (1710–76), who, as “Wheelwright of the Heavens” has a chapter devoted to his life and work. This development on the home-front of astronomy gave rise to transparent demonstrational orreries for large audiences, and, therefore, of maximum size—a development which ultimately led to the projection planetarium.

This work germinated a quarter of a century ago as a modest project to compile a list of extant models of the Solar System. In 1970 its scope expanded so as to include astronomical clocks and biographical notes. The result bears traces of this gestation. It is a somewhat uneven, but readable account of the elements of the early history of astronomy, horology and instrument making for the interested amateur, to whom the geometry of the various models is explained especially well. On the other hand, the historian of science will find this book useful too, because it has retained its original character of a catalogue; none other exists. He will recognise some obvious shortcomings—rather heavy reliance on secondary literature, occasional inaccuracies and the absence of a firm terminus to the references. As stated before, the findings of J. D. North—also referred to as J. and J. G. North—on the clock designed and executed by Richard of Wallingford—also referred to as Robert—are rather inaccurately reproduced. The book by Leopold of 1974 on the Reinhold astronomical table clock is not among the references, nor is Maurice's authoritative book on German geared clocks, although an article by the same author in the September issue of the *Connoisseur* of the same year (1976) is included.

These, however, are only minor flaws in this wide-ranging book, which offers much at a reasonable price. □

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Meticulous archaeological surveys

J. E. Wood

Megalithic Remains in Britain and Brittany. By A. Thom and A. S. Thom. Pp. 192. (Clarendon/Oxford University Press: Oxford, 1978.) £8.95.

THE senior author of this book, Alexander Thom, is a mechanical engineer and was Professor of Engineering Science at Oxford University for 16 years until his retirement in 1961. For 35 years or more he has devoted much of his life to the study of the many stone settings which are found in the wilder parts of Britain and in Brittany and date from the late Neolithic and Early Bronze Ages. Thom is not an archaeologist and his research is done, not with the spade, but with the steel tape and the theodolite. He has applied his scientific training to the precise measurement and surveying of these remains. His work was originally published in scientific papers, but at intervals he has collected his results together for publication in book form. *Megalithic Remains in Britain and Brittany* is the third book; the earlier ones, *Megalithic Sites in Britain* and *Megalithic Lunar Observatories*, were published in 1967 and 1971.

The new book was written jointly by Alexander Thom and his son Archibald S. Thom, who has been actively working with his father for many years. About 80% of it is based on a series of papers which were published in *Journal for the History of Astronomy* between 1971 and 1977. Some of the paragraphs and most of the diagrams are indeed identical to those previously published in the *Journal*, but apart from the convenience of having Thom's results in one volume, their work gains in significance when it can be studied as a whole. The book is particularly valuable for giving a comprehensive view of their work on the Carnac megaliths; this is the main topic and accounts for about one third of the text. They describe their surveys of the stone rows, give geometrical interpretations and postulate the existence of two prehistoric lunar observatories. Avebury, Stonehenge and the Ring of Brodgar are also analysed in detail, and there are short notes on several lesser sites. There is also a chapter on the geometry of cup and ring marks.

One's first reaction on reading the book is a feeling of admiration and

awe. The whole of its contents is the original work of one dedicated man and his team of assistants. Only someone who has walked along the stone rows of Carnac and Kermario, and seen the vast numbers of stones, the almost impenetrable thickets of gorse which cover the smaller ones and the clouds of voracious midges in midsummer, can appreciate the enormous amount of hard work and discomfort that underlies the Thoms' detailed plans of the alignments. They have raised the standards of archaeological survey work to a far higher level of accuracy than earlier surveyors of stone rows, and the archaeological community should be ever grateful.

In the course of his studies, Professor Thom arrived at three conclusions: (a) that the non-circular stone rings were not merely accidental departures from circles but were intentionally laid out in other geometrical figures, such as ellipses, flattened circles and egg-shapes; (b) that a precise unit of length, the Megalithic yard of 2.722 feet, was used in setting out the stone rows and circles, over the whole area from Shetland to Carnac and for the whole period of Megalithic buildings; and (c) that the function of many of the stone settings was astronomical, either indicating sightlines for observations of the azimuths of the rising and setting points of the Sun and Moon, or as calculators for predicting lunar eclipses.

Thom's conclusions have been hotly debated by archaeologists for more than a decade, and are not fully accepted at the present time. (Many archaeologists, for example, would accept the idea of a local unit of length, but not the universality of Thom's proposal.) In their book there is barely a hint of the controversy. The starting point is the assumed correctness of the elder Thom's ideas, and sites are individually interpreted in that light. There are no alternative explanations and in the minds of the authors there are no doubts.

Thus, the authors' approach is largely to disregard conventional archaeology, and they do not discuss whether their conclusions are reasonable in the light of current archaeological knowledge. The stone circle at Avebury, for example, is analysed by the Thoms as a compound ring consisting of six circular arcs drawn from six centres which are geometrically related to a 3, 4, 5 Pythagorean triangle. It is the most complicated ring in Great Britain. However, archaeologically there are grounds for believing Avebury is earlier than most rings in the British Isles, that early rings were geometrically simple, and that the more complicated rings are the later ones. The Thoms' interpretation does not fit

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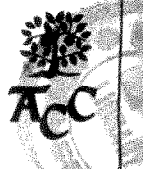
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comfortably into the archaeological framework, and archaeologists are bound to ask if simpler constructions are possible. The robustness of their interpretation, such as the possible range of values for the radii of the arcs, is not discussed.

Another example of their conclusions being in conflict with the archaeology is the supposed distant foresight at Stonehenge, called 'Peter's Mound', which was suggested as an indicator for midsummer sunrise. This looked unlikely archaeologically for several reasons, and indeed was shown by excavation in 1977 to be modern, though this fact does not appear in the book.

These criticisms are not meant to detract from the authors' work, for which I personally have considerable

admiration, but are intended to indicate the nature of the book. It is not a balanced account of megalithic astronomy and geometry, but a compilation of original work, the field reports of a team of meticulous surveyors, to which the serious student of the period will turn and which no specialist in the late Neolithic and Early Bronze Ages can ignore. Some of their interpretations may be proved incorrect in the course of time, but this is not particularly important; *Megalithic Remains in Britain and Brittany*, together with Alexander Thom's two earlier works, will be an invaluable source book for researchers for very many years. □

J. E. Wood is a Deputy-Director of the Admiralty Surface Weapons Establishment, Portsmouth, UK.

Star formation

Bart J. Bok

Stellar Formation. By V. C. Reddish. Pp. 287. (Pergamon: Oxford, 1978.) Hardback £15; paperback £7.50.

THIS is an interesting volume and I learned much from reading it with care. At the same time, I express deep regret that it came out in print effectively four years after the writing was finished. It covers adequately and thoughtfully the literature available to the end of 1974 and should therefore have been published by the end of 1975. It does not include the new results reported at IAU Symposium No. 75, which was held at Geneva in September, 1976 (International Astronomical Union Symposium No. 75, *Star Formation*, D. Reidel: Dordrecht, Netherlands, 1977), nor does it include researches that were discussed and analysed at the Tucson Colloquium of January, 1978, papers that are now in print (T. Gehrels, *Protostars and Planets*, University of Arizona Press: Tucson, 1979; *The Moon And The Planets*, 19, No. 2, 1978).

There are several very good chapters in the present volume. Chapter 3 deals in a masterly fashion with the determination of the initial luminosity function and the initial mass function. The three chapters that follow it are a bit on the meagre side, but the concluding chapter of the observational

half of the book gives a well-rounded review of the necessary environment for fostering star building from interstellar clouds. Professor Reddish defends strongly the point of view that star formation results almost exclusively as a by-product of the formation of H_2 molecules on very cold solid particles in interstellar clouds. I agree that this is a major contributing process, but there are regions in the heavens, notably in the Magellanic Clouds, in which cosmic dust particles and even H_2 molecules seem to have played less of a part than neutral atomic hydrogen.

Professor Reddish's independent and honest scholarly considerations have led him to visualise ahead of his time one major process contributing to star formation, but he ignores others. If I were asked to list my favourite processes for star formation, I would list five, which seem to be at work in our galaxy within 1,500 parsecs of the Sun. These are: (1) The formation of OB stars within large dark complexes such as the Ophiuchus Complex, the Taurus Complex, the Greater Orion Complex and the southern Gum Nebula. (2) The Elmegreen-Lada process according to which a large molecular cloud acts as a feedback for the continuous formation of OB stars in the vicinity of established H II regions and associated young OB groupings such as Messier 8 and Messier 17. (3) Supernovae and pulsars, whose high-energy charged particle emissions, along with the Lin-Shu spiral density waves, contribute materially to the condensing of interstellar clouds, thus leading to star formation. (4) Cloud-cloud collisions and interactions between passing clouds, which may further assist condensation

processes. (5) Collapsing large globules (Barnard objects), of low temperature and chockerbloc-full with CO (and thus with molecular hydrogen), provide a good basis for the building of less massive stars; two Herbig-Haro Objects have been found associated with a southern globule (see *Publ. Astron. Soc. Pacific*, October, 1978). Professor Reddish alludes vaguely to most of these processes, which were, however, not generally recognised as being as basically important in 1974-75 as they are today. I wish we might persuade him to add an additional volume to his present offering and bring us up to date.

The second half of the book presents the more theoretical aspects of star formation. It is an excellent and comprehensive treatment but it too is dated. He shows nicely that the physical properties of interstellar gas and dust are such that an interstellar cloud structure is inevitable. Chapter 9 is a most readable presentation of the Lin-Shu density wave theory of spiral formation and its effect on interstellar cloud evolution. There follow very fine chapters on cloud contraction, especially on fragmentation and its effects on the distribution of protostar masses. The chapter on the rate of star formation is regrettably brief and the concluding chapter on the evolution of galaxies did not stir this reviewer.

The book is nicely produced and the medium-priced paperback edition handles well. There are a few places in which a little more care in proof-reading would have been helpful; I noted that the word "asymmetry" is consistently misspelled (pp153, 164, 165). On page 171, there is a reference to (Roberts, 1969); one finds in the bibliography two such references—one to M. S. Roberts, the other to W. W. Roberts—the second one being the one that is indicated.

Because of its underlying unified personal approach of Professor Reddish, this is a book that should be read by everyone who works in the field of star formation. Once one accepts the obvious limitations, we can all learn from his treatment of star formation. This is a good book to assign to one's students, provided it is supplemented by the other volumes mentioned in my review; it is a good treatment for the beginning graduate student, and it should also appeal to advanced undergraduate students in the physical sciences. Professional physicists will find that this book has much to offer, as will informed amateur astronomers. □

Bart J. Bok is Emeritus Professor of Astronomy at the Steward Observatory, University of Arizona, Tucson, Arizona.

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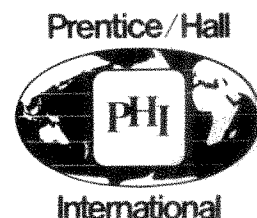
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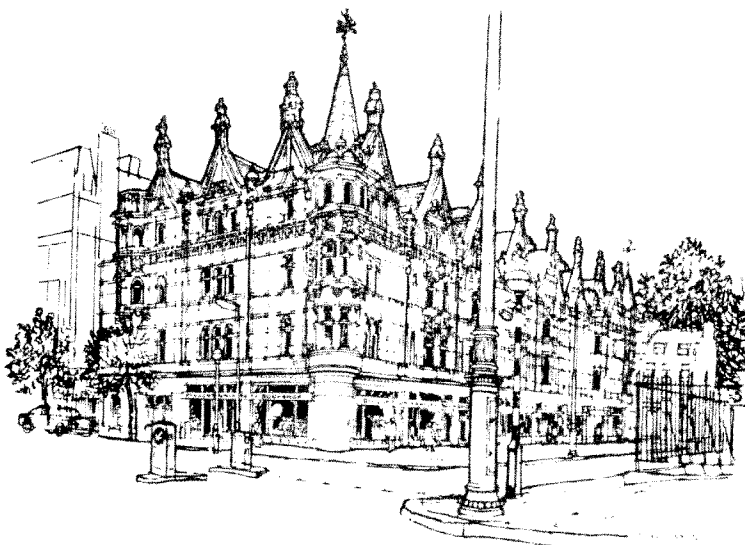
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Stellar evolution

R. J. Tayler

Stellar Evolution. Second edition. By A. J. Meadows. Pp. 171. (Pergamon: Oxford, 1978.) Hardback £7.50; paperback £2.50.

THE subject of stellar structure and evolution is believed to be the branch of astrophysics which is best understood. Because most parts of physics—such as nuclear physics, atomic physics, thermodynamics and statistical physics, electromagnetism and gravitation—are involved in determining the structure of a star, it is a very suitable topic in applied physics to introduce to undergraduates. It is also possible to present the subject to an audience with a slighter knowledge of physics and this is the concern of Professor Meadows.

When the first edition of this book was published in 1967, it received a warm welcome because of the manner in which it bridged the gap between very superficial discussions of stellar structure and evolution in books for the layman and complex mathematical texts for the professional astronomer. Twenty years earlier George Gamow's book *The Birth and Death of the Sun* had served a similar purpose, but by 1967 this was seriously out of date in detail. The first edition of Meadows' book gave a clear account of the observed properties of stars and the manner in which they are obtained. It then discussed the principles underlying the structure of stars, the source of the energy radiated from stellar surfaces, the manner in which this energy travels from the centre to the surface of a star, and the physical state of the matter inside stars. The remainder of the book was concerned with the evolution of stars from their birth as dense condensations in interstellar gas clouds, through the major part of their life as main sequence stars (the present state of the Sun) and subsequently as red giants, to their death as white dwarfs, possibly after a supernova explosion. It was then difficult to see how our knowledge of stellar evolution could be treated in a significantly clearer manner without the introduction of mathematical detail, which was completely absent from Meadows' book.

The first edition has now been revised to take account of developments in the subject. Most of the book has been photographically reproduced from the first edition and in most places it is possible to see where changes have been made because of a slight variation in

contrast of the type. In the first half of the book there are only a small number of detailed alterations but later there are much more substantial changes, particularly in chapters 6, 7 and 8. The book is essentially unchanged in length, the addition of new material being balanced by the removal of speculative or unimportant material.

Substantial changes in the new edition include the following. A section has been added on the solar neutrino experiment, the results of which are at present the major question mark against the standard view of stellar structure. There is a completely revised discussion of the evolution of low mass stars following the helium flash, the explosive onset of the conversion of helium into carbon. The major change concerns stellar old age and death. Shortly after the first edition appeared, pulsars were discovered and it was

soon realised that they are neutron stars. It now seems certain that neutron stars rather than white dwarfs are a normal end-product of a supernova explosion. In addition it has been realised that sufficiently massive stellar remnants will be neither white dwarfs nor neutron stars but will be black holes. Rather surprisingly in view of the great current interest in close binary stars and mass exchange between their components, the section on this topic has been slightly reduced in length, although there is a brief mention of the possible detection of a black hole in Cygnus X-1.

The revised edition of this book remains a very good non-mathematical introduction to stellar evolution and the paperback edition is very reasonably priced. □

R. J. Tayler is Professor of Astronomy at the University of Sussex, Brighton, UK.

Microcosm of astrophysics

F. Graham Smith

The Crab Nebula. By Simon Mitton. Pp. 194. (Faber and Faber: London; Scribners: Totowa, New Jersey, 1979.) £6.50; \$14.95.

THE 900th birthday of the Crab Nebula was celebrated by radio astronomers in July 1954. At that time it was almost the only celestial object, apart from the Sun, where a close link between optical and radio astronomy had emerged. In these days, when such links have appeared throughout astronomy, the Crab still has a unique importance, and annual celebrations of the supernova explosion would still be appropriate. Geoffrey Burbridge once said that astronomy could be divided into two parts: the astronomy of the Crab Nebula and the astronomy of everything else. There are indeed links between the Crab and almost everything else in astronomy, so that a discourse on the Crab can be used as an introduction, for example, to astronomy, stellar evolution, cosmology and interstellar matter; including the Crab Pulsar extends the list to the physics of condensed matter, high energy electrodynamics, superconductivity, and many other aspects of quantum physics.

This microcosm of astrophysics can therefore be used as a general introduction to astronomy, which should appeal to any scientist and which may persuade some that astronomy is a very useful means of education in basic physics. Popular books on astronomy

often do not succeed in presenting the more serious aspects of the subject, and their readers might not realise the important part which is played by astronomy in postgraduate physics education. Mitton is a physicist, and emphasises the basic physics throughout, but his book is nevertheless a popular book. He eschews mathematics, and uses not a single equation. The reader need only be moderately numerate, so that he can appreciate the large powers of ten involved in any branch of astrophysics.

The decision to exclude mathematics reduces the astronomy of the Crab to less than half of the total of astronomy; and further choices have been made. Mitton has chosen to make the book readable, straight through, for anyone with an undergraduate level of physics or astronomy. Others, with less expert knowledge, will enjoy at least the historical accounts of the supernova explosion, gleaned from Chinese and Japanese records of the "guest star", and the more recent history of discoveries—notably of the pulsar which remains the centre of the nebula, providing the powerhouse for the energy to keep the nebula shining. For the physicist, the exposition of the basic concepts of degeneracy in white dwarfs and neutron stars, and of superconductivity, will have a strong appeal, as they show most clearly the connection between terrestrial and celestial solid-state physics.

Mitton is an enthusiast. In conveying his enthusiasm he does sometimes overstep the mark, in such purple passages as his description of synchrotron radiation: "photon screeches from very energetic electrons as they wrap themselves round the ambient magnetic field". He refers to the giant pulses from the pulsar as "jumbo", he tells us

that the "youngest pulsars are the jazziest". He also obtrudes a view on space research: the X-ray observations were "made at truly vast public expense". Colourful presentation, but nothing incorrect. Colour is a strong advantage in places—for example, where the energy of one mosquito is given as a million million electron volts.

The astrophysics is sufficiently complete and correct that one can regret quite small omissions, such as the optical polarisation of the pulsar, and

wish to dispute the conclusions on the birthrate of neutron stars in the Galaxy. The serious reader is encouraged to consult a useful but rather short bibliography. This will certainly not be adequate for astrophysicists, and the index is also far too short. Nevertheless, the book is strongly recommended. □

F. Graham Smith is Director of the Royal Greenwich Observatory, and Visiting Professor of Astronomy at the University of Sussex, Brighton, UK.

Anatomy of a stellar murder

Donald D. Clayton

Stars: Their Birth, Life and Death. By I. S. Shklovskii. Pp. 442. (Freeman: San Francisco and London, 1978.) £10.70.

ARE you a practising astrophysicist unable to keep up with the literature, too fatigued by your own specialty? Are you worried that you are becoming too narrow while an exciting flood of astrophysical discovery flows all about you? Then this book is for you. Or are you a physicist wondering what the physics of modern astronomy is all about? This book is for you too. It is also for the amateur astronomer or the student who wants to hear the real issues of astrophysics from one who wrestles with them and who can also describe them without needless complication on the one hand or sterilising simplifications on the other.

Iosif Shklovskii writes incisively about the full range of what I would call stellar astrophysics. Not addressing extragalactic astronomy or cosmology on the one extreme, nor the origin and evolution of the Solar System at the other, Shklovskii concentrates on "stars, the most interesting and important objects in nature." But what stars! Not the serenely twinkling objects we see above us, but rather the collapsing, masering, exploding, bouncing, pulsing, bursting, spinning, extincting labour of birth and violence of death in the heavens. What makes his "serious popular book" (in the author's own words) so exciting is the closeness of many thrilling discoveries to Shklovskii's own career. His brilliance as a thinker and writer is evident throughout, not at all dampened by what must be a very good translation from the Russian, in which language the book was first published in 1975. This English edition has been updated by the author

to render it even more current, although currency is not the main objective.

Perhaps the book should have been called *Anatomy of a Stellar Murder*, so vividly described are the clues marking the birth, ageing, and death throes of its central character. The reader feels like a detective on the case. The book unashamedly emphasises the newer areas of astrophysics, especially the evidence of birth in molecular clouds and the phenomena associated with neutron stars. Shklovskii correctly credits radio astronomy with the leading role in these discoveries, perhaps somewhat under-representing the contemporary excitement in optical astronomy because it is older and, alas, just as taken for granted as a wife of several decades. It would have been Shklovskian to have paused to conjecture how

different it would all have been had we by accident evolved within an optically opaque atmosphere.

This book is quantitative, but not mathematical, displaying only important equations. A strong point is the presence of the many clarifying figures, which seem almost as abundant as the equations. Not designed as a textbook it would nonetheless fascinate final-year astronomy undergraduates or beginning research students, and could be supplemented by physics derivations in class. I would use it for such purposes without hesitation. In fact, the publisher should for this market consider issuing a separate companion paperback of derivations and problems at this level. This is, however, a personal book with the author as its main attraction. It has no references, except a general list at the end, but Shklovskii is liberal with text credits to pioneering researchers. The fact that half of these salutes are to Russians and to himself seems, in this case, justifiable by the author's own major role and by a general lack of awareness in the US of research in Russia.

The book is in four parts, having a total of 24 chapters. Some of the chapters, especially those on Cosmic Masers, Evolution of Protostars, the Crab Nebula, Neutron Stars and Pulsars, and X-ray Stars, are so fascinatingly written as to be pinnacles of astronomical exposition. Don't tell me that when it comes to writing serious popular books there is no difference between a great thinker and merely a great writer. Shklovskii is in the former league with Fred Hoyle and Carl Sagan. Only in the chapters on normal stars does Shklovskii lose the torch. In discussing white dwarves, for example, he seems to slip from his role as excited guide to that of teacher, beginning to wax pedagogical. Stellar structure and evolution suffers also in comparison, results of calculations tending to supplant discussions of physical mechanisms. Nucleosynthesis in stars is not described at all. A few controversial positions are taken up, such as that the admixture of overlying ³He excesses is the resolution of the solar neutrino problem. But maybe it is only in the eyes of this reviewer that this part of the book seems less stimulating than the mystery of the death screams of the heavenly moribund.

All in all, this is a wonderful addition to the astronomical literature, not only as an introduction to modern issues in stellar astrophysics but also as a chronicle of the way of thinking and the temperament of one of its most colourful practitioners. □

Donald D. Clayton is Professor of Astrophysics at Rice University, Houston, Texas.

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Origin of the Solar System

Fred L. Whipple

The Cosmogony of the Solar System. By Fred Hoyle. Pp. 168. (University College Cardiff Press/ Christopher Davies Publishers: Swansea, UK, 1979.) £7.95.

THIS book is not for the general public nor even for the serious amateur astronomer. The physics and the mathematical equations are critical to much of the discussion and, even though painstakingly explained, remain high hurdles for readers without advanced training in these subjects. On the other hand, Sir Fred writes down to research students and to his scientific peers. Had he published the book a decade or so ago as a scientific monograph with proper referencing to the host of contributors to the field, it would have been a useful summary of Sir Fred's imaginative thinking on the subject. It could have stimulated progress in understanding the complex processes that combined to form the Solar System and helped explain how we came into being. Today *The Cosmogony of the Solar System* serves chiefly as an historical document which can be seen to illustrate the formidable intricacy of the problem as measured in masses of data, in diverse physical processes and in time sequences of events.

Nearly everyone agrees on the gross simple picture. Collapse within a black interstellar cloud of dust and gas formed the Sun, the planets, the asteroids and the comets. Since 1950, when the comets were recognised as dirty ice balls, it has been apparent that temperature was the controlling factor in the evolution of the Solar System. The temperature decreased with increasing solar distance. Near the Sun only rocky material could freeze, so that within Jupiter's orbit dust aggregated to form planetesimals and the rocky terrestrial planets. Beyond Saturn icy cometesimals were the building blocks that formed Uranus, Neptune, probably Pluto, and some of the satellites of the great planets. Jupiter and the Sun are considered by some investigators as a double star system and in a certain sense Sir Fred agrees. This all happened 4.6 Myr ago. The details remain obscure and therein lie the controversies. Some of these complications are elaborated in *The Cosmogony of the Solar System*.

Sir Fred makes the basic assumption that the black cloud collapsed to the

radius of Mercury's present orbit, then expanded as a rotating disk star to the limits of the planetary system. His concept fails in at least three major respects, each of which is well documented in the literature and each of which negates most of his thesis. First, a collapsing cloud in the interstellar medium cannot collapse as a simple entity. Nature really does abhor a vacuum; the collapse brought with it an influx of outlying gas and dust, horribly complicating the situation. Secondly, Sir Fred utilises "magnetic brushes" from the rapidly rotating central mass to expand the dust. He assumes that the expanding gas will carry along solid particles that condense as the temperature falls with distance. In fact, the gas in the disk carries an outward radial pressure that reduces the effective central gravity field. The solid particles feel only the gravity field and try to revolve faster than the gas. Thus, the gas acts as a resisting medium causing the particles to spiral inward. No rocky material, therefore, should progress beyond Jupiter's orbit, contrary to the evidence from comets and probably Uranus, Neptune and some outer satellites. Thirdly, Sir Fred adopts the "minimum mass" concept which assumes that the only matter "wasted" from the system is gas that could not condense into the planets, asteroids and comets. The other strongest proponents of the "minimum mass" concept (to whom he does not refer) have long since abandoned the

idea. Also Sir Fred's ingenious removal process—interaction with interstellar clouds—is marginally possible at best. It fails completely if nature is really as wasteful as most investigators now believe.

The accounts of the aggregation of condensates into planets are lucid and Sir Fred presents clearly the problems of explaining rotations. His thinking here follows current conventional patterns except for Jupiter and Saturn. Great uncertainty, however, remains as to the processes which formed these giant planets. Some of the geophysical presentations will surely raise eyebrows and comments among specialists, particularly the concept that radioactive elements such as potassium, uranium and thorium were concentrated in the upper mantle of the Earth as a late time-sequence layering process rather than by magmatic differentiation. Related concepts of lunar evolution and structure are also novel and highly controversial.

As for Sir Fred's proposal that life originated in comets, I am charmed but not impressed by the picture of life forms developing in "warm little ponds," protected in their icy igloos from the cruel cold and near vacuum of open space and falling to primitive Earth at speeds exceeding eleven kilometres per second. □

Fred L. Whipple is Phillips Professor of Astronomy Emeritus at Harvard University, and Director Emeritus of the Smithsonian Astrophysical Observatory, Cambridge, Massachusetts.

Heroes on the climatic stage

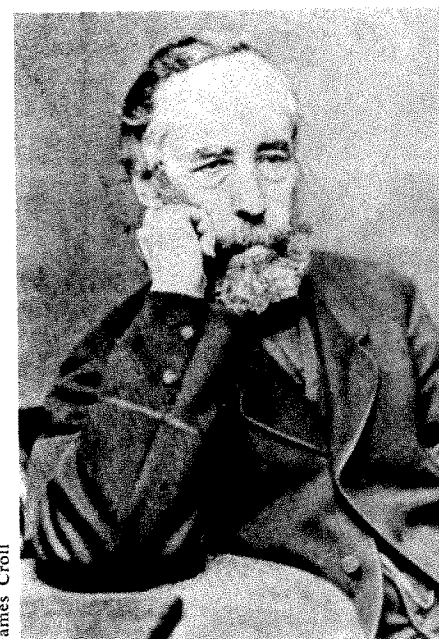
W. S. Broecker

Ice Ages: Solving the Mystery. By J. Imbrie and K. Palmer Imbrie. Pp. 224. (Enslow: Short Hills, New Jersey; Macmillan: London, 1979.) \$12.95; £6.95.

ANY hit parade of prominent problems in the Earth sciences would have to include among its top ten the cause of the glaciations which have plagued the Pleistocene. Despite the prominence of this problem relatively little has been written about the history of the endeavour to explain the instability in our planet's climate. The Imbries have not only plucked this plum but have done a superb job. They provide a very readable insight into not only the development of scientific thinking on this subject but also into the person-

alities of the key participants.

It is a book of heroes. Croll, from the Imbries' ancestral Scotland, receives top billing for his role in advancing the orbital theory Milankovitch, with whose name the orbital theory is



James Croll

normally linked, is portrayed as a patient and confident mathematician who devotes his life to calculating the geographical and temporal variations in monthly isolation. John Imbrie's fellow detectives in the current investigation aimed at tracking down evidence in support of the orbital theory are given strong supporting roles.

The Imbries' account shows how personality conflict and serendipity complicate the path towards scientific discovery. Rarely does the key evidence emerge from a carefully planned strategy. More often it emerges from arguments between parties holding divergent views or from measurements made for some other purpose. Perhaps one of the greatest contributions of books like this is to demonstrate to the public the necessity of supporting basic research. The carefully designed pert charts of government agencies rarely produce major discoveries. Creativity cannot be programmed.

The Imbries' account shows the incredible breadth of evidence and technique which have contributed to our understanding of climatic history. The stage is successively dominated by the field geologist observing grooves and erratics, the oceanographer looking

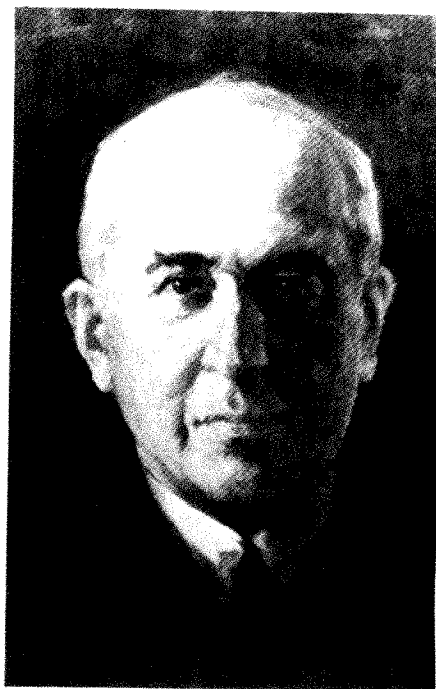
down the microscope at microfossils from deep sea cores, the isotope geochemist with his mass spectrometers and radioactivity detection systems, by the paleomagnetician, and yet again by the field geologist and by the micropaleontologist. Interwoven with field observation are mathematical calculations involving celestial mechanics, isolation dynamics, factor analyses and spectral analyses.

The Imbries are evangelists actively involved in the revival of a once prominent and then much maligned hypothesis. Although they wish the reader to believe that like continental drift the Milankovitch hypothesis is being swept back into fashion by a landslide of new evidence, this reviewer suspects that the CLIMAP *blitzkrieg* will be slowed by a wave of resistance. Like Agassiz, Imbrie and his colleagues will encounter the Bucklands and Lyells of modern meteorology. They are steeped in the dogma that the orbital changes are far too weak to be responsible for the awesome changes from a glacial to an interglacial world. Thus, rather than constituting the final report on solution of a major scientific problem, the Imbries' book will more likely prove to be a status report on a continuing investigation. Many new heroes will likely occupy the climatic stage before the mystery is solved.

The Imbries' enthusiasm for the isolation hypothesis in no way detracts, rather, it gives the book an excitement which allows it to transcend most treatments of the history of science. Their book does for climate what J. D. Watson's *The Double Helix* did for

molecular biology. It allows the reader for a few hours to participate in the intrigues, the mystery, and the excitement of scientific discovery. I heartily recommend this book not only to my fellow Earth scientists but also to their wives, children, neighbours and acquaintances who find it difficult to comprehend our preoccupation with the past. □

W. S. Broecker is Newberry Professor of Geology at Columbia University, and Associate Director of the Lamont-Doherty Geological Observatory, Palisades, New York.



Milan Milankovitch in 1943

Portrait by Paja Jovanovic



Portrait by Alfred Berthoud in the University of Neuchâtel

Scientific journalese

Joe Schwartz

Experiencing Science. By Jeremy Bernstein. Pp. 275. (Basic Books: New York; Burnett Books/André Deutsch: London, 1978.) £5.95.

JEREMY BERNSTEIN could be called the C. P. Snow of the 1960s. Writing with an authority and confidence characteristic of Snow's novels Bernstein communicated a literate insiders view of the high energy physics community during the easy money days of ten years ago. The present collection of articles, published from 1969 to 1978 reflects the strains induced in science by the financial stringencies of the seventies and a different social and political cli-

mate. Bernstein begins to show signs of struggling with the changed circumstances, but the results are not as satisfying as his earlier work.

His evaluation of Nora Sayre's book on Rosalind Franklin suffers from an insensitivity to the very real difficulties women experience in a male dominated profession. By concentrating on the facts of the case as presented in Robert Olby's book, *The Path to the Double Helix*, Bernstein chooses to avoid the underlying issues of patriarchy and male domination that prompted Ms Sayre to write her book in the first place. This attitude intervenes to weaken an interesting attempt to describe Bernstein's personal struggle to understand Goedel's Theorem (there exist true mathematical statements that are unprovable). Bernstein introduces a lady friend, K., with whom he will discuss his difficulties. Unfortunately all she does is cook him a meal, listen to a lecture and go to bed. She has no lines of her own. K.

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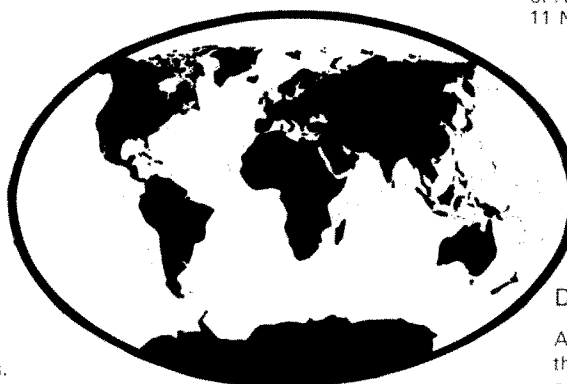
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could have been a mathematician and Bernstein the listener which would have given him a chance to show more directly where his understanding breaks down. Instead we end up hearing a lecture rather than seeing the kind of effort it takes to understand the complexities of metamathematics.

But the long piece on US physicist I. I. Rabi is a gem. This is Bernstein at his best. We have a fascinating story of the shift of the centre of gravity of physics research from Europe to the US in the inter-War years as seen through the eyes of Rabi, a Nobel Prizewinner in 1944 for work on molecular beams. Bernstein succeeds in presenting a fresh account of the most romantic period in the history of physics. Here is Rabi criticising the

present division of labour between theorists and experimentalists in physics: "You do an experiment because your own philosophy makes you want to know the result. It's too hard, and life is too short, to spend your life doing something because someone else has said it's important. You must feel the thing yourself—feel that it will change your outlook and your way of life. You must bring it back to the human condition, the human expression—much closer to what the artist is supposed to feel." In the present period there are not many physicists who can experience this kind of emotional commitment to their research. □

Joe Schwartz is on leave from the City University of New York.

Gravitation and the expansion of the Earth

T. C. Van Flandern

Cosmology and Geophysics. By P. S. Wesson. Pp. 240. (Adam Hilger: Bristol, UK, 1978.) £17.50.

It is not immediately evident that the book's two subjects, cosmology and geophysics, have very much to do with each other. The primary connection is through expansion of the Earth, a predicted consequence of several recently proposed cosmological theories and a favourite theme of the author. If the Earth does indeed expand with time, this provides a driving mechanism for continental drift, a simple understanding of how the Earth's surface became partitioned into continents and ocean basins, and in general a deeper understanding of the origin and evolution of the surface features of planetary bodies. The evidence that the Earth has, in fact, been expanding in the past is still debated; but the author presents a credible case for his interpretation. The evidence ranges from the suggestion that all of the continents would fit together nicely on a globe having one-fourth the Earth's present surface area, to palaeomagnetic determinations of the Permian radius, and arguments from logic: How could Antarctica become almost surrounded by an ocean ridge, with which it was once supposedly in contact, unless the globe has expanded?

Two mechanisms for producing the

expansion are offered, and this leads us immediately into cosmology. The first is that the universal gravitational constant may be decreasing; the second is an expansion of all macroscopic bodies and the space they occupy relative to the size of the atomic particles which comprise them, at the same rate as the observed large scale expansion of the Universe. These two mechanisms can now be unified into a single theory, which has been the direction that most theoretical work has taken since this book was written.

The book opens with an excellent survey of variable-*G* cosmologies and what motivated them; Milne, Dirac, Jordan, Gilbert, Brans and Dicke, and Hoyle and Narlikar are all reviewed. There follows a general discussion of post-Einstein gravitational theories, of the various attempts to incorporate Mach's Principle of Inertia into these theories, of space variability of the gravitational constant, vacuum elasticity, possible anisotropy of gravitation and the limits placed by the Eötvös experiment, and additional related topics. There is then an entire chapter devoted to the Eddington numbers, which form the basis of the Large Numbers Hypothesis and the motivation for the original 1937 proposal of Dirac that gravitation must be weakening with time. This chapter also discusses experimental limits on the variations of other fundamental quantities, such as the fine structure constant, and discusses the physical and astrophysical consequences of the proposed changes in fundamental constants of physics.

This summarises the first half of the book. It is a truly excellent review with a very complete bibliography. Moreover I was pleased to see explicit mention of the assumption underlying this entire discussion, that there may be as yet undiscovered laws of physics. This

has always seemed to me an obvious point as long as our understanding of the true nature of phenomena such as gravitation is so limited (Why do masses attract only? Is there a quantum of gravitation? Is shielding possible?); yet it is an assumption few physicists seem to make. The only criticism to this point is that reading would not be easy going for the novice, as specialised terms are sometimes introduced with only very sketchy definitions and no real attempt to provide understanding of the concepts. There is also confusion on p42 about just what information ancient eclipses provide.

The second half of the book deals with experimental evidence on changes in gravitation and Earth expansion, and an attempted reconciliation of such concepts with Relativity Theory. The author very much favours the idea that the Earth has nearly doubled its radius in its lifetime, rather than the more modest expansion of perhaps 10% allowed by a weakening of gravity. I think he has overlooked a valuable argument. We know that the Moon must have been quite close to the Earth in the past; the best estimates place the closest approach at about 3×10^8 yr ago. Tidal friction under these circumstances must have been enormous, and surely must have produced considerable internal heating and expansion of the Earth. Moreover, if the Earth had a much smaller radius than at present for most of this early period, this would help explain why the tidal coupling coefficient has averaged less than half its present value since the Moon was close. Whether the expansion continues today would then depend on whether gravitation is weakening; but the bulk of the expansion would have taken place early in the Earth's history. Moreover, it is important to keep in mind that expansion from any cause would surely occur in discrete events rather than continuously, as radial strain, like horizontal strain, would have to reach a threshold before the Earth would yield. It is interesting to note the recent discovery of a huge surface fissure on Venus, comparable to the largest known on Mars. Taken with the large Van de Graff lunar farside surface depression, it is probably not premature to infer that expansion may indeed be a regular feature of planetary evolution. If so, Wesson deserves much of the credit for advocating the idea while it was still unpopular, and for placing it in a reasonable theoretical and observational perspective. □

T. C. Van Flandern is Chief of the Celestial Mechanics Branch of the Nautical Almanac Office, US Naval Observatory, Washington, DC.

Key to the mysteries

John M. Charap

Einstein's Universe. By N. Calder. Pp.147. (Viking: New York; BBC Publications: London, 1979.) \$10; £6.25.

SOME of the very best popularisations of science to have been published in the past decade have had their origin in BBC television productions: these have included Nigel Calder's own *Violent Universe* and *The Key to the Universe*. And now comes, in celebration of the centenary of Albert Einstein, *Einstein's Universe*. As in his earlier books, Mr Calder has drawn upon the resources deployed by the BBC in their original telecast form and has made a brave effort to meet the challenge inherent in explaining the intellectual achievements of fundamental physics without either scandalising the experts, boring the lay readers for whom he writes, or still worse misleading them. That there is a very significant desire for information, a craving for a simple route to understanding, a key to the mysteries, cannot be doubted. Any physicist who has been button-holed at a cocktail party and asked to 'explain' quasars or lasers, quarks or black holes, can testify to that. But he can also give evidence of the difficulties: how to explain modern physics to an intelligent, inquisitive but non-numerate questioner whose understanding of classical physics is likely to be non-existent. It's not just making the progression from nineteenth-century science to quantum physics and relativity, but rather going from Aristotle to Einstein leapfrogging Newton.

The millions who watched the television broadcasts may have been satisfactorily distracted by the glamour of the hardware, or entertained by Peter Ustinov's German accent, or fascinated by the contrasting personalities of the contributing scientists. And in his earlier book Mr Calder allowed himself some glamorous packaging, beautiful colour photographs, coffee-table format. This time his approach is more austere: indeed he seems to have taken his task to "make relativity plain" almost literally and *Einstein's Universe* has few illustrations, none in colour. I would even question whether the photographs chosen actually illustrate anything very significant to the purpose of the book, but am grateful for two of Einstein

himself, neither of them over-familiar. Einstein thought visually, as do many creative scientists, and the book would have been better, and the author's task easier, if there had been more and better diagrams. One of the few which actually relate directly to the text (p47) is annoyingly wrong, and even if it were corrected it would still be less informative than might have been possible with better draftsmanship and captioning.

The sequence of topics chosen by Mr Calder to lead the reader right up to today's research is unusual and I think effective. Instead of the conventional historical approach, from special relativity to general, the order is reversed. Moving swiftly from the transverse Doppler effect to $E=mc^2$ and then to gravitation, the basic



Einstein in 1916

Radio Times Picture Library

themes are blocked in. At the same time opportunity is taken whenever it is presented to make reference to the most recent experimental or observational confirmation of the consequences of the ideas. So it is that very early in the book the evidence from astronomy for the existence of black holes, as collapsed stars (Cygnus X-1) or as galactic nuclei (M 87) is woven into the argument. Einstein's contributions in fields other than relativity are also brought in to further the main development of ideas. So his notion of stimulated emission leads through atomic clocks to the gravitational effect on clocks and on time. The 'time-shells', successive zones in which clocks run at greater rates as one goes further from a massive object are skilfully introduced and lead without strain to the gravitational red-shift.

The equivalence principle, so central

to Einstein's own way of thinking is ably introduced in a chapter on weightlessness: the advantage an author who can draw on the reader's experience, if only vicarious, of free-fall in a space-capsule is very substantial and is well-exploited.

The effect of gravity on time is then used as a bridge to the effect of gravity on space. The over-worked, and to my mind misleading analogy with the distortion of a rubber sheet by heavy objects is used, but more telling is the difference in surface area of a sphere enclosing a mass from the $4\pi r^2$ of Euclidean geometry—the surface of the Sun has a 'missing' area roughly the area of the USA.

The famous classic tests of general relativity, and their modern counterparts are all described; not just the perihelion advance of Mercury, but the periastron advance of the binary Pulsar 1913+16. The book went to press when it was only possible to speculate that that same binary pulsar might give evidence through Joseph Taylor's careful and continuing observations for gravitational radiation; that confirmation was announced last December. The deflection of star-light by the Sun observed by Eddington's 1919 expedition is explained, and the counterpart annual observation of the same effect on 3C 279 also described. The so-called 'fourth classic test', the radar echo delay, is here too.

I think the treatment of the observational consequences of general relativity and the present status of such observations, is very good indeed. I'm less happy, however, with the discussion of special relativity, which is often made less than clear by the author's attempts to verbalise the mathematics. However, the reasoning for the apparent rotation of a fast-moving object (and hence its foreshortening) is really very well handled, and overall I think it fair to say that many much fuller accounts of special relativity, in popular or technical treatments, succeed far less ably in getting the basic points across.

The book's last main theme is an exploration of relativistic cosmology; inevitably and quite properly this leads into more speculative areas. In the course of the book we have already had our attention directed to quantum effects, to Hawking's quantum radiation of black holes, to gravitons (there's one sentence I shall try to remember for its explanation of the non-linear effects of quantum gravity: "This incest among the gravitons produces the curvature of space"). And now we meet supergravity and gravitinos, twistors and foamy space.

I enjoyed reading the book. I was occasionally irritated by infelicities like "At its birth light accelerates infinitely

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rapidly" or "Even more fundamental than the speed of light is energy". (What exactly can that mean?). More serious a criticism is the worry that in spite of his best efforts the reader may fail to grasp the logic of the theory rather than be convinced by evidence in its favour. But Mr Calder has made

a valuable contribution to the demystification of relativity, and one which is most worthy to appear in this year of Einstein's centenary. □

John Charap is Professor of Theoretical Physics at Queen Mary College, University of London, UK.

Einstein's charisma

W. B. Bonnor

Einstein: A Centenary Volume. Edited by A. P. French. Pp. 332. (Heinemann: London; Harvard University Press: Cambridge, Massachusetts, 1979.) £10.50; \$25.

THE personality of an intellectual giant like Einstein exerts special fascination. How far is his genius apparent in everyday behaviour? Does he have special insight into non-scientific matters? How does he get on with his ordinary fellows? It is fortunate from this point of view that Einstein's life and opinions have been so well documented: indeed so much has been written about him that it is quite hard to say anything new.

Einstein: A Centenary Volume was written at the instigation of the International Commission on Physics Education, bearing especially in mind teachers and students of physics at secondary and undergraduate levels. It consists of four parts. Part I contains 18 reminiscences and reflections on Einstein, half of them reprinted from elsewhere. Part II is the main part of the book containing thirteen articles on Einstein's scientific work and political and social ideas. Twelve of these seem to be new, and specially commissioned for the book. Part III contains a number of letters from and to Einstein: it is not made clear which (if any) of these are being published for the first time. In Part IV a number of Einstein's writings are reprinted and a pitifully inadequate bibliography is given. The book contains many interesting photographs and drawings and is lavishly produced. However, it certainly does not offer a rounded account of Einstein's work or his personality, and the statement on the dust-cover that the book is "a total assessment of the life and works of one of the intellectual giants of our age" is unjustified. To mention one important omission, there is no description of

Einstein's work on cosmology.

In the commissioned essays there are several worthy of serious attention. Silvio Bergia gives a scholarly history of the discovery of special relativity, starting from the relativity of Newtonian physics. His article contains a careful judgement of the parts played in the discovery by Lorentz, Poincaré and Einstein, with special reference to the assessment of E. T. Whittaker, who called special relativity "the theory of Poincaré and Lorentz". Bergia concludes that Whittaker's view cannot be sustained, and that the credit usually given to Einstein is justified. There are competent accounts of Einstein and the quantum theory by Martin J. Klein, and of the observational verification of general relativity by A. P. French.

The highlight of the book for me was the article, "Relativity theory and gravitation", by Hermann Bondi. Bondi presents a derivation of general relativity which differs from that of Einstein, and which he attributes to Fock. He starts with the notion of gravitation as a tidal force only, so that a uniform gravitational field is a zero gravitational field; and he discusses the nature of mass, describing the three different sorts and commenting on the non-existence of negative mass (of the reason for which relativity gives no clue). A somewhat

sophisticated treatment of Newtonian gravitation theory is set up for later generalisation. Gravitation is introduced into special relativity by a thought-experiment using the gravitational red shift. A simple introduction to non-euclidean geometry leads painlessly to the Riemann tensor and the equation of geodesic deviation, which is neatly tied up with the Newtonian treatment and the tidal approach. It is beautifully done and should be read by all those interested in general relativity. One feels that Einstein, with his eagerness to explain relativity to the man in the street, would have enjoyed this article.

Among the essays on social and political matters are "Einstein and world affairs" by A. P. French and "Einstein and Zionism" by Gerald E. Tauber. Here Einstein emerges as the idealist, working to put the world right, but almost as much at sea as the rest of us. He espouses utopian ideas such as world government, disarmament and democratic communism; he writes to Roosevelt in 1939 calling attention to the possibility of building an atomic bomb, but later he is horrified when one is dropped; he writes to the President of the USSR Academy of Sciences asking for contributions to a book *One World or None*, and gets a dusty answer. All this was well meant, of course, but how naive and ineffective compared with the majesty of his achievements in physics!

In this field there emerges too a side to Einstein somewhat at variance with the saintliness which his reputation usually enjoys. Although he wrote that he passionately hated "all the loathsome nonsense that goes by the name of patriotism", yet, unable to detach himself emotionally from his Jewish origin, he became a Zionist. He went on fund-raising tours and let himself "be exhibited like a prize ox". Even before the Nazi era, Einstein disliked the Germans and after World War II he developed a pathological hatred of them, calling them mass-murderers. This attitude contrasts ill with that of his Jewish colleague Max Born who showed a gentle forgiveness for Germany after World War II.

Although, as we ought to expect, Einstein had his weaknesses, the book brings out many of his fine qualities. One is his modesty and unpretentiousness—illustrated by the story of how, at a dinner in his honour at which a too fulsome speech was being made about him, he whispered to his neighbour, referring to himself, "But he doesn't wear any socks!". □

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Einstein broadcasting at Balboa Park, San Diego

Camera Press

Special relativity

Bernard F. Schutz

Special Relativity: The Foundation of Macroscopic Physics. By W. G. Dixon. Pp. 261. (Cambridge University Press: Cambridge and London, 1978.) £14.50.

DON'T let the title deceive you: this is not another introduction to special relativity. This is a book for those of us, working physicists or advanced students, who know a few things about relativity, thermodynamics, and electrodynamics but have not bothered to examine too closely how all these things fit together and on what sorts of assumptions they are based. Graham Dixon's aim is to isolate these assumptions and to derive from them the macroscopic laws we know so well, making full use of the spacetime viewpoint. On the whole he succeeds very well, and the book should prove rewarding to the careful and patient reader.

The first chapter studies the foundations of relativity (special and Galilean). Dixon discards the usual postulates of relativity (that the laws of physics are the same in all frames) and of the invariance of the speed of light (or for Galilean relativity, the usual velocity-addition law) in favour of two other postulates. One is the Principle of Uniformity, that in a given frame, space and time are homogeneous and space is isotropic. The other is the Extended Principle of Inertia, which not only extends the usual principle of uniform motion in the absence of forces to macroscopic bodies but also postulates that a clock's rate may depend on its speed but not on its position or direction of motion. From these two principles and a careful discussion of the nature of measurements, Dixon arrives essentially at the conclusion that the transformation law between the preferred reference frames picked out by the principles must be linear and must preserve the line element $(\delta x)^2 + (\delta y)^2 + (\delta z)^2 - C(\delta t)^2$, for some constant C . The case $C > 0$ is special relativity, and $C < 0$ is excluded by a rather weak argument which would have been unconvincing before the days of particle accelerators. The limiting case, $|C| \rightarrow \infty$, is actually treated separately and is, of course, Newtonian theory. (The case $C=0$ is not discussed but is in fact easy to exclude.) Later Dixon remarks that it seems as if we have got something—a fundamental constant with the dimensions of velocity—from postulates

which seem to have no scale, and that this velocity may originate in the fundamental velocity $\hbar/e^2$ by way of quantum-mechanical nature of the measuring rods one uses. Although there may be something in this, a more direct explanation seems to me to be that his postulates already imply the consistency of using universal units for time and distance, and the only question is what value C has in these units. As the postulates do not restrict C , they do not themselves give a universal velocity. This restriction comes from outside the postulates, by adding that the cases $C < 0$, $C=0$, and $|C|=\infty$ are contrary to experiment. But this is a minor quarrel, which should not obscure the fact that this chapter makes the useful point that special relativity and Newtonian physics need not be derived from such disparate postulates as one usually finds in elementary treatments.

This unity of treatment of relativistic and Newtonian theories is developed further in chapter 2 and then carried through the whole book. Chapter 2 begins by developing affine tensor calculus. The point of view is algebraic rather than geometric, with tensors defined by their transformation properties rather than as linear functions of vectors. Integration on arbitrary surfaces in an affine space E_n is discussed, with a full and clear treatment of antisymmetric tensors, volume elements, orientation and Stokes' theorem. Lie derivatives don't appear here but are introduced very briefly in chapter 4. In order to treat relativity and Newtonian theory in a unified manner, their respective spacetimes are discussed and fundamental tensors defined: the metric for relativity and both a 3-metric and the universal time co-vector in Newtonian theory. (Throughout the book Dixon uses 'Newtonian' to mean a gravity-free spacetime, which others often call Galilean.) A particularly nice feature is a demonstration of how to contract the Lorentz group to get the Galilean group.

There follows a discussion of particle and continuum dynamics, conservation laws, and an especially clear discussion of the effects of forces on mass and energy in both theories. One can also find here in chapter 3 a careful treatment of the 'Newtonian limit', which deserves a careful treatment where mass and energy are concerned.

In chapter 4, Dixon picks up his assertion that the laws of physics are seen most simply from a relativistic point of view by showing how much of thermodynamics and phenomenological hydrodynamics can be derived from exceedingly simple assumptions. His starting point is the entropy-flux four-vector s^μ , which by the second law of

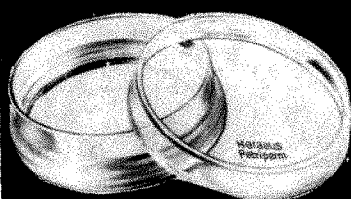
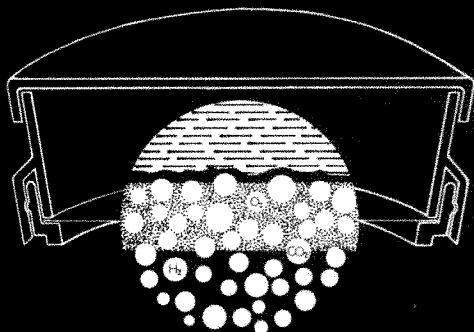
thermodynamics satisfies $s^\mu_{;\mu} \geq 0$, the equality obtaining in equilibrium (but not only there). He defines a simple fluid as one in which s^μ is a function only of the stress-energy tensor and the particle-flux vector, at least in and near equilibrium. From this starting point and using Lorentz invariance he shows that, in equilibrium, s^μ is parallel to the flux vector and the stress-energy tensor is $\text{diag}(\mu, p, p, p)$ in the reference frame defined by s^μ . Near equilibrium the usual phenomenological laws governing heat conduction, bulk viscosity, and shear viscosity come out as first-order corrections, whereas second-order corrections give the time-derivatives in the diffusion laws which are necessary to limit the diffusion to speeds less than that of light. The treatment of the second-order terms seems to follow that of W. Israel (*Ann. Phys. New York*, **100**, 310–31, 1976) to which Dixon refers, but Israel's "cross-coupling coefficients" don't appear here and it is unfortunate that Dixon does not comment on this. It is also sad that Dixon does not refer earlier in this chapter to the often-overlooked work of van Dantzig in the 1930s, who seems first to have introduced "thermasy" ($T\tau$, where τ is proper time), a variable often used by Dixon as a parameter along the world lines of his fluid.

The final chapter gives, for fluids which interact with electromagnetic fields, a treatment largely parallel to the one above, except that here of course there is no point taking any Newtonian limits. It contains a good discussion of the Abraham and Minkowski stress-energy tensors in view of the arbitrariness of any separation between 'fluid' and 'field' for a polarisable fluid. The second-order terms now include other phenomenological terms associated with the Debye and Langevin effects.

Throughout the book Dixon maintains a clear and methodical style. The book is well-produced and admirably free of misprints, the worst of which I found was a statement (p87) that no timelike vector can be expressed as a linear combination of spacelike vectors, in which it is clear from the context that "orthogonal" is intended before "spacelike". It assumes a fair degree of sophistication of the reader, and I would not send a student to it who did not already know special relativity and relativistic thermodynamics from other sources. But I would recommend it strongly for libraries and for anyone who wants to bring a little more order to that jumbled mixture of theory and phenomenology that most of us carry around in our brains. □

Bernard F. Schutz is Reader in General Relativity at University College, Cardiff, UK.

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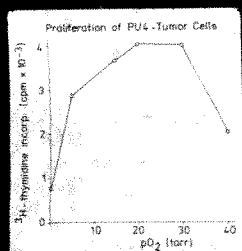
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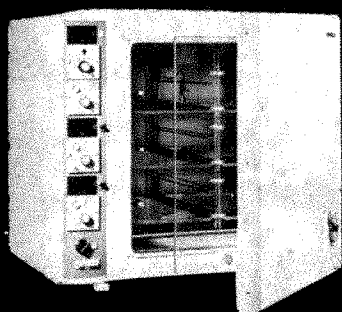
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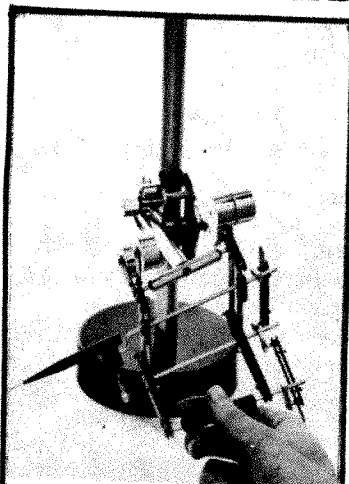
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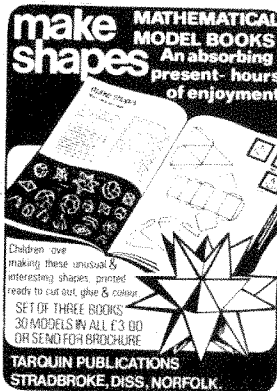
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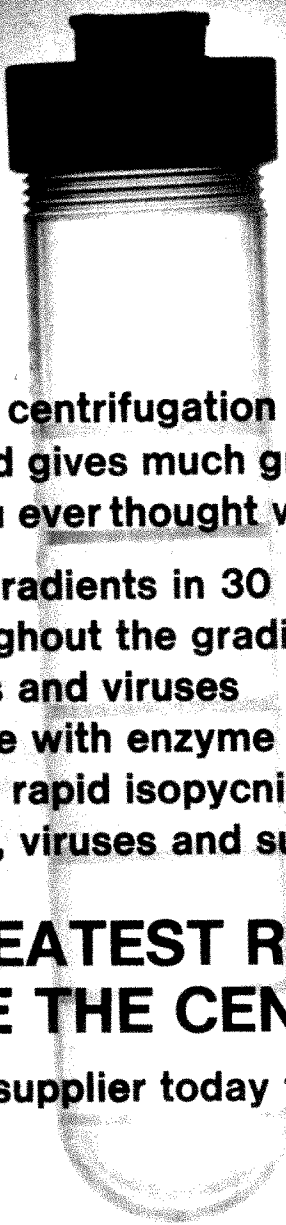
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review article

Dilepton production in hadronic collisions

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Quarks play a central part in particle physics. They were introduced as three fundamental, irreducible building blocks whose combinations reproduced the vast spectroscopy and static properties of hadronic states. Here we are concerned with the extension of the quark concept to collisions: the quark-parton model provides a description of the weak, electromagnetic and strong scattering processes.

A GAUGE invariant quantum field theory of quarks and gluons, quantum chromodynamics (QCD) aspires to the fundamental theory of hadron dynamics. This theory, coopting the successes of the quark-parton model, should account for the quark-quark forces which lead to structure functions (momentum space wave functions) of the bound quarks, including the properties of the 'hydrogen-atoms' of quark-quark forces: the $\bar{q}q$ states of massive quarks. Examples of these systems are the recently discovered particles J/ψ and ψ . We also want this theory to explain 'confinement', why quarks are apparently permanently imprisoned in hadrons.

The field has been enriched by the necessity to postulate an increasing number of quarks. The original three have now been joined by a fourth (charm, 1974) and a fifth (beauty, 1977). I consider here one experimental approach which has been particularly fruitful on two fronts: (1) the enrichment in the number of quarks and (2) on sensitive determinations of the properties of the old quarks which are the main constituents of protons and neutrons.

Dilepton production

There has been much theoretical and experimental concern about the high energy collisions of protons with nuclei in which two leptons appear in the final state:

$$p + N \rightarrow \mu^+ + \mu^- + \text{anything else} \quad (1)$$

(In some experiments the muons are replaced by electrons.) The emerging leptons are the only particles observed in these experiments; their momenta and angles are measured and the 'effective mass' of the pair is calculated:

$$m^2 = 2p^+ p^- (1 - \cos [\theta_+ + \theta_-]) \quad (2)$$

As the quantum numbers of the lepton pair are those of a photon, a compilation of the spectrum of effective masses is equivalent to an observation of the (virtual) photon flux generated in high energy collisions.

The effective mass of the muon pair, (that is, the mass of the parent photon) acts as a probe of small distance structure of the colliding hadrons—and the Heisenberg Uncertainty Principle teaches us that the higher the mass, the smaller the detail probed. This was the motivation for the first such experiment carried at the Brookhaven 30 GeV accelerator in 1968¹.

Muons are extremely penetrating, so we can use very intense incident proton beams and protect the detection equipment by thick filters through which only the muons penetrate. Thus one

can select reaction (1) out of processes which are millions of times more probable, and follow the reaction out to large effective masses where the probability of emission is extremely small.

The intuitive idea of probing for small distance structure was quantified by the quark model.

The dynamical property of quarks (the quark picture of high energy collisions) came of age in the Feynman² (partons) and Bjorken³ models in the late 1960s. The first success was the explanation of scaling observed (or so it seemed) in deeply inelastic electron-nucleon scattering. In these models, the hadron is treated as a bound state of elementary, point-like objects, called partons. In the interaction with virtual photons and with the (weak) intermediate bosons, the partons behave as if they are 'almost free'. These ideas are applied to the following reactions:

$$l + N \rightarrow l + \text{anything} \quad (3)$$

$$l + l \rightarrow \text{anything} \quad (4)$$

$$p + N \rightarrow l^+ + l^- + \text{anything} \quad (5)$$

where l can be an electron, muon or a neutrino. 'Anything' is an integration over all possible final states. Reaction (3), already referred to as deeply inelastic scattering has been extensively examined experimentally, beginning with pioneering experiments at SLAC and progressing to much higher energies at Fermilab where the electrons are replaced by muons. In the constituent model, the charged lepton interacts with the quark constituent through the emission and absorption of a photon (virtual) of momentum Q . In this view equation (3) can be written

$$l^+ + q \rightarrow l^- + q \quad (6)$$

The experimental result that most significantly advanced the quark model was the confirmation of Bjorken's prediction of scaling. Scaling describes a particularly simple behaviour of the scattering in which only a particular combination of supposedly independent variables are adequate to describe the data: the combination is written as

$$x \equiv \frac{2m_p \nu}{Q^2}$$

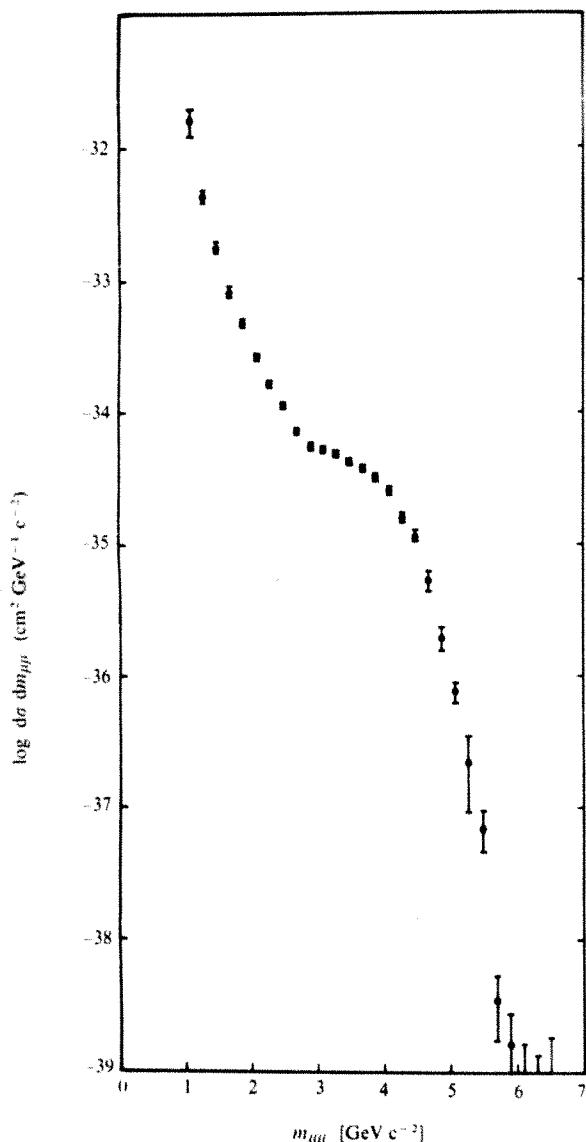


Fig. 1 Data obtained at BNL in 1968 (See ref. 1).

where ν is the lepton's energy loss in the scattering and Q is the momentum transferred, so that the scattering is described by functions:

$$F(x \text{ only}) \quad (7)$$

The scaling behaviour will be shown later to be only approximately true.

Reaction (4) is investigated in electron-positron storage rings. These included facilities at Orsay (France), Frascati (Italy), Novosibirsk (USSR) and, most notably at SLAC's SPEAR and DESY's DORIS. The quark view of equation (4) is:

$$e^+ + e^- \rightarrow q + \bar{q} \quad (8)$$

Given these interpretations, Drell and Yan⁴ interpreted reaction (5) as:

$$q + \bar{q} \rightarrow e^+ + e^- \quad (9)$$

the hard collision of two hadrons to produce a massive lepton pair is viewed as the annihilation of a quark in one with an anti-quark in the other. At first sight it would appear that equations (8) and (9) are simply reversed forms of the same reaction. However, in practice, they teach us quite different things. Quarks do not emerge from collisions but somehow fragment into normal hadrons such as pions, and kaons. Thus reaction (8) informs us about the fragmenting process. Reaction (9) is dominated by the density and motions of the quark and

anti-quark content of the colliding particles and so informs us about the quark structure of hadrons. This would then be the main thrust of dilepton physics except that from time to time, the continuum distribution of muon pairs is interrupted by the resonant production of a massive particle which decays into lepton pairs. This distorted the original Brookhaven spectra¹ but was not perceived because of the poor mass resolution which smeared the mass peak at 3.1 GeV into the shoulder seen in Fig. 1. This peak was found in an improved experiment run in 1974 and named the J/ψ particle⁵. Its interpretation as evidence for the anticipated new quark (the charmed quark) has been discussed in detail elsewhere. In the higher energy experiment carried out at Fermilab in 1975–78, the J/ψ peak is shown in Fig. 2 along with a second interruption to the rational study of the continuum—the upsilon peak near 9.5 GeV. Here again it is generally accepted that a new quark is operating^{6–8}.

Thus, the physics of dilepton production has two components both relevant to the constituent theory of hadrons: (1) the study of the structure functions of the hadrons, dominated by old quarks and (2) the existence, as a very small component, of heavier quarks bearing new internal quantum numbers (flavours).

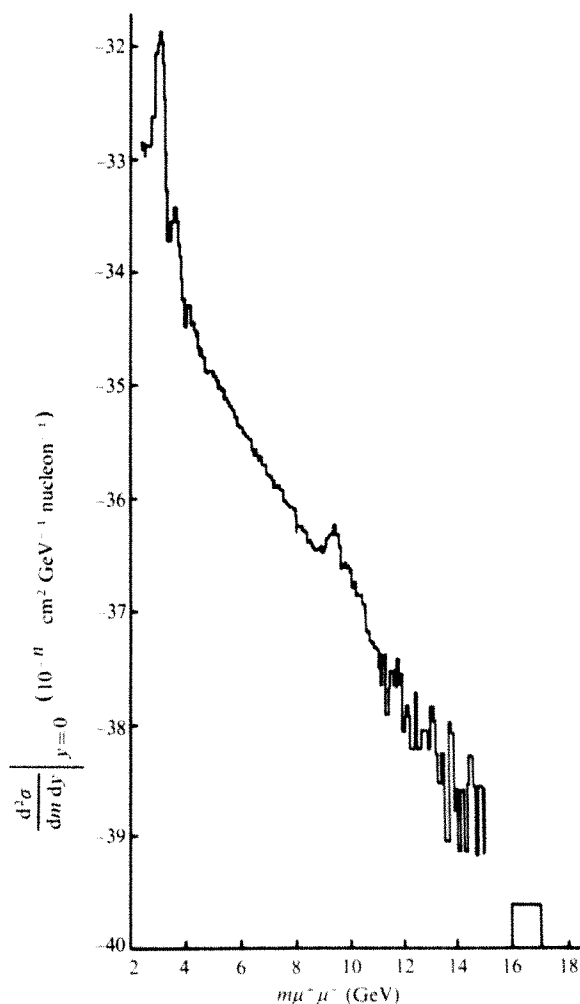


Fig. 2 Dimuon mass spectrum obtained at FNAL in 1977 showing J/ψ family bumps near 3 GeV and the upsilon peaks near 10 GeV. $-0.3 \leq y \leq +0.3$.

Experimental

After several experiments^{9–10} in which electron pairs were detected^{11,12}, the leptons of choice are muons, principally because of the ease of filtering the enormous flux of hadrons. The earliest muon experiments were hampered by poor resolu-

tion—the result of multiple scattering of the muons in the iron that served as a filter. In the most recent Fermilab research⁶⁻⁸ iron was replaced by beryllium, a compromise which led to a great increase in sensitivity (combination of intensity and resolution). The experiment then consists of a double arm spectrometer which identifies and measures the vector momenta of the two emerging muons. The effective mass is calculated as in equation (2). Other variables of interest are the longitudinal momentum of the parent virtual photon:

$$P_{11} = P_+ \cos \theta_+ + P_- \cos \theta_- \quad (10)$$

and its transverse momentum:

$$P_1 = P_+ \sin \theta_+ + P_- \sin \theta_- \quad (11)$$

We include here data from three groups of experiments. The Fermilab experiments of a Columbia-Fermilab-Stony Brook (CFS) collaboration used 200, 300 and 400 GeV protons on a copper target, a Fermilab experiment which used 225 GeV pions on a carbon target and a group of experiments at the CERN ISR in which protons colliding with protons gave an effective energy of 2,000 GeV proton-proton collisions.

Resonance production

When the bumps are ignored, the data of Fig. 2, are well fitted with a straight line having a mass distribution of the form

$$\frac{d\Gamma}{d\sigma} = A e^{-bm} \quad (12)$$

Subtracting the fitted curve gives Fig. 3. The fitting program then establishes the existence of two peaks Y and Y' at masses of 9.4 and 10.0 respectively. Evidence for a third peak, Y'' at 10.4 is present and has recently been confirmed. A detailed analysis of the experimental resolution established that the peaks are 'narrow'—the observed width is consistent with the experimental smearing of a peak which must then have a width < 100 MeV and could have essentially zero intrinsic width. This single fact requires a new law of nature. A particle as massive as 10 GeV should decay with extremely short lifetime, corresponding to a width of several GeV unless inhibited by a new law. The standard example of an uninhibited decay, is the rho meson which, at 700 MeV of mass, has a width which is 15% of its mass. A detailed investigation of various candidates found in the theoretical literature ruled out all but one possibility: the upsilon is made of new quarks—and is then strongly inhibited from decaying into pions, kaons and so on—particles made of the old up, down and strange (u, d, s) quarks. This essentially repeated the lesson learned in the case of the J/ψ . There, the new (charm) quark hypothesis was resisted until higher energy e^+e^- collisions produced particles in which the charm quantum number was exhibited. (In the J/ψ , it is hidden by a cancellation—the J/ψ is a $c\bar{c}$ bound state.)

Here we should remember the quantum numbers of the old quarks. They are arranged in pairs:

$$\begin{pmatrix} u \\ d \end{pmatrix} \quad \begin{pmatrix} c \\ s \end{pmatrix} \quad (13)$$

The basic distinction is called the flavour—in these pairings, the upper quark has an electrical charge of $+2/3$, the lower one $-1/3$. A proton is primarily a three-quark system (uud), a neutron is (udd). The upsilon changes the picture to:

$$\begin{pmatrix} u \\ d \end{pmatrix} \quad \begin{pmatrix} c \\ s \end{pmatrix} \quad \begin{pmatrix} ? \\ b \end{pmatrix} \quad (14)$$

where the designation b (bottom or beauty) is assigned to the newest quark. The upsilon is then $b\bar{b}$ bound state in this interpretation. The evidence in favour of this interpretation may be summarised as follows:

(1) We have already noted that the FNAL data on the narrowness of the upsilon peak ($\sigma < 100$ MeV) proves the need for a new quantum number. Weak bosons and Higgs particles were ruled out experimentally^{9,10}.

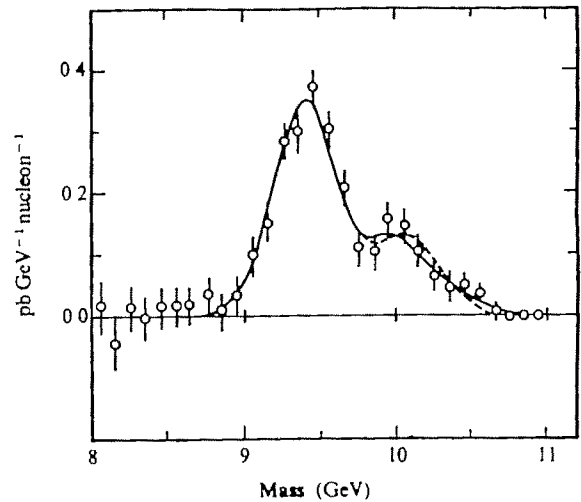


Fig. 3 Upsilon peak with the continuous background subtracted.

(2) The force between quarks, as a basic premise, is supposed to be independent of flavour and carried by quanta of the quark-quark force called gluons. The J/ψ system of particles carrying the c-quark has been analysed in terms of a potential model by Eichten and Gottfried¹³. Using this potential, they calculated that a quark of mass, $m \sim 5$ GeV should give rise to three bound states $1S^3$, $2S^3$ and $3S^3$ of the $b\bar{b}$ system with spacings which are crudely that observed.

(3) Other calculations¹⁴⁻¹⁸ using the $b\bar{b}$ model successfully accounted for the relative strengths of the three levels.

(4) The above conclusions have recently been confirmed in the reaction (4) at the DORIS storage rings. Here, the partial width of the decay

$$Y \rightarrow e^+ + e^- \quad (15)$$

is measured and¹⁹⁻²¹, when compared to theoretical expectations²² establishes the charge $|e_b| = 1/3 e$.

The expectation of partners to the b-quark to replace the ? in equation (14) (called t for top or truth) is very strong. The CFS group has extended the search by collecting ~ 10 times the data that led to the discovery of the upsilon. No more bumps were found and therefore a limit of

$$m_t \geq 8.5 \text{ GeV}$$

could be set by the absence of a bound state, $t\bar{t}$, up to masses of 17 GeV.

Having established the structure of the upsilons as a set of bound states of an 'atom' composed of a massive quark and antiquark, we can learn about the force that produces the binding. This is the primordial strong force, as (see below) our present view of matter is that hadrons are made of quarks glued together by this quark-quark force. (The strong force between protons and neutrons, in this view, becomes a complicated Van der Waal's-type interaction.) Analyses of the upsilon is simplified by the fact that non-relativistic potential theory may be used^{15-18,22}. Literature data indicate that there is a narrow range of potential models that can fit the spectroscopy of both the charmonium and the upsilon energy levels. A very incisive set of data will come from the spectroscopy in the upsilon region and from heavier quark bound states.

The hadrons we know are predominantly composed of u and d quarks. There are traces of s, c, b in decreasing amounts as the masses increase. Nevertheless, without a complete picture of the quark content, an understanding of hadrons is not possible. Clearly an inflation in the number of quarks raises basic questions about elementarity—perhaps six quarks will settle the problem—but already speculations of subquark structures are appearing.

Old quarks: structure functions

The Drell-Yan model (equation (9)), has a simple formulation if we integrate over the motion of the dilepton. As the mass depends only on the momenta of the colliding quarks conservation of energy and momenta permit us to write:

$$x_1 x_2 = \frac{m^2}{s} = \tau \quad (16)$$

$$x_1 - x_2 = x_F \quad (17)$$

$$x_1 = \sqrt{\tau} e^y; x_2 = \sqrt{\tau} e^{-y} \quad (18)$$

Here x_1 is the fraction of the hadron's total momentum carried by the quark 1. Similarly for x_2 . x_F is the longitudinal momentum of the dilepton, m is the mass and s the square of the energy of the collision, evaluated in the CM.

The variable y is often used in place of longitudinal momentum and is known as the rapidity, defined by equation (18).

The aim of the analysis of continuum data is to obtain the structure functions $V(x)$, that is the probability distribution for finding a quark of fractional momentum x . Since there are u and d quarks in a proton, we need $u(x)$, $d(x)$. We note that the proton consists mainly of uud quarks. However, in the old picture of the proton, there was a meson cloud, the virtual presence of mesons, was needed to account for the properties of the proton. In the quark version, the cloud is condensed to a 'sea' of quark-antiquark pairs created by the very strong forces between the main (or valence) quarks. As the sea appears in pairs with no net quantum numbers, we can have $u\bar{u}$, $d\bar{d}$, $s\bar{s}$, $c\bar{c}$, and so on. This accounts for the possibility of producing objects with strange quarks, charmed quarks and so on, in high energy collisions. It also accounts for the presence of antiquarks, required for the Drell-Yan process. Now then, the probability of the production of a dilepton can be written as:

$$\frac{d\sigma}{dm} \propto [V(x_1)S(x_2) + V(x_2)S(x_1)] \quad (19)$$

subject to the condition (16). Here we abbreviate the valence quark distributions by $V(x)$ and the sea quark distributions by $S(x)$.

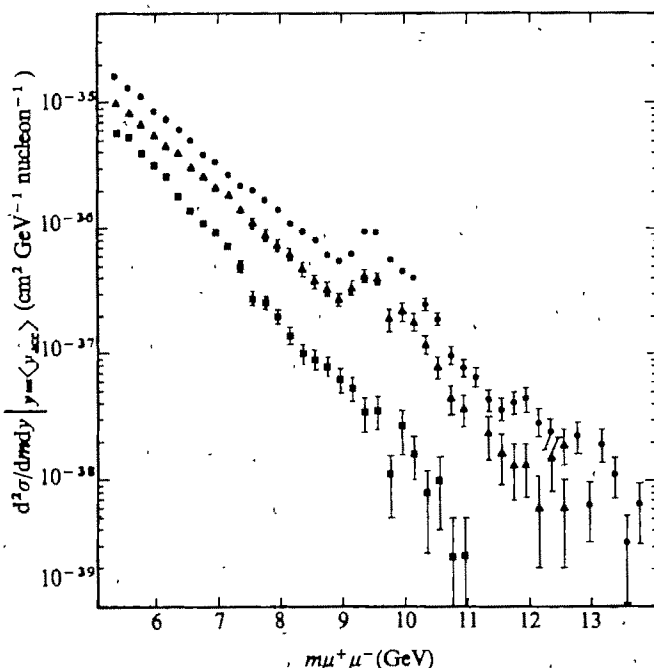


Fig. 4 Dimuon spectra produced by 200 (■), 300 (▲) and 400 (●) GeV proton collisions

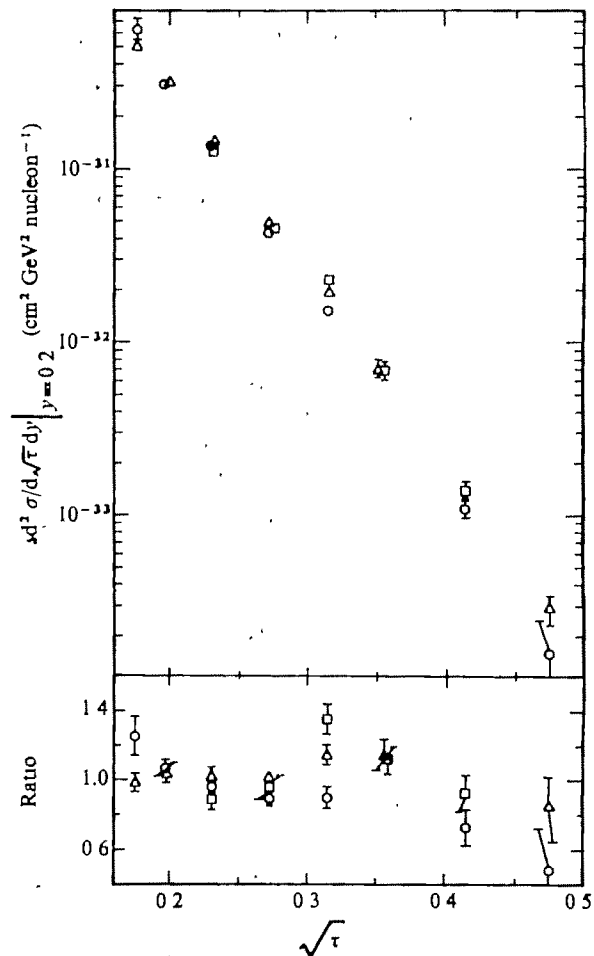


Fig. 5 Test of the scaling hypothesis: at 200 (□), 300 (△) and 400 GeV. All three energies give identical results when $m^2 d\sigma/dm$ is plotted against the dimensionless variable m^2/s .

Now reaction (3), deeply inelastic scattering, also measures $V(x)$ and therefore, the dilepton data of Fig. 2 can be used to determine the sea distribution. A typical result of the fitting program yields a distribution:

$$S(x) = 0.54 (1-x)^{8.5} \quad (20)$$

so that the sea momenta are crowded towards very low values of the fractional momentum. This would be valuable information but the universality of the quark model leads to an even more important conclusion.

There is a reasonable amount of experimental support for the notion that the weak and electromagnetic forces are 'unified'. In older forms, a conserved vector current (CVC) theorem was proposed and tested. The modern version is the theory of Weinberg and of Salam which group the mediators of the weak force, W^+ , W^- , Z^0 and the mediator of the $e-m$ force, γ together into a family. Thus all reactions mediated by a virtual photon should have analogues mediated by the weak boson. Support for this was originally found in weak decays and more recently in neutrino collisions.

The analogue of equation (3) is then

$$\nu + N \rightarrow \mu^- + \text{anything} \quad (21a)$$

or

$$\nu + q \rightarrow \mu^- + q' \quad (21b)$$

for example,

$$\nu + d \rightarrow \mu^- + u$$

Thus neutrino (and anti-neutrino) scattering is also sensitive to quark distributions. Data from Fermilab and CERN have also

been analysed to give the sea distribution. The data agrees well with the results of dilepton production. Here is a subtle detail of the constituent model, the sea distribution of anti-quarks as viewed by two observations (1) neutrinos bringing in virtual W 's to probe the quarks and (2) colliding two hadrons one of which provides a quark to seek out an anti-quark in the other. This is a substantial triumph for the fundamental picture of quark constituents.

The model has another success which has 'practical' applications. One can write the cross-section:

$$\frac{d\sigma}{dm} = \frac{1}{m^3} F(m^2, s) \quad (22)$$

From dimensional arguments F must be dimensionless. Scaling is the assertion that:

$$m^3 \frac{d\sigma}{dm} = F(m^2/s) = F(\tau) \quad (23)$$

that is there are no parameters with energy dimensions which enter. The practical value is that one can use this to predict the yield of masses at future accelerators from results at hand. Example: At FNAL, $s = 750 \text{ GeV}^2$ and the yield of 15 GeV dileptons is known. Thus at a future accelerator, such as ISABELLE, $s = 160,000 \text{ GeV}^2$, one would expect the same value of F for $m = 200 \text{ GeV}$. Because m is the mass of a virtual photon, the predictions can be applied to all states which can connect to photons: pairs of massive leptons, W bosons, quarks, monopoles, and so on.

The test of the scaling hypothesis is indicated in Fig. 4 where a fit to the Fermilab data at 200, 300 and 400 GeV (consistent with scaling but over too small a range to be decisive) is extended to 2,000 GeV and compared to ISR ($s = 3,600 \text{ GeV}^2$) results^{23,24}. To the level of ~20% the agreement is good. The fit that was found is:

$$F(\tau) = 44.4 \exp(-26.6 \tau^{1/2}) \times 10^{-30} \text{ cm}^2$$

Another success of the model becomes evident in the Fermilab experiments of a Chicago-Princeton collaboration²⁵ who use pions as the incident particle. A negative pion is a tid combination of quarks. Thus the anti-quark is part of the valence population. Qualitatively this means that dilepton production should be much easier there would be no need to search the sea for antiquarks. Indeed the probability for continuum dilepton production near 10 GeV of mass is more than 200 times bigger for incident pions. As a further benefit, the structure function for the valence quarks of the pion have been determined from the same experiment—so a photograph of the pion is now at hand. As the pion lives only 10^{-8} s and is created in high energy collisions, this is another major achievement. The form of the pion structure function (in momentum space) is:

$$u(x) \approx (1-x)^{1.13} \quad (24)$$

the quark momenta in the pion fall only linearly with x .

Quantum chromodynamics

Given this quantitative success (at the 20% level), it is clear that the next few years will lead to refinements and more incisive tests of the model. At this point we should note that the model has become a theory: quantum chromodynamics (QCD) deals with the quantum field theory of the quark-quark strong force in close analogy to QED. Thus the force between quarks is carried by vector gluons which are also an ingredient of the sea. Being neutral they do not interact directly with photon probes but their ability to scatter with quarks and among themselves leads to corrections to the quark model. Some of these effects have been observed in deeply inelastic scattering as violations of scaling in those experiments. That is, experiments at Fermilab²⁶ using high energy muons have established that reaction (7) is not true but that, in the higher Q^2 domain one must write $F(x, Q^2)$. These same corrections, in the dilepton production data we have

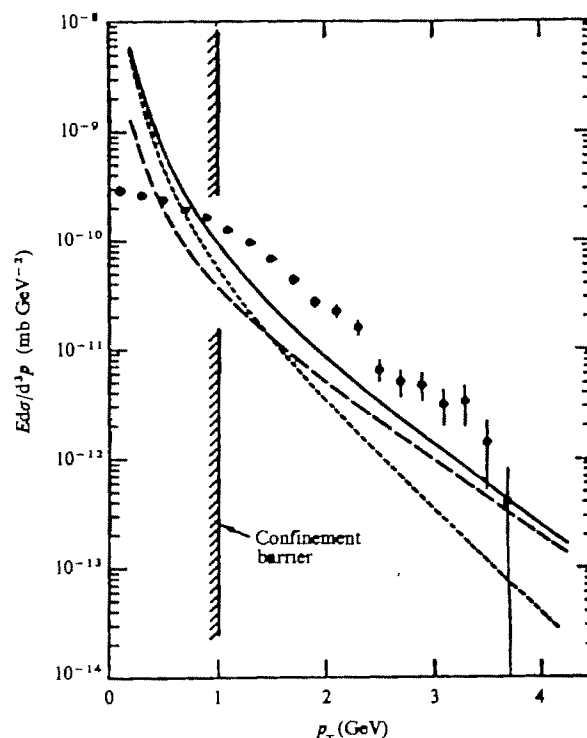


Fig. 6 A comparison of QCD calculations (due to Berger) with the data on transverse momentum of dileptons. We see the infrared difficulties of the present status of the QCD theory, also the theory needs an additional component: intrinsic quark motion: $7 \leq M \leq 8 \text{ GeV}$, $y = 0$; —, total; — — —, Compton; — — — —, annihilation.

discussed are small. However, they show up in a surprising way. There is one aspect of the data which is wholly determined by the QCD effects.

High transverse momentum dileptons

In the quark-parton model the hadron is viewed as a cluster of parallel moving point-like partons. If two annihilate, the resulting dilepton should also move longitudinally. Even the earliest observations¹ indicated that there is a large transverse momentum component to the dilepton. In the context of the model this implies that the annihilating quarks must bring in the transverse momentum. In the confrontations of theory and data, (see Fig. 6) and in the subsequent evolution of the applications of QCD it became clear that QCD contained just the necessary ingredients: one source of quark transverse momentum has to do with dynamical processes contributing to the production of the lepton pair²⁷⁻³³. These processes involving both quarks and gluons could account qualitatively for the observations. Quantitative comparisons with experiment were used to tune the parameters of the theory—most sensitively, the momentum behaviour of the gluons. However, not all the observed effects were produced until an intrinsic transverse momentum was involved. This microcosmic Fermi motion is closely related to the size effect of the confining force.

Thus the transverse momentum of dileptons provides both a basic challenge to the theory and a source of information on the constituent behaviour. Although it is not clear how quantitatively valid the QCD calculations are, we know qualitatively that they give a good account of the high P_T dilepton data, using terms which are required to understand scaling violations observed in reaction (3). The prospects are very encouraging and the issues are crucial. QCD is the first quantum field theory of hadrons. Progress is sporadic and problems in the calculations abound. For example, Fig. 6 shows the infrared divergence of the P_T calculations. Nevertheless, the stakes are high and the advance of the experimental techniques in dilepton physics will provide even more incisive data to confront and guide the developing theory.

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articles

Geochemistry of basalts from the Tyrrhenian Sea

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Comparison between the Tyrrhenian basalts and oceanic basalts coming from different geodynamic environments shows that Tyrrhenian basalts have a clear organic signature with a transitional character.

THE genesis of the western Mediterranean oceanic floor is still poorly understood¹, and the origin of the Tyrrhenian Sea (Fig. 1) is a matter of conjecture. What kind of basement lies underneath—thin continental crust, intermediate crust, or true oceanic crust? Recent studies^{2,3} have interpreted the Tyrrhenian Sea as a marginal one⁴.

The DSPD leg 42 A site 373 A drill hole provided, for the first time, fresh material from the Tyrrhenian Sea floor. This material is basaltic and has recently been described from a petrological point of view^{5,6}. This article presents the results of a geochemical study including strontium and lead isotope compositions and trace elements (including rare earths) concentrations of seven samples representing different stratigraphic positions⁷. These results will permit comparison with the basalts erupted in different geodynamic environments, and are identical to patterns expected for transitional basalts.

Experimental techniques

The strontium isotopic compositions, as well as K, Rb and Sr concentrations, have been determined by mass spectrometry and isotope dilution using the method described elsewhere⁷. The concentrations of these elements are known with an accuracy of 1%. We used the NBS 987 standard and our data are normalised to a ⁸⁷Sr/⁸⁶Sr ratio of 0.7014 ± 0.00007 (2σ) for this standard.

The lead isotopic composition was measured using the Manhes microtechnique⁸. The blanks range from 0.9 to 3.5 ng per 1 g of sample powder. Accuracy of the isotopic measurement is limited by mass fractionation which was found to be 0.1% per mass unit by repetitive analyses of the SRM 982 standard. Data are normalised to the accepted ratios of this standard which are: ²⁰⁴Pb/²⁰⁷Pb = 0.05845, ²⁰⁶Pb/²⁰⁷Pb = 2.1433, ²⁰⁸Pb/²⁰⁷Pb =

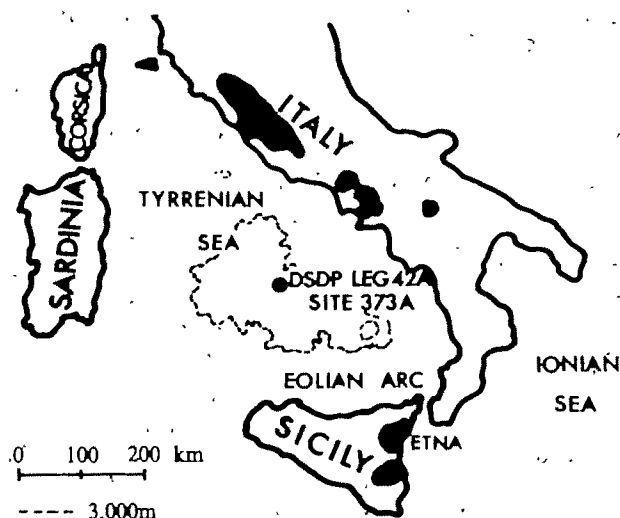


Fig. 1 Location of leg 42 site 373 A. Darkened regions are areas of Italian and Sicilian volcanism.

Table 1 Trace element abundances (p.p.m.) in Tyrrhenian basalts

	1 Core 7-3 130-135	2 Core 2-1 136-142	3 Core 12-1 120-124	4 Core 4-1 87, 5-92	5 Outer Barrel	6 Core 7-2 94-100	7 Core 7-3 100-105
La	4.5 ±0.01	8.0 ±0.1	7.3 ±0.1	5.4 ±0.1	7.5 ±0.2	5.5 ±0.2	4.5 ±0.1
Ce	10.3 ±0.4	18.8 ±0.4	18.5 ±0.3	13.1 ±0.3	18.1 ±0.4	12.8 ±0.3	10.3 ±0.3
Nd	7.3 ±0.5	11.5 ±0.9	12.0 ±1.0	9.2 ±0.9	9.6 ±0.9	8.1 ±0.8	7.2 ±0.8
Sm	2.13 ±0.01	3.60 ±0.02	3.15 ±0.02	2.54 ±0.01	3.5 ±0.02	2.4 ±0.01	2.03 ±0.01
Eu	0.84 ±0.02	1.26 ±0.04	1.22 ±0.03	1.07 ±0.03	1.25 ±0.03	0.93 ±0.04	0.84 ±0.04
Gd	2.6 ±0.2	4.1 ±0.2	4.0 ±0.2	3.0 ±0.2	4.0 ±0.2	2.9 ±0.2	2.5 ±0.2
Tb	0.47 ±0.01	0.75 ±0.01	0.70 ±0.01	0.58 ±0.01	0.74 ±0.01	0.52 ±0.01	0.45 ±0.01
Yb	1.91 ±0.03	2.75 ±0.04	2.75 ±0.04	2.34 ±0.04	2.86 ±0.04	1.94 ±0.03	1.74 ±0.03
Tm	0.35 ±0.03	0.49 ±0.03	0.50 ±0.04	0.40 ±0.04	0.50 ±0.03	0.35 ±0.02	0.31 ±0.02
Lu	0.38 ±0.01	0.54 ±0.01	0.54 ±0.01	0.45 ±0.01	0.56 ±0.01	0.38 ±0.01	0.34 ±0.01
U	0.35 ±0.01	0.23 ±0.01	0.15 ±0.01	0.12 ±0.01	0.20 ±0.01	0.15 ±0.01	0.15 ±0.01
Th	0.49 ±0.01	0.74 ±0.01	0.71 ±0.01	0.43 ±0.01	0.71 ±0.01	0.60 ±0.01	0.46 ±0.01
Zr	50	125	125	84	137	96	67
Hf	1.62	3.07	2.88	2.02	3.00	1.88	1.57
Ta	0.22	0.58	0.54	0.34	0.58	0.29	0.24
Ba	62	65	66	57	57	74	61
Ca	0.13	0.04	0.04	0.41	0.04	0.05	0.17
Sr	0.098	0.036	0.048	0.33	0.038	0.018	0.062
Cr	182	124	125	151	131	213	213
Co	38.6	35.5	33.8	35.5	32.2	40.6	33.0
Ni	86	49	53	47	54	114	83
Sc	28.6	27.7	26.8	33.7	27.8	22.5	24.0
Tb	0.47	0.75	0.70	0.58	0.74	0.52	0.45

Uncertainties, estimated by reproducibility and standard measurements, are about 3% for Sc, Th and Ce, 6% for Ca, Eu, Hf, Ta, Cr, Co, La and Sm, and 10% for the others, except Zr (15%). Quoted errors represent statistical error in counting.

Table 2 K, Rb and Sr contents and strontium and lead isotope ratios in Tyrrhenian basalts

	1 Core 7-3 130-135	2 Core 2-1 136-142	3 Core 12-1 120-124	4 Core 4-1 87, 5-92	5 Outer Barrel	6 Core 7-2 94-100	7 Core 7-3 100-105
K (p.p.m.)		2,700	3,300		2,900	2,500	2,900
Rb (p.p.m.)		2.5	2.96	2.2	3.1	2.8	3.7
Sr (p.p.m.)			175	200	177	214	
K/Rb		1,080	1,114		935	893	783
K/Ca		67,500	82,500		72,500	50,000	17,059
$^{87}\text{Rb}/^{86}\text{Sr}$			0.048	0.031	0.050	0.057	
$^{87}\text{Sr}/^{86}\text{Sr}$	0.70321	0.70292	0.70313	0.70299	0.70300	0.70300	0.70306
$^{206}\text{Pb}/^{204}\text{Pb}$	±0.00010	±0.00009	±0.00012	±0.00012	±0.00010	±0.00009	±0.00007
$^{207}\text{Pb}/^{204}\text{Pb}$	18.874	18.657				18.774	18.848
$^{207}\text{Pb}/^{204}\text{Pb}$	±0.013	±0.020				±0.023	±0.015
$^{207}\text{Pb}/^{204}\text{Pb}$	15.613	15.567				15.567	15.600
$^{207}\text{Pb}/^{204}\text{Pb}$	±0.015	±0.021				±0.022	±0.017
$^{208}\text{Pb}/^{204}\text{Pb}$	38.62	38.47				38.52	38.59
$^{208}\text{Pb}/^{204}\text{Pb}$	±0.02	±0.08				±0.02	±0.05

Quoted errors for lead data are $(\sigma^2 + (\delta_m R)^2 \cdot \sigma_s^2)^{1/2}$ where σ is the uncertainty in the run, and σ_s the error on the discrimination factor, estimated from repeated measurements of SRM 981 standard, R is the measured ratio and δ_m the mass difference in this ratio. Measured ratios of one basalt from the Lau basin are: $^{206}\text{Pb}/^{204}\text{Pb} = 18.322$; $^{207}\text{Pb}/^{204}\text{Pb} = 15.521$; $^{208}\text{Pb}/^{204}\text{Pb} = 38.020$.

2.1393. No measured value differs from this average by more than 0.4% for $^{204}\text{Pb}/^{207}\text{Pb}$, which can be considered as an upper limit for error.

The trace element concentrations (other than K, Rb and Sr) have been measured by epithermal neutron activation analysis, following the technique described by Treuil *et al.*⁹. The rare earths were measured after chemical separation, the other elements by direct instrumental analysis. Results and uncertainties for each element are given in Table 1.

Results and interpretations

The $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic ratios are fairly constant (0.7031) (Table 2) and higher than the typical mid-oceanic ridge basalt values, which are between 0.7022 and 0.7028 (refs 10, 11); they are also higher than the few values obtained on interarc basin tholeiites (Lau Basin¹¹ and Mariana Trough^{12,13}). These results are very

similar to the 'abnormal or transitional MORB', like the Reykjanes Ridge near Iceland^{14,15} or the FAMOUS area^{16,17}. On the other hand, the values are much lower than those of the Eolian basalts (0.7049 to 0.7065)¹⁸ and Italian volcanic rocks analysed by Vollmer¹⁹, although similar to the values on the eastern Sicilian volcanics²¹ (Etna and Iblean basalts).

The lead isotopic compositions (Table 2, plotted on a $^{207}\text{Pb}/^{204}\text{Pb}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ diagram in Fig. 2) fall just above the 'MORB quadrangle' defined by Sun²², as do the FAMOUS area samples (Dupré and C.J.A., in preparation, and Cumming²³). These basalts have $^{206}\text{Pb}/^{204}\text{Pb}$ ratios similar to those of the Icelandic basalts, but slightly higher $^{207}\text{Pb}/^{204}\text{Pb}$ ratios^{15,24}, and are also within the domain of some island arc volcanics^{25,26}. Moreover, they are clearly different from the Italian volcanic rocks¹⁹, as well as from the Sicilian and Etna basalts²¹, which show the high $^{207}\text{Pb}/^{204}\text{Pb}$ typical of rocks

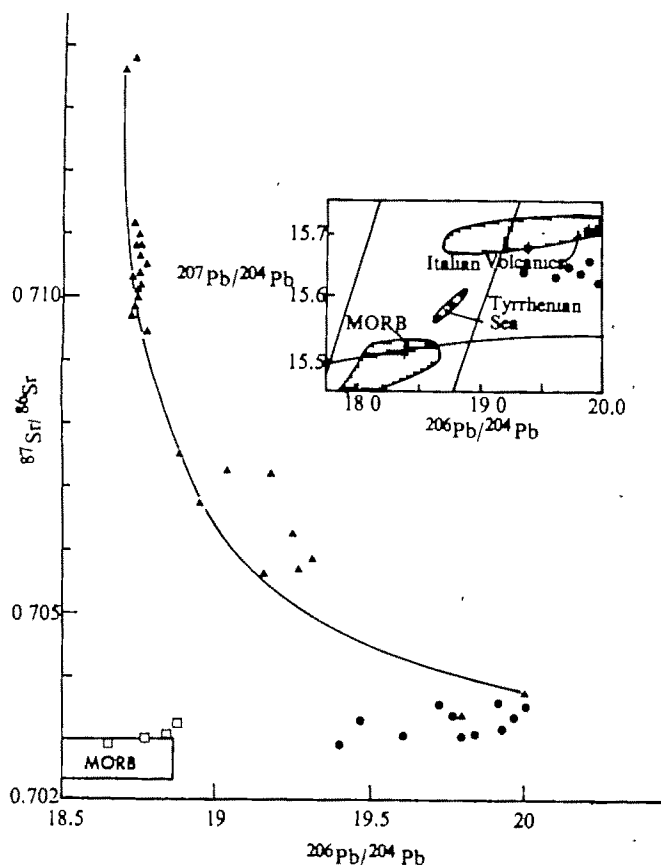


Fig. 2 Lead-strontium correlation diagram discussed by Vollmer¹⁹. The Tyrrhenian basalt results (□) are clearly off the mixing trend between Italian volcanic rocks (Δ) and Sicilian and Etna basalts (●).

including crustal material. The few data obtained on marginal basins (Mariana Trough¹³, and one sample from the Lau Basin, Table 2) give typical ridge values.

The Ni and Cr content of basalts which are direct products of partial melting of the mantle are around 220 to 350 p.p.m. for Ni, and 500 p.p.m. for Cr (refs 27, 28). Such values decrease rapidly when the basalts suffer shallow fractionation²⁸. The values of Ni, Cr and Co (Table 1) show clearly that these basalts are not very primitive and have undergone some fractional crystallisation before their eruption on the ocean floor. Therefore, data on trace element levels should be used with caution in attempts to infer the nature of the primitive material. However, findings for the magmatophile elements²⁹, which are the least affected by fractional crystallisation, lead to several interesting points. The K/Rb ratios are similar to those of MORB (~1,000) and clearly higher than values for alkali basalts³⁰: this is true in spite of the high content in Cs (for at least three of the analysed samples), which is a very good alteration indicator. Alteration could have lowered the K/Rb and K/Cs ratios, and raised the K content and ⁸⁷Sr/⁸⁶Sr ratio³¹ (see, for example, sample 7). It is likely that these characteristics have not been greatly affected,

however, as we observe no correlation between the Cs and K contents and K/Rb or Sr isotopic ratio, and as these values are the same for the samples with low Cs content.

The rare earth patterns are very constant, although the absolute concentrations vary. The patterns are light rare earth enriched, very different from typical MORB which show light rare earth depletion. Such a distribution is very similar to the transitional MORB type, like some Icelandic tholeiites or the basalts dredged in the FAMOUS area³². On the other hand, the Tyrrhenian basalt patterns are very similar to those found by Hart *et al.*¹³ in the Mariana Trough.

Th and Ta are incompatible elements and therefore their ratios are not greatly affected by fractional crystallisation and partial melting²⁹. One of the very interesting properties of this ratio is the clear distinction in signature between subduction zone volcanics and oceanic volcanics. The Ta/Th ratio of subduction zone volcanics, like Japan or the Eolian islands, is very low (0.1) whereas the oceanic type like the mid-Atlantic Ridge, Red Sea or FAMOUS area, is between 0.6 and 1.2. The basalts from the Tyrrhenian Sea are clearly of the 'oceanic' type.

The Tyrrhenian Sea is a relatively recent (7.3 Myr)³ and rather small sea, bordered by two important cratons, so these results suggest that the development of small or marginal seas may generate oceanic crust containing basalts of transitional character. Such characteristics are different from the marginal sea types associated with subduction zones which are, so far, not very different from typical MORB.

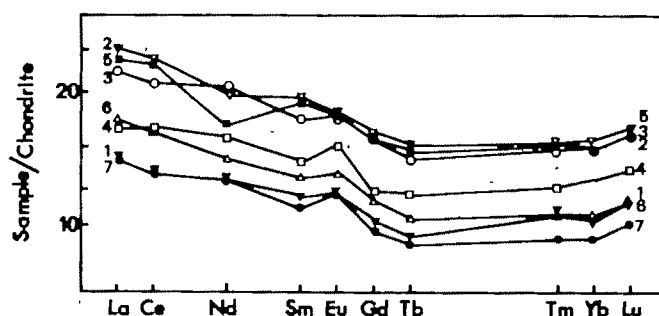


Fig. 3 Chondrite normalised rare earth abundance. The values are similar to those of transitional basalts, like those of the FAMOUS area³². The positive Eu anomaly can be attributed to the presence of plagioclase phenocrysts³.

We can try to incorporate our results into the isotopic picture given by Vollmer¹⁹ for the Italian province. As shown in Fig. 2, the Tyrrhenian basalts do not follow the general trend found for the Italian volcanics. We can perhaps define a trend between Etna and Tyrrhenian basalts. Therefore, the petrogenesis of the Italian volcanic province may be explained by the mixing between oceanic island type magma and the continental crust¹⁹ (such an interaction is very similar to a rift valley-type volcanism, for example), although it may not be due to a subduction process. The Etna volcanism may be a result of interaction between the same oceanic island type magma and a mid-oceanic ridge type one.

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Local aggregation of hormone–receptor complexes is required for activation by epidermal growth factor

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An analogue of epidermal growth factor (EGF) which is virtually devoid of biological activity retains receptor binding activity but cannot form cell surface clusters or patches. Bivalent anti-EGF antibodies restore both bioactivity and patch formation. The sensitivity of fibroblasts to native EGF can also be enhanced greatly by these antibodies, especially in hormone-resistant cell lines.

EPIDERMAL GROWTH FACTOR (EGF) is a single-chain polypeptide hormone of molecular weight ~6,000. The hormone was isolated from the male mouse submaxillary gland¹ and found to be a potent mitogen of various epidermal and epithelial tissues both *in vivo* and *in vitro*^{2,3}. It can stimulate the proliferation of various cultured cells such as human fibroblasts⁴, glial cells⁵, granulosa cells⁶ and mouse 3T3 cells⁷. Carpenter and Cohen have presented data which suggest that the binding of EGF to human fibroblasts is followed by internalisation and degradation, presumably by lysosomal enzymes⁸. Using fluorescently labelled, biologically active analogues of EGF and insulin⁹, it has been shown by fluorescent photobleaching recovery that the surface-bound hormone molecules can diffuse laterally in the plane of the membrane with a diffusion coefficient (D) of $3-5 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ (refs 9, 10). Fluorescence microscopy studies have shown that these hormones are initially bound diffusely on the cell surface, and at 4 °C they remain dispersed for at least 90 min (ref. 11). Within a few minutes of warming the cells to 37 °C, the hormones aggregate into patches. The visible, clustered hormone molecules are subsequently (within half an hour) internalised by endocytic vesicles. Although it has been tempting to try to relate these processes of redistribution, internalisation and degradation to the mode of action of these hormones, the physiological significance of these events has not been established.

We have recently presented data which indicate that the stimulation of DNA synthesis in fibroblasts by EGF depends on the persistent occupation of a relatively small fraction of the total measurable, externally located binding sites for the hormone¹². The enhancement of DNA synthesis observed 24 h after the addition of EGF is completely blocked by adding anti-EGF immunoglobulin at any time during the first 8–10 h of incubation, indicating the need for the continued presence of externally located molecules¹².

The studies described here suggest that hormone–receptor complexes must undergo some type of cell surface redistribution and self-aggregation to elicit a biological response. Cleavage of EGF with cyanogen bromide results in a unique hormone derivative (CNBr-EGF) which is virtually devoid of biological activity but which nevertheless retains substantial (10%) binding activity. The biological activity of this inactive analogue can be restored by cross-linking with specific bivalent antibodies to EGF. Also, concentrations of native EGF far below those that

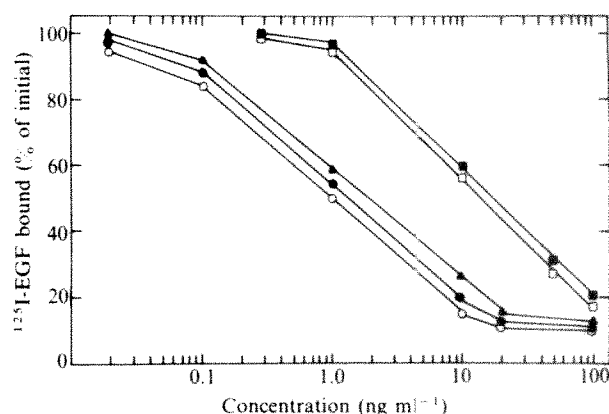


Fig. 1 Competition of binding of labelled native EGF by EGF and various derivatives. 3T3 fibroblasts in monolayers (approximately 3×10^5 cells, 100 μg of protein) and ^{125}I -EGF (1.4 ng ml^{-1} , 300,000 c.p.m. per ng) were incubated with various concentrations of native EGF (purified from mouse submaxillary glands as described in ref. 13) or its derivatives in 0.5 ml medium containing 0.1% bovine serum albumin (BSA) for 45 min at 37 °C. Unbound radioactivity was then removed by washing four times with cold Hank's solution–0.1% BSA. The cells were solubilised with 1.0 ml of 1.0 M NaOH and the radioactive content was determined. The amount of bound radioactivity was 6,000 c.p.m. per well in the absence of unlabelled hormone. ○, Native EGF; ●, native EGF which had been incubated in 70% formic acid for 24 h; ▲, R-EGF; □, CNBr-cleaved EGF; ■, R₂-CNBr-cleaved EGF.

normally show activity can elicit dramatic responses on addition of anti-EGF antibody. Furthermore, cells that are relatively refractory to EGF, despite unimpaired binding, can be markedly 'sensitised' to respond by cross-linking the cell-bound native EGF with antibody. Clearly, hormone binding alone is a biologically unproductive process without a subsequent step of micro-redistribution, aggregation or cross-linking which seems to be a limiting, determining process.

CNBr-EGF: a biologically inactive analogue antagonistic for EGF binding but not activity

EGF has a single methionyl residue at position 21 (ref. 1), and cleavage with cyanogen bromide results in the formation of two polypeptide chains connected by the disulphide bonds of the protein molecule. The binding and biological properties of CNBr-EGF are presented in Fig. 1 and Table 1. Cyanogen bromide-cleaved EGF inhibits the binding of ^{125}I -EGF to 3T3 fibroblasts at concentrations approximately one order of magnitude higher than the native hormone. However, CNBr-EGF only has 0.3% or less of the activity of the native hormone in stimulating thymidine incorporation (DNA synthesis) into

confluent 3T3 fibroblasts. The analogue therefore exhibits at least a 30-fold disparity between its affinity for binding and for biological activation.

The biological and binding properties of the other derivatives used in the present studies are also presented in Table 1 and Fig. 1. Labelling of CNBr-EGF with two molecules of rhodamine (R), to produce R₂-CNBr-EGF, does not result in significant further changes in the properties of this derivative. Exposure of native EGF to the same conditions used in the preparation of CNBr-EGF slightly reduced the binding affinity and biological activity (Table 1).

Cyanogen bromide-cleaved EGF retains 10% of its antigenic activity as determined by radioimmunoassay (see legend to Fig. 2). Half-maximal inhibition of ¹²⁵I-EGF precipitation in this assay is obtained at 50 ng per assay compared with a value of 5 ng per assay obtained with the native hormone.

Anti-EGF antibodies restore the activity of CNBr-EGF

When 3T3 cells are incubated with CNBr-EGF and anti-EGF IgG is then added, full restoration of biological activity can be obtained. Figure 2 shows a typical experiment in which the cells were first incubated with 5 ng ml⁻¹ of the derivative and then with various concentrations of anti-EGF IgG. Full restoration of activity is limited to a narrow range of concentration of the antibody (1:10,000–1:8,000 of the antibody solution, A₂₈₀ = 3.1). No restoration of activity occurs at higher or lower concentrations or when monovalent Fab' derived from anti-EGF IgG is combined with the hormone derivative (Fig. 2). This fragment has about 80% of the capacity of anti-EGF IgG to precipitate ¹²⁵I-EGF in a double antibody immunoassay. Anti-EGF IgG has no effect on DNA synthesis in the absence of the hormone derivative, and an IgG fraction from normal rabbit serum is inactive either alone or in combination with CNBr-EGF.

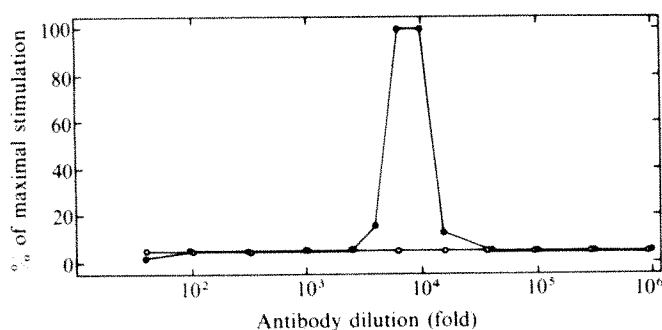


Fig. 2 Biological activity of CNBr-cleaved EGF in the presence of various concentrations of anti-EGF-IgG. Thymidine incorporation into confluent 3T3 monolayers was carried out in the presence of CNBr-cleaved EGF (5 ng ml⁻¹) and the indicated antibody dilutions prepared from purified anti-EGF immunoglobulin fraction (A₂₈₀ = 3.1). The cells were incubated with the hormone derivative for 20 min at room temperature before adding the antibody. The cells in this experiment produced half-maximal stimulation at EGF concentrations of 0.15 ng ml⁻¹. Maximal thymidine incorporation was 400% of control values in the absence of added mitogen. Monovalent Fab' was prepared by papain digestion of anti-EGF-IgG (ref. 15). The Fc fragment and the undigested IgG fraction were removed by a Sepharose-protein A affinity column (Pharmacia) and the non-retarded fraction was subjected to reduction with 5 mM dithiothreitol at pH 8.5 for 30 min. Iodoacetamide was then added (0.01 M final concentration) and after 20 min the sample was dialysed overnight against 0.05 M sodium phosphate (pH 7.5) at 4 °C; CNBr-EGF combined with anti-EGF-IgG (A₂₈₀ = 3.1) (●); CNBr-EGF combined with monovalent Fab' fragment derived from anti-EGF-IgG (○). By radioimmunoassay CNBr-EGF retains 10% activity, as determined by a standard 'double antibody' radioimmunoassay using anti-EGF-IgG fraction (A₂₈₀ = 3.1) as the 'first antibody' at a final dilution of 1:5,000. Anti-EGF-IgG was purified¹² from rabbit anti-EGF serum; this was used rather than anti-EGF serum as the latter has intrinsic DNA-stimulating activity¹².

Table 1 Binding and biological activities of various EGF derivatives

EGF or derivative	Binding affinity to cells		Biological activity	
	50% ¹²⁵ I-hormone displacement (ng ml ⁻¹)	Relative binding potency (%)	ED ₅₀ (ng ml ⁻¹)	Relative activity (%)
EGF	0.8	100	0.13	100
EGF treated with 70% formic acid	1.0	80	0.15	87
R-EGF	1.3	61	0.24	54
CNBr-EGF	10	8	50	0.3
R ₂ -CNBr-EGF	12	7	60	0.2

3T3 fibroblasts were plated at 10⁵ cells per dish in 1 ml of modified Eagle's medium with HY-Clone 10% fetal bovine serum (Sterile System) in 35-mm plastic tissue culture dishes. Incubations were carried out at 37 °C under 90% air, 10% CO₂ humidified atmosphere. The cells were fed after 24 h and used after 96–120 h. Thymidine incorporation into confluent monolayers was measured as described previously¹⁴ using a 2-h pulse with 1 μCi ml⁻¹ of [methyl-³H]thymidine started 24 h after the addition of the mitogen. CNBr-EGF was prepared by treating EGF (1 mg ml⁻¹) containing a trace quantity of ¹²⁵I-EGF in 70% formic acid with 400–500 equivalents of crystalline cyanogen bromide (Fluka) for 24 h at room temperature. The solution was then evaporated under vacuum and the residue redissolved in H₂O and lyophilised. Native EGF, with no cyanogen bromide added, was treated similarly. An acid hydrolysate of the derivative contained no methionine and about 1 mol of homoserine or its lactone. Details of the preparation of the fluorescent derivatives is given in the legend to Fig. 3. Experimental values for binding affinity were obtained from Fig. 1. The relative binding potency is the concentration of native EGF or derivative that inhibits 50% of the binding of ¹²⁵I-EGF. The ED₅₀ is the concentration of EGF that produces half-maximal stimulation of DNA synthesis. R-EGF = native EGF labelled with one molecule of rhodamine; R₂-CNBr-EGF = CNBr-EGF labelled with two molecules of rhodamine.

These data suggest that the hormone receptors occupied by CNBr-EGF are ineffective because of their inability to induce receptor redistribution and aggregation, and that this deficiency can be corrected by passively cross-linking the CNBr-EGF-occupied receptors with bivalent (but not monovalent) antibody. This hypothesis was explored further by fluorescence microscopy studies.

Distribution of fluorescent analogues of EGF on the cell surface

It was previously reported that in 3T3 cells exposed to rhodamine-labelled EGF (R-EGF) the hormone-receptor complexes are homogeneously distributed at 4 °C whereas at 37 °C they rapidly cluster and form visual patches on the cell surface^{11,16}. When 3T3 cells are incubated with 200 ng per ml of CNBr-EGF labelled with two molecules of rhodamine (at each of its two amino-terminal groups) (R₂-CNBr-EGF) for 45 min at 4 °C, the hormone complexes are also diffusely distributed on the cell membrane (not shown). In contrast to the results with R-EGF, incubation at 37 °C for 1 h or more does not cause any visible clustering, and staining remains diffuse (Fig. 3a, b). However, visual patches are readily seen when anti-EGF antibodies are added to cells labelled with R₂-CNBr-EGF (Fig. 3c, d). These visual patches are observed with antibody dilutions of 1:10–1:100 but not at higher (1:120–1:20,000) dilutions.

These data further confirm that, unlike the native hormone, CNBr-EGF has lost its ability to self-aggregate spontaneously at 37 °C, even at high receptor occupancy and after prolonged periods of incubation. This deficiency can be at least partially corrected by antibody to EGF. Visible patches are not, however, observed at the range of antibody concentration in which the biological activity of CNBr-EGF is restored (see previous section). It is thus possible that microaggregates (which may be composed of two or several hormone-receptor complexes), rather than large clusters or aggregates, are the 'state of microaggregation' responsible for triggering biological activity. It is not possible, however, to determine whether some macroaggregates of the type visualised at high receptor occupancy with R-EGF can also form at low receptor occupancies.

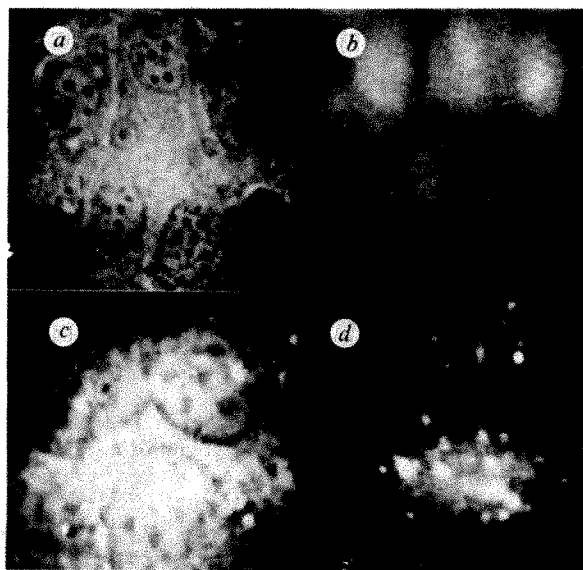


Fig. 3 Distribution of R_2 -CNBr-EGF on the cell surface of 3T3 fibroblasts in the presence and absence of anti-EGF-IgG. *a* and *b* describe the phase and fluorescence micrographs, respectively, of the same field ($\times 1,950$) of 3T3 cells which were incubated for 30 min at 37°C with 200 ng per ml of R_2 -CNBr-EGF. The cells were then washed with phosphate-buffered saline (PBS)-1% BSA, fixed with 1% formaldehyde, and observed with an image-intensified video fluorescent microscope as described previously¹¹. The level of nonspecific fluorescence was determined as described¹¹, and found to be about 30% of the total fluorescence intensity. R_2 -CNBr-EGF was prepared by reacting CNBr-EGF (1 mg) containing a trace quantity of ^{125}I -CNBr-EGF in 1 ml of 0.2 M sodium carbonate buffer (pH 9.3) with three aliquots (20 μl each) of a solution (10 mg ml^{-1}) of rhodamine isothiocyanate (Research Organic Chemicals) in dimethylformamide. Excess reagent was removed by gel filtration on a Sephadex G-15 column. R-EGF was prepared in a similar manner, starting from native EGF (1 mg) containing a trace quantity of ^{125}I -EGF. The ratio of $^{125}\text{I}/A_{545}$ was used to determine the extent of the reaction as previously described⁹. R-EGF and R_2 -CNBr-EGF contained about 1 and 2 mol of rhodamine per mol EGF, respectively. Their biological properties are given in Table 1. *c* and *d* describe the phase and the fluorescence micrographs, respectively, of the same field ($\times 1,950$) of cells prepared as described above except that the cells were incubated for an additional 30 min at 37°C with anti-EGF-IgG (final dilution of 1:50 from a stock solution of the antibody, $A_{280} = 3.1$) before fixation with formaldehyde.

Antibody enhancement of native EGF activity in sensitive and resistant cells

In view of the above findings, we examined the possibility that antibodies might enhance the activity of very low, sub-optimal concentrations of native EGF. In these conditions, it is theoretically possible that the density of active EGF-receptor complexes is sufficiently low to limit the probability and extent of cross-linking in a way that may be enhanced by bivalent antibody. Indeed, although antibodies have virtually no enhancing effects on low but slightly active concentrations (1 pg ml^{-1}) of native EGF, a definite effect on inducing the emergence of activity can be observed when lower concentrations (0.1 pg ml^{-1}) of the hormone are used (Fig. 4*a*).

As these studies were carried out on cells which are highly sensitive to EGF and whose receptors are not expected to be defective in the property of self-aggregation when occupied by the hormone, we examined cells containing unaltered numbers of EGF binding sites but which were relatively insensitive to the hormone (Table 2). It was reasoned that the receptors in these cells could theoretically be relatively defective in the property of

self-aggregation, so that artificial, exogenous cross-linking with antibody might induce even more dramatic effects. As we consistently observed that 3T3 cells of low passage are more sensitive to the effects of EGF, and that with increasing passage there is a progressive loss of sensitivity, despite the fact that the numbers of EGF binding sites do not change, high-passage or 'poorly responsive' 3T3 cells were studied (Table 2, Fig. 4*b*). In these cells addition of anti-EGF IgG results in a dramatic restoration of the activity at all concentrations of the hormone. Highly significant biological effects are observed at EGF concentrations as low as 0.1 pg ml^{-1} , which is three to four orders of magnitude lower than the concentrations normally required to produce half-maximal effects in these cells. As observed for CNBr-EGF, monovalent Fab' is without effect (Fig. 5*b*). Note that quantitative differences exist between the restoration of activity of CNBr-EGF (Fig. 2) and that observed with submaximal doses of the native hormone (Fig. 4). The restoration is more complete for CNBr-EGF and it is restricted, for unknown reasons, to a narrower range of concentration of antibody. It is also pertinent that substantially lower concentrations of EGF are required to elicit activity in sensitive compared with resistant cells (Table 2). These results also suggest that the loss of hormone responsiveness observed with increased cell passage may be related to the modification of a process which restricts hormone-receptor mobility or self-aggregation.

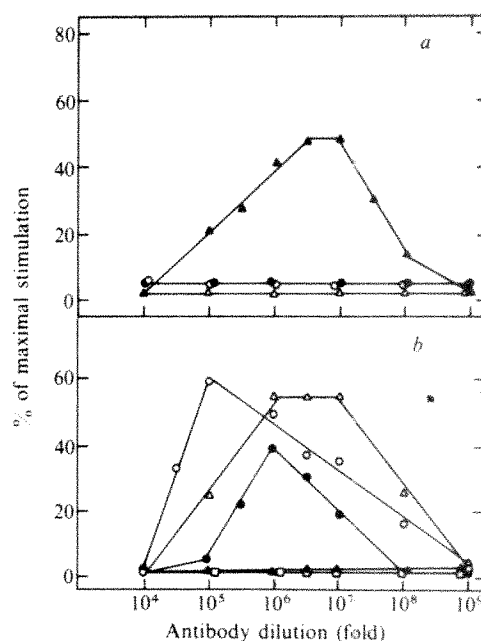


Fig. 4 Biological activity of submaximal concentrations of native EGF using various concentrations of anti-EGF-IgG in 'highly responsive' (*a*) and 'poorly responsive' (*b*) 3T3 cells. *a*, Thymidine incorporation was carried out with 0.1 pg per ml ($\blacktriangle$) and 1 pg per ml ($\bullet$) of EGF and the indicated dilutions of a solution of purified anti-EGF-IgG ($A_{280} = 3.1$). The cells were incubated with the hormone for 20 min before the addition of antibody. The cells in this experiment (passage 2) produced half-maximal stimulation at EGF concentrations of 7–10 pg ml^{-1} . Maximal thymidine incorporation was 650% of control values without added mitogen. 0.1 pg per ml ($\triangle$) and 1 pg per ml ($\circ$) of EGF in the absence of anti-EGF-IgG. No effect of the antibody was detected at higher concentrations of the hormone (5 and 10 pg ml^{-1} , not shown). *b*, Thymidine incorporation was carried out with 0.1 pg per ml ($\triangle$), 1 pg per ml ($\bullet$) and 10 pg per ml ($\circ$) of native EGF and the indicated dilutions of purified anti-EGF-IgG ($A_{280} = 3.1$). The cells in this experiment (passage 14) produced half-maximal stimulation at EGF concentrations of 1 ng ml^{-1} . Maximal thymidine incorporation was 250% of control values without added mitogen. $\square$, Native EGF (10 pg) in the absence of anti-EGF-IgG; $\blacktriangle$, EGF (10 pg) plus monovalent Fab'.

Table 1 Comparison of some properties of 3T3 cells 'poorly responsive' or 'highly responsive' to EGF

Cell type	Passage no.	No. of binding sites	Binding affinity	ED ₅₀	% Thymidine incorporation
Highly responsive	2-5	40,000-70,000	2.5-4.0	5-20	600-1,100
Poorly responsive	12-26	40,000-70,000	2.5-4.0	1,000-2,000	170-250

Binding affinity is the concentration of ^{125}I -EGF ($\times 10^{10}$ M) required to achieve half-maximal binding, determined by incubating 3T3 cells in monolayer with increasing concentrations of ^{125}I -EGF (300,000 c.p.m. per ng) for 45 min at 37 °C, as described in ref. 14. The ED₅₀ is the concentration (pg ml⁻¹) of EGF producing half-maximal stimulation of DNA synthesis. Thymidine incorporation is expressed as the per cent stimulation with maximal concentrations of EGF compared with that in the absence of the mitogen (100%).

Mechanisms which involve self-aggregation require lateral mobility of receptor sites in the plane of the plasma membrane. This was predicted earlier^{17,18} and demonstrated recently for EGF and insulin receptors of 3T3 fibroblasts^{9,10}. The significance of receptor-receptor aggregation has previously been suggested in receptor-mediated processes as well as in other biological systems. Kahn *et al.* have shown that antibodies to insulin receptors (obtained from patients with insulin-resis-

tant diabetes) can mimic insulin in stimulating glucose oxidation in adipocytes^{19,20}. The Fab' fragment derived from these antibodies competes with ^{125}I -insulin for binding but does not stimulate glucose oxidation. However, cross-linking with anti-Fab' antisera restores activity²⁰. Antibodies raised against the purified insulin receptor²¹, and plant lectins which are known to bind to insulin receptors²² may also produce biological responses by cross-linking insulin receptors. Conditions which cause degranulation of mast cells and basophils also cause clustering of Fc receptors²³. When B lymphocytes, which contain immunoglobulin molecules as an integral component of the plasma membrane, interact with bivalent antigens, they are triggered to proliferate and differentiate²⁴.

The present studies could have important applications to other polypeptide hormone systems. For example, in certain types of insulin-resistant diabetes, or other similar states, defects may exist that impair receptor self-aggregation.

Note added in proof: Anti-insulin antibodies have now also been shown to enhance substantially the biological effects of this hormone on fibroblasts²⁵. Although this effect is accompanied by an increase in the apparent affinity of binding of insulin, in the case of EGF, antibodies do not significantly increase the binding of this hormone to fibroblasts or liver membranes²⁵.

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letters

Circular polarisation and the magnetic dipole model

THE magnetic dipole model for a compact extragalactic radio source proposed by Sanders^{1,2} and discussed by Milgrom and Bahcall³ and by Sanders and DaCosta⁴, accounts for one of the features observed in a few of these sources—the apparently superluminal separation of two components (see ref. 5). However, the magnetic dipole model requires a strong, well-ordered magnetic field and predicts that the radio emission will have a substantial degree of circular polarisation unless the double source appears highly symmetric to the observer, or the radiation is by positrons and electrons in nearly equal numbers. For the past five years we have been monitoring extragalactic variable radio sources for circular polarisation at 8 GHz, and we have recently begun observations at 4.8 GHz. We present evidence here that the circular polarisation observations made by us and by other observers conflict with the predictions of the magnetic dipole model.

Our circular polarisation monitoring program and the observing procedure has already been described⁶. Averages for all our observations of several sources at 8 GHz between

October 1973 and October 1978 are presented in Table 1. The standard deviations are based on the scatter of the observations about their mean values and therefore reflect any possible

Table 1 8.0 GHz circular polarisation measurements October 1973 to October 1978

Source	V (%)	σ_v (%)	N†
0235+164	0.040	0.096	30
3C84	-0.075	0.015	109
3C120	0.031	0.030	64
0J287	0.056	0.090	42
4C39.25	0.031	0.024	80
3C273	-0.056	0.010	110
3C279	0.148	0.017	63
3C345	-0.058	0.021	127
2134+004	0.065	0.031	39
BL Lac	0.168	0.078	20
3C454.3	0.036	0.022	68

* Significant variations in V were detected during the observing period⁶.

† Number of days observed.

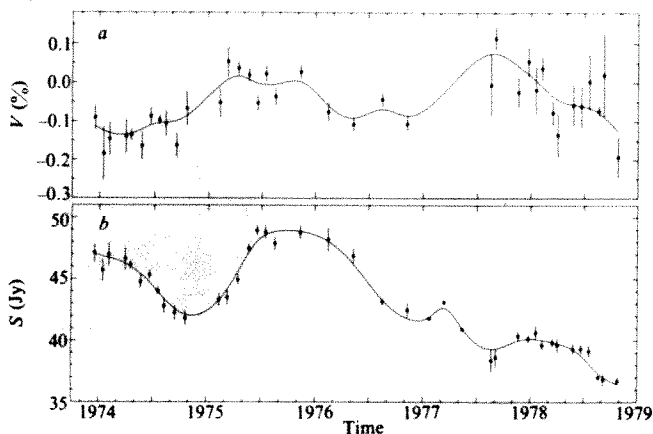


Fig. 1 Monthly averages of the degree of circular polarisation (a) and total flux density (b) for 3C273 at 8 GHz.

variations. Four bright radio sources have exhibited superluminal expansion⁵: 3C120, 3C273, 3C279 and 3C345. The degrees of circular polarisation which we have observed at 8 GHz for these and other sources have been less than or about 0.1% (ref. 6 and Table 1). Degrees of circular polarisation up to a few tenths of a per cent have been detected by other observers at lower frequencies (see refs 7–9). We have detected variations in the circular polarisation from at least two sources, 3C84 and 3C273 (ref. 6). Figures 1–3 show monthly averages of the total flux densities and degrees of circular polarisation at 8 GHz for 3C273, 3C279 and 3C345 respectively. Smoothing cubic splines¹⁰ have been fitted to the data. Throughout the period of observation the degree of circular polarisation did not rise substantially above ~0.1%.

In the magnetic dipole model^{1–4} the relativistic electrons stream out along the magnetic field lines with moderately small pitch angles during an outburst. The required magnetic field strengths must be at least the equipartition value to maintain the geometry of the field. The four sources which have shown superluminal expansion have estimated equipartition magnetic field strengths of about 0.1–1 G (ref. 11). Fields of this strength and pitch angles of 20°–30° yield 1–3% circular polarisation at 8 GHz for a transparent synchrotron source¹². (Sanders and DaCosta⁴ suggest larger magnetic fields and smaller pitch angles, which would increase the degree of circular polarisation to 10–15%.) In contrast, very long baseline interferometry (VLBI) indicates typical values of $\leq 10^{-3}$ G for the transverse component of the magnetic field $B_{\perp}$ in these sources¹³. This can be reconciled with the equipartition values if the pitch angles are quite small, $\leq 10^{-3}$ rad, which would produce degrees of circular polarisation approaching 100%¹⁴.

As Sanders and DaCosta⁴ pointed out, no circular polarisa-

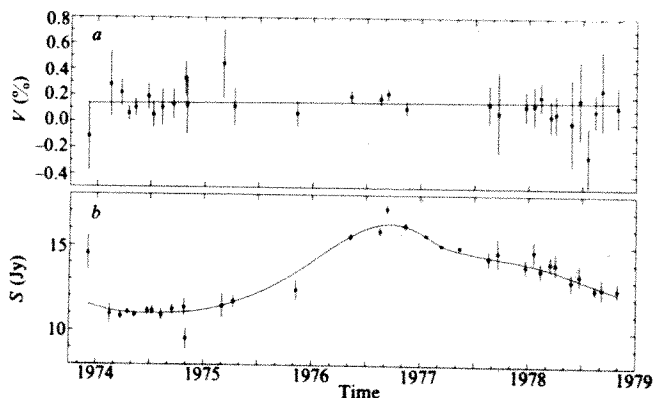


Fig. 2 As Fig. 1 but for 3C279.

tion would be produced if the radiation were generated by comparable ensembles of electrons and positrons. The following discussion considers the case when the radiation is primarily by electrons, so that the individual source components would be highly circularly polarised.

A significant degree of cancellation of the circular polarisation is expected in the magnetic dipole model because the magnetic field points towards the observer in one component and away from the observer in the other component, so that the sense of circular polarisation will be opposite in the two components^{1,4,12}. The net circular polarisation will, in general, still be non-zero because the total flux densities and magnetic field strengths of the two emitting regions are not expected to be identical. For an observer out of the equatorial plane of the dipole (as is required for separation rates in excess of 4.4c), the magnetic fields and pitch angles in the two components would be different even if the dipole geometry were perfectly symmetrical³. The polarised flux densities of the two components must differ by less than about 10% to be consistent with our observations, but the possibility that the known superluminally expanding sources are highly symmetric is excluded by VLBI^{15–18}. For example, Shaffer *et al.*¹⁵ found that the two

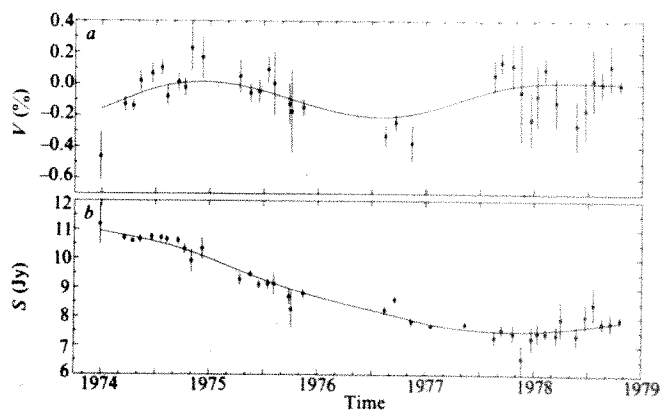


Fig. 3 As Fig. 1 but for 3C345.

components of 3C345 differed by more than 10% in total flux density and that one component had $B_{\perp} \sim 1$ mG where the other had $B_{\perp} \sim 100$ mG.

The conflict between the model and the observations can be reduced if both source components are opaque to the observer. The degree of circular polarisation from an opaque synchrotron source is about one-third as much as from a transparent source¹⁹. However, unless the observer is in the equatorial plane of the dipole, the two components will become transparent at different times (because of the different distances of the emitting regions from the dipole centre), thus producing a large imbalance between the components some time during the development of the burst. Our observations (see Figs 1–3) show no evidence for periods of large circular polarisation. Except for the possibility that the radiation from extragalactic sources is generated by both positrons and electrons, the asymmetries observed by VLBI together with our measurements of low degrees of circular polarisation in these sources do not seem to be reconcilable with the magnetic dipole model.

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Tropospheric circulation and interplanetary magnetic sector boundaries followed by MeV proton streams

TRANSITS of some interplanetary magnetic sector boundaries across the Earth were shown by Svestka *et al.*¹ to be followed by streams of protons with energies in the range of a few MeV. These proton boundary transits were associated with a characteristic decrease²⁻⁴ of the vorticity area index (VAI) during Northern Hemisphere winter that was about twice as large as the decrease associated with all the other boundary transits during the same interval. I show here that the MeV proton streams associated by Svestka *et al.*¹ with certain sector boundary transits apparently select boundary transits associated with unusually active conditions in the solar wind. These proton boundary transits were associated with a larger change in tropospheric circulation than were the other boundary transits observed during the same interval of time. New information in the present study of sector boundary transits relating changes in solar wind velocity, density and magnetic field to the resulting terrestrial changes in vorticity area index, geomagnetic activity and cosmic ray flux at Earth may provide a test for proposed physical mechanisms.

Svestka *et al.*¹ summarised what they observed: "There are certain magnetic sectors in interplanetary space in which the density of low-energy protons is increased. The increased proton flux is first observed when the western boundary of the sector passes across the Earth. After that time the flux remains enhanced for two or more days. In some cases the duration of the enhancement corresponds to the width of the magnetic sector, but generally this is not the rule; the duration is usually shorter, but it can be even longer than the sector width. In some cases the enhancement is observed once again when the sector returns to Earth after 27 days. More often, however, the proton increase is not recurrent."

Table 1 of Svestka *et al.*¹ gives a list of the transits of magnetic sector boundaries associated with MEV proton streams. The overlap in time between this table and the interval studied in refs 2-4 includes the winters in 1963-69 during which there were 18 proton boundary transits. During the same winter months there were 62 other well defined boundary transits tabulated by Svalgaard^{5,6}.

Figure 1a shows a superposed epoch analysis of the classical VAI of Roberts and Olson⁷ computed at 500 mb in the Northern Hemisphere for the 18 proton boundary transits described above (solid line) and for the 62 other boundary transits in the same interval of time (dashed line). The proton boundary transits were associated with a narrower minimum that was about twice as deep as the minimum associated with the other boundary transits.

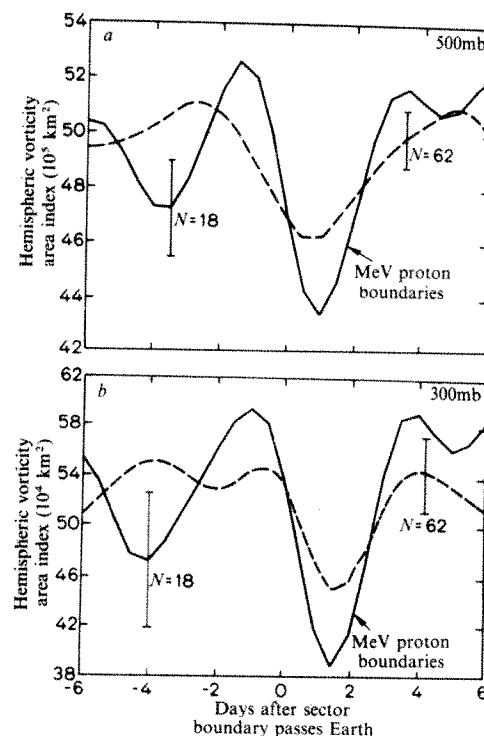


Fig. 1 a, Superposed epoch of the vorticity area index during Northern Hemisphere winters 1963-69 about sector boundary transits. The solid curve represents MeV proton boundary transits (see text) and the dashed line represents all other boundary transits in the same time interval. The VAI was computed at 500 mb as the sum of the area in which vorticity was greater than or equal to $20 \times 10^{-5} \text{ s}^{-1}$ plus the area in which vorticity was $\geq 24 \times 10^{-5} \text{ s}^{-1}$. In all figures the error bars indicate $\pm \text{s.e.m.}$ b, The same as (a) except that the vorticity area index was computed at 300 mb for the area in which vorticity was $\geq 28 \times 10^{-5} \text{ s}^{-1}$. Note that the ordinate in (b) is about 10 times smaller than that in (a).

As the depth of the minimum in the VAI may depend on the number of boundary transits considered, an analysis was made using 1,000 groups of 18 boundary transit times selected at random from the 62 'other' transits. A quantitative measure of the depth D of the minimum is defined as the average VAI at day -2 and at day 4 minus the VAI at day 1. (This definition is based on the minimum observed from 162 transits during the winters from 1 November 1963 to 31 March 1976.) For each of the 1,000 groups of 18 transit times the value of D is computed. For the 1,000 values of D the mean is $3.54 (\times 10^5 \text{ km}^2)$ and the standard deviation is 2.04. For the solid curve in Fig. 1a the value of D is 7.32, which is almost two standard deviations larger than the mean. This is consistent with the fact that only 30 (3%) of the 1,000 random values of D were larger than 7.32.

Figure 1b is the same as Fig. 1a except that the VAI was computed in a different part of the troposphere, namely at a height of 300 mb for vorticities $\geq 28 \times 10^{-5} \text{ s}^{-1}$. The VAI in Fig. 1a was computed at 500 mb for vorticities $\geq 20 \times 10^{-5} \text{ s}^{-1}$ plus the area with vorticity $\geq 24 \times 10^{-5} \text{ s}^{-1}$. Note that the area of the troposphere computed in Fig. 1b is about 10 times smaller than in Fig. 1a, but in Fig. 1b the minimum associated with MeV proton boundary transits is again at least twice as deep as the minimum associated with the other boundaries. A very similar effect was thus observed in rather different conditions in the troposphere.

Figure 2 shows a superposed epoch analysis of geomagnetic activity (Mudryaud's index⁸ am) associated with the proton boundary transits (solid line) and the other boundary transits (dashed line). There was a large increase of geomagnetic activity following the proton boundary transits and no significant change

following the other boundary transits. Figure 3 shows a superposed epoch analysis of the magnitude of the observed interplanetary magnetic field associated with proton boundary transits (solid line) and the other boundary transits (dashed line). The field magnitude showed a large increase after the transit of the proton boundaries and no significant change associated with the transit of the other boundaries. The shapes of Figs 2 and 3 are very similar, as would be expected from earlier discussions of the response of geomagnetic activity on this time scale to the solar wind and interplanetary magnetic field⁹. A measure of the information content in the present analysis can be gained from the close similarity of the peaks in the solid lines of Figs 2 and 3.

The MeV proton streams discussed by Svestka *et al.*¹ are now thought to have been accelerated in the solar wind beyond 1 AU in co-rotating interaction regions¹⁰ that are formed when high-velocity solar wind streams are catching up with lower velocity solar wind. The MeV proton boundary transits were thus associated with unusually active solar wind conditions.

Some information about possible physical mechanisms may be inferred from the analysis reported here. The magnitude of the interplanetary magnetic field had a large increase after proton boundary transits and no significant change after the

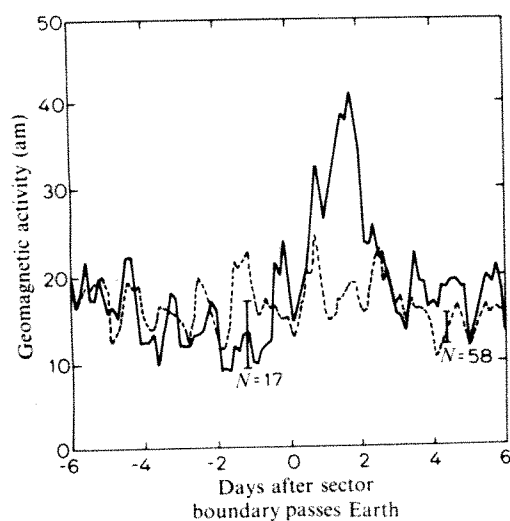


Fig. 2 Superposed epoch analysis of geomagnetic activity associated with the transit of proton sector boundaries (solid line) and the other boundaries in the same time interval (dashed line).

other boundary transits, while the minimum in VAI after proton boundary transits was twice as deep as the minimum after the other boundary transits. But the minimum in VAI after the other boundary transits was still well-defined. Thus, a simple relationship between the increase in the magnitude of the interplanetary magnetic field and the decrease in VAI following boundary transits seems to be excluded.

Geomagnetic activity had a large increase following proton boundary transits and no significant change associated with the other boundary transits, while the minimum in VAI following proton boundary transits and following the other boundary transits was well defined. We may infer that an increased in geomagnetic activity following a boundary transit is not a necessary condition for a minimum in VAI following a boundary transit. Olsen *et al.*¹¹ have reported a relationship between minimum VAI and maximum geomagnetic activity, but the large increases in geomagnetic activity studied by Olson *et al.*¹¹ were associated with transient solar flares (and/or plagues¹²) while the smaller increases in geomagnetic activity discussed in the present paper were associated with the quasi-stationary

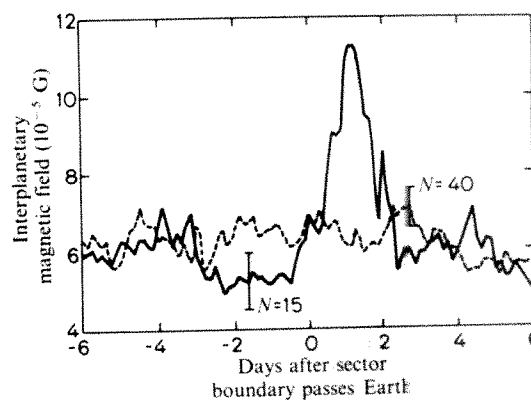


Fig. 3 Superposed epoch analysis of the magnitude of the interplanetary magnetic field associated with the transit of proton boundaries (solid line) and all other boundaries in the same interval (dashed line). Note the similarity between Figs 2 and 3.

co-rotating solar and interplanetary magnetic sector structure¹³. Physical processes may be different in these two cases.

The streams of MeV protons used by Svestka *et al.*¹ to select the proton boundaries analysed in the present work are not necessarily involved in the physical cause of the decrease of VAI following boundary transits. The proton streams may simply mark the presence of unusually disturbed solar wind conditions. Superposed epoch analysis (not shown) of solar wind velocity and of the density showed the characteristic increase¹³ in these quantities following proton boundary transits and following the other boundary transits, but the maximum values were larger after the proton boundary transits than after the other boundary transits. Thus, the increase in solar wind velocity and/or density that followed boundary transits may have been associated with the minima in VAI that followed boundary transits. The galactic cosmic ray intensity observed at Earth (Deep River neutron monitor) decreased by about 1% after the proton boundary transits and did not have a significant variation associated with the other boundary transits. Thus, a decrease in galactic cosmic ray intensity following boundary transits does not seem to be a necessary condition for a decrease in VAI following boundary transits.

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Effect of humidity on monolayer desorption at the air-water interface

THE effect of atmospheric humidity on the physicochemical properties of lipid monomolecular films at the air-water interface has been thought to be of secondary importance, and has never been studied experimentally. Yet, the physical situation encountered at the air-water interface may be strongly dependent on the humidity in the gas phase^{1,2}. Harkins³, while not specifically investigating this effect, took painstaking measures to ensure saturation of the air above the interface in an effort to suppress any temperature difference between the interior of the aqueous phase and its free surface. Obviously, below the saturation point of the air, the free surface of water is expected to be at a lower temperature than the interior by virtue of the evaporation of liquid. Thus, in some instances, the temperature of the outermost layer of the free water surface was reported to drop by 15°C or more relative to the temperature of the bulk from measurements of the surface tension during evaporation⁴. In other investigations the mean temperature, measured by thermistors, of a thin region immediately below the free surface of a water pool was reported to be several degrees lower than that in the interior during evaporation¹. The data presented here constitute a clear indication of the decisive effect of atmospheric humidity on the kinetics of monolayer desorption from the air-water interface. Figure 1 illustrates the dramatic difference in the observed decrease of the surface pressure of myristic acid monolayers in three different conditions of atmospheric humidity.

Unstable density and temperature gradients may thus be expected along the depth of a water layer undergoing evaporation. The main effect of these gradients is the generation, at a critical value, of the well known convective flows^{5,6} in which liquid at the surface is driven, by buoyancy or surface tension effects, to the interior and replaced by liquid from below. The intensity of these hydrodynamic effects obviously should be a function of the rate of evaporation (or heat flux) and hence, in the case of water pools, of the relative humidity of the air above

the free liquid surface. As the motion of fluid involves exchange of surface liquid for bulk liquid it could, in principle, affect studies of the dynamics of surfactant molecules both along the interface, such as surface diffusion, or across the interface, such as adsorption-desorption phenomena.

The apparatus used in this study consisted of a 1.6-mm deep Teflon trough, a Wilhelmy-type film balance, three thermistor probes for scanning the temperature of both the liquid and gas phases, and small relative humidity probes placed 5 mm above the interface. The entire system was enclosed in a Lucite cabinet, and the experiments were conducted in a controlled environment room. The cabinet was fitted with small pluggable access ports which served two purposes: first, to allow spreading of the monolayer from *n*-hexane through long capillary tubes, thus minimising disturbances of the physical conditions of the system, and second, to allow for adjustment of the humidity levels in the cabinet which also contained moist filter paper. In every instance spreading of the monolayer was accomplished only after complete stabilisation of the temperature and relative humidity. Every effort was made to check for and eliminate experimental artefacts due to film leakage, contact angle hysteresis, and solvent effects.

It is believed that curve *a* in Fig. 1 represents the rate of monolayer desorption in a convection-free aqueous layer (pH 2.0). The increase in the apparent desorption rate at 68% relative humidity (curve *b*) is presumably due to the convective motion in the liquid which causes a more rapid depletion of surfactant molecules from the surface, perhaps even during the spreading process. Interestingly, if the relative humidity is allowed to increase from 72 to 84% (curve *c*), the apparent film pressure first decreases rapidly, due to enhanced surfactant depletion by convection, and then increases gradually as a result of surfactant readorption which could be aided by the well known suppression of convective motion in liquids by the presence of solvent-free monolayers at the surface. The behaviour depicted also was found to persist for lauric acid monolayers in the liquid-expanded state and, except for curve (*c*), the results for a given surfactant were independent of trough dimensions or construction material.

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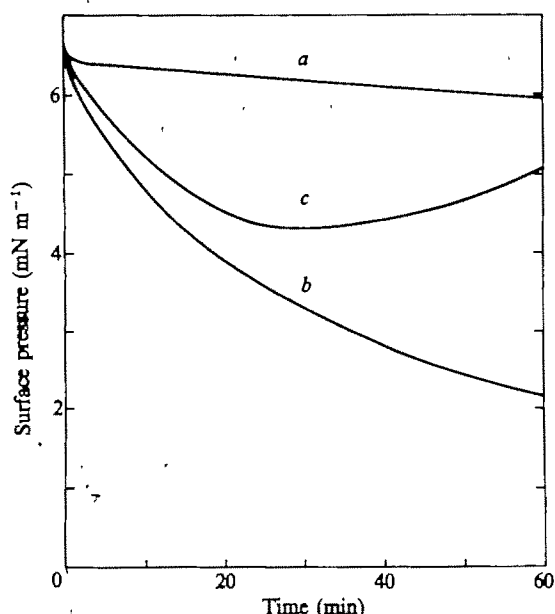


Fig. 1 Monomolecular film behaviour of myristic acid on 0.01M HCl at constant surface area. Relative humidities are 100% (*a*), 68% (*b*) and increasing 72 to 84% (*c*). The surface pressure was measured within 30 s of spreading and compressing the film. Each curve is reproducible to within at least 0.1 mN m⁻¹. Temperature 21.2 °C.

Ice elevation map of Queen Maud Land, Antarctica, from balloon altimetry

THE technique and expected coverage of ice elevation mapping using radio altimetry data from the Twerle balloons¹, have already been discussed². We present here preliminary results obtained after analysing 18 balloon traverses, with 1,900 elevation points. A map of the entire continent agrees very well

with existing maps, suggesting the validity of the method. A larger-scale, more detailed map of Queen Maud Land permits better delineation of the drainage systems of that region than has previously been possible.

In Fig. 1 the entire continent is separated into 30×38 km rectangles. The digit printed in the rectangle is the 100-m digit of the unweighted average of all elevation readings obtained in that rectangle (S represents the elevation range of 20–99 m). Contour lines at 500-m intervals are plotted where data coverage permits. Figure 2 is an expanded scale of the 330° – 90° E section, with the balloon trajectories and contour lines every 100 m.

The contour lines on both maps are based on the balloon altimetry data alone. The elevation accuracy, has been shown² to be better than 60 m. The data gathered thus far does indicate a change in that figure of accuracy.

Although the map of the entire continent (Fig. 1) does not show any new features of the general pattern of elevations on the Antarctic ice sheet it does demonstrate the validity of the balloon technique by its close correspondence with standard American and Soviet maps of surface elevation of the Antarctic ice sheet based on data from ground traverses and aircraft flights^{3,4}. An example is the occurrence in central East Antarctica of a closed 4,000 m contour similar in size and shape to that previously observed from Soviet over-snow traverses, showing the good agreement between the data from different sources in this region of maximum elevation above sea level and maximum distance from the coast.

The more detailed map (Fig. 2) is of particular interest from the glaciological standpoint, as Queen Maud Land is a region in which there were very few previous elevation data. Although mapping is not yet complete, there are already some interesting features.

For example, a 3,700-m closed contour appears at about 77° S 40° E, indicating a fourth 'dome' along the central East Antarctic Ridge ('Dome A', 'Dome C', to the east, are already well-known). This feature does not appear on either of the standard Antarctic maps^{3,4}, although it is on a map drawn from the measurements on the Japanese traverse in 1968–69 from Syowa to Plateau Station⁵. The contour in Fig. 2 has been shaped to agree with the Japanese map. However, an additional balloon traverse, only partially analysed, hints that this 3,700 m contour is considerably broader, and may extend as far as 34° E.

On the other hand, the absence of a major dome (higher than the contour interval) centred at about 75° S 12° E, that is shown on the American map³ but not on the Soviet map⁴, is confirmed by the present map. Previous data spacing was wide enough to permit either interpretation.

Of more interest than the absence or presence of domes along the summit ridge may be the indication of valleys inland from several coastal glaciers, showing the convergence of ice flow from substantial drainage basins into those glaciers, and in some cases suggesting the occurrence of ice streams. One such valley, presumably feeding into Recovery Glacier, shows clearly around 81° – 82° S, 0° – 10° W, some 350 km inland. Crevasses as



Fig. 1 Elevation map (polar azimuthal equidistance projection) for the whole Antarctic continent from balloon altimeter data. Small numbers denote the hundreds digit in the elevation figure. Contour interval is 500 m.

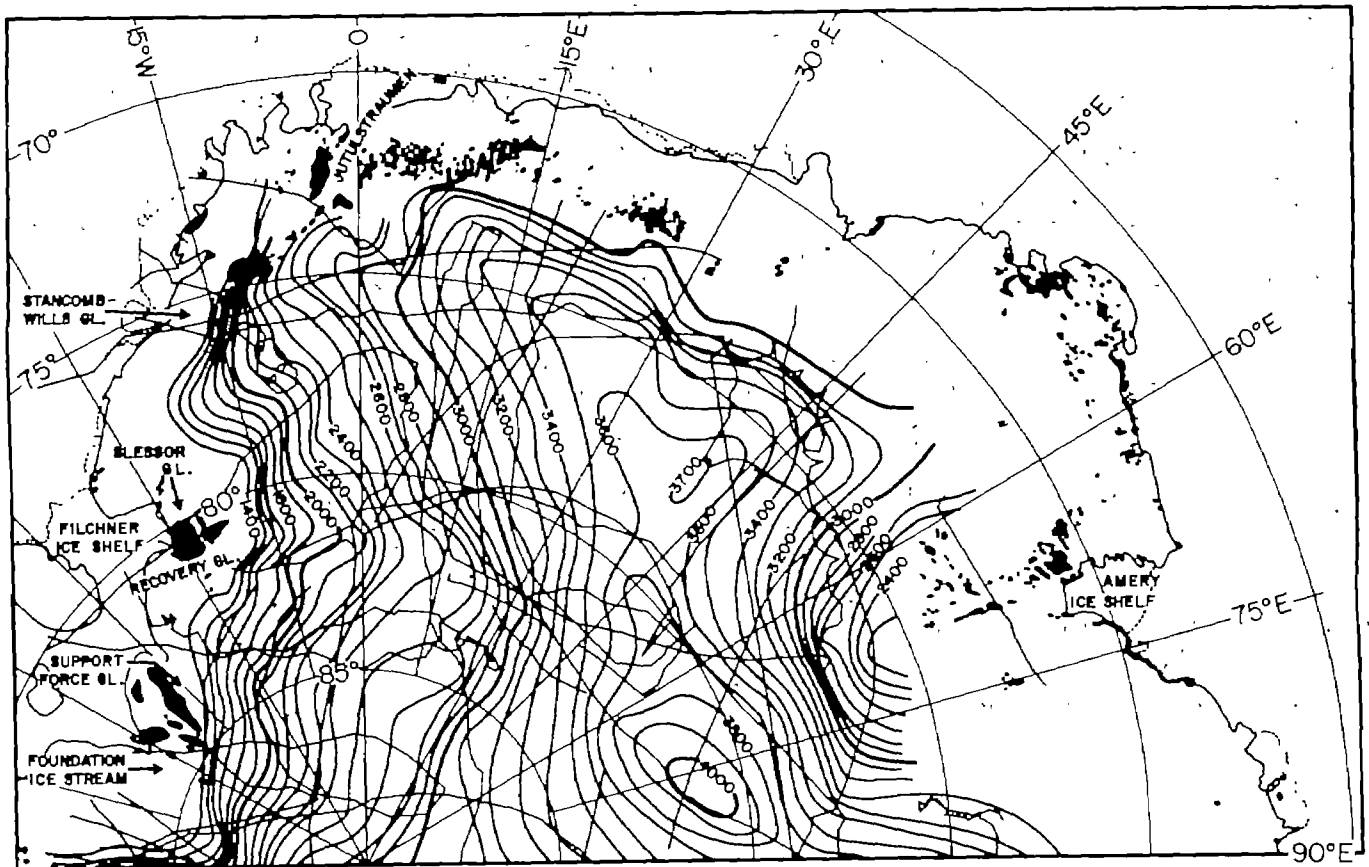


Fig. 2 Elevation map (polar azimuthal equidistance projection) of Queen Maud Land and neighbouring regions. Thin lines are individual balloon tracks. Contour interval is 100 m.

far inland as $83^{\circ}\text{S } 15^{\circ}\text{E}$ (ref. 6) suggest a deeply penetrating ice stream⁷.

Another valley can be seen around $77.5^{\circ}\text{S } 10^{\circ}\text{W}$. Contour lines have been interpolated between rather widely-spaced balloon tracks on the assumption that this valley is associated with Slessor Glacier. Although that is not definite, the shape of the Coats Land coast necessitates the pronounced westward bowing of the contours north-east of Slessor Glacier (Fig. 2) unless the ice sheet profile in that region is most exceptionally flat. Furthermore, massive outflow through the Recovery Glacier-Slessor Glacier system is consistent with the high movement rates of the Filchner Ice Shelf front around 40°W ⁸. As drawn, this drainage system is the largest in East Antarctica, exceeding that feeding the Amery Ice Shelf by ~20%.

Stancomb-Wills Glacier (Dalgicah Ice Stream) is very fast-moving—the ice shelf it feeds is advancing at almost 1.5 km yr^{-1} (ref. 10). In the light of this, the drainage area that seems to feed it is surprisingly small.

A clear indentation of contours, reflecting a major region of outflow through the mountains of New Schwabenland by Jutulstraumen⁹ appears just north-east of $75^{\circ}\text{S } 0^{\circ}\text{E}$. This valley shows good agreement with elevations from the single traverse line in the region, that of the Norwegian-British-Swedish Antarctic Expedition, 1949–52.

In contrast, there appears from fairly good coverage to be little contour line indentation inland of the Pensacola Mountains, where one might otherwise suspect concentrated drainage through either Support Force Glacier, or Foundation Ice Stream, or both. The total area of the drainage basin feeding the southern Filchner Ice Shelf, formerly thought to be the main source of the ice shelf input¹¹, is now seen to be only about half as large as the Recovery Glacier-Slessor Glacier system.

Work on the mapping of the Antarctic ice sheet from the

balloon data continues, and several of the more speculative features mentioned here clearly require closer examination as the data coverage increases. Nevertheless, the map presented here for Queen Maud Land is already substantially more detailed than any previously available.

The information on which this report is based was obtained during the Tropical Wind, Energy Conversion and Reference Level Experiment (Twerle), supported by NASA and NSF. The analysis is supported by a grant from the US-Israel Binational Science Foundation (BSF), Jerusalem, Israel.

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Depth of curie temperature in continental shields: a compositional boundary?

MUCH attention is being given to estimating the depth to the bottom of the crustal magnetised layer. The latter can be interpreted as the surface where ferrimagnetic minerals, which are responsible for the magnetisation of crustal rocks, reach their Curie temperatures ~ 1.5 . The depth of this surface is therefore temperature dependent and it can be used to locate thermal anomalies within the continental crust. One application would be in the search for shallow intercrustal heat sources (such as large magmatic reservoirs or cooling plutons) which would be of economic interest as natural energy sources^{3,6}. In all these works the bottom of the crustal magnetised layer is assumed to correspond to an isotherm whose temperature is approximately the Curie temperature of pure magnetite (580 °C). Haggerty⁷ cautioned against assuming a single Curie temperature for the continental crust and pointed out that Curie temperatures as low as 300 °C may exist in the crust because of the low temperature-oxidation of titaniferous magnetite. Furthermore, he suggested that depths of Curie temperatures greater than 20 km may be due to serpentinisation of mafic and ultramafic components of the continental lower crust with consequent formation of Fe-Co-Ni alloys with Curie temperatures in the range 620–1,100 °C. We show here that depths of Curie temperatures computed for continental areas characterised by normal and low heat flow cannot be interpreted as isothermal surfaces with temperatures at or above 580 °C. We suggest that the bottom of the crustal magnetised layer may correspond to vertical compositional changes within the crust.

This idea occurred to us while we were interpreting the results of the computation of the depth to the bottom of the crustal magnetised layer in southeastern Minas Gerais, Brazil⁸. This is a Precambrian area where extensive outcrops of high grade metamorphic rocks occur. This metamorphic basement was formed 2,000–2,600 Myr ago⁹ and it is locally covered by metasediments of Upper Precambrian age and sediments formed during the last orogenic event which affected this area, the Brazilian orogenesis (600–650 Myr)^{9,10}. A crustal model was computed for a part of this area from gravity data¹¹ also using the information obtained from an unreversed deep refraction profile¹². This model indicates a normal crustal thickness of

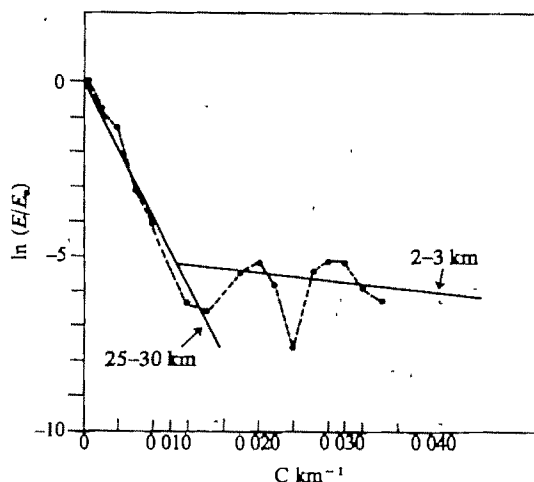


Fig. 1 Power spectrum of the total intensity anomaly field in southeastern Minas Gerais, Brazil. Continuous lines are regression lines relative to depth of anomalies at 25–30 km and 2–3 km, respectively.

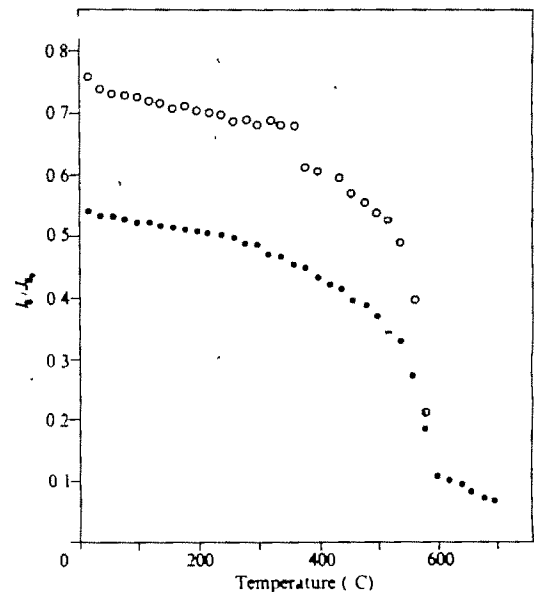


Fig. 2 Saturation magnetisation plotted against temperatures for two acidic charnockites outcropping along the coast of Sao Paulo State, Brazil. Chemical and petrological data about these rocks are available²⁰. Measurements were carried out on the Curie balance of the Istituto di Geologia e Geofisica of the University of Napoli, Italy²⁷. O, MP003; □, MP035.

38–40 km with local thickening to 45 km (see Fig. 3). Furthermore, predominantly acidic rocks seem to be the crustal constituents at least to a depth of 22–25 km. The gravity derived model requires a lower crustal layer with density between 2.8 and 3.3 g cm⁻³. If a density of 3.0 g cm⁻³ is assumed, the normal depth to the top of this layer is about 23–22 km, with local thickening to 25 km. Seismic data¹² indicate a continuous increase of P-wave velocities in the lowermost 15 km of crust, with an average velocity of 7.4 km s⁻¹. Preliminary heat flow data in this area are in the range of 40–50 mW m⁻² with temperature gradients in the range 10–16 °C km⁻¹ (ref. 13).

The depth to the bottom of the magnetized crustal layer was computed from a very detailed aeromagnetic survey which was carried out in 1973 by the German company Prakla and the Brazilian Companhia de Pesquisa de Recursos Minerais¹⁴. The map of the total intensity anomalies was averaged on a 10 × 10 km grid and was then submitted to Fourier analysis. A typical power spectrum is given in Fig. 1. The decay of the energy content with frequency¹⁵ indicates a distribution of anomaly sources at 25–30 km at a depth of 2–3 km. A low pass filter with cut-off at 0.013 cycles per km (wavelength 80 km) was applied. The resultant residual anomalies were inverted by using two-dimensional polygonal bodies of uniform magnetisation whose upper surface corresponds to the ground level and the depth and morphology of its bottom is computed by iterative procedures which compare the theoretical field produced by the model with the observed field. The computed depths are in the range 22–27 km⁸.

These depths are in the range of 'normal' depths reported in the literature for areas of normal and slightly higher than normal heat flow^{4,5}. The average depth of Curie temperature in continental areas was estimated to be about 20 km (ref. 1). A linear extrapolation of shallow temperature gradients would actually predict temperatures higher than 500 °C at a depth of 20 km in areas of normal heat flow. However, a constant temperature gradient within the continental crust is not allowed by the energy balance between the measured heat flow and the radiogenic heat production determined on rocks which are believed to be the main constituents of the upper continental crust. Heat flows and radiogenic heat productions measured in

continental areas require a strong depletion of radioactive elements with depth¹⁶⁻¹⁸. This is also supported by geochemical considerations¹⁹. The main consequence of an upward concentration of radioactive elements is that the temperature gradient decreases with depth. Figure 3 shows the highest temperatures which can be reached within the crust of a normal heat flow area¹⁷. This temperature profile was calculated by assuming a stepwise distribution of radioactive elements, that is, a 5-km thick upper crustal layer with concentrations typical of acidic intrusive rocks and a 35-km layer with concentrations typical of high grade metamorphic rocks¹⁷. The highest temperatures which can be reached at 20–25 km of depth are about 250–300 °C. Temperatures above 500 °C can be reached only below the crust–upper mantle boundary. Only in areas with heat flow higher than 80 mW.m⁻², temperatures of 500–580 °C are possible at a depth of about 20 km. Different models of depletion of radioactive elements with depth do not significantly change these conclusions^{18,20}. Therefore the bottom of the crustal magnetised layer computed for areas of normal and low heat flow cannot represent an isothermal surface at 500–580 °C.

Geophysical models of the continental crust of typical Precambrian areas, such as the Canadian Shield²¹ or the Baltic Shield²², are similar to that inferred for Minas Gerais (Fig. 3). P-wave velocities of 6.1–6.3 km.s⁻¹ and densities of 2.8–2.9 g.cm⁻³ are determined down to a depth of 15–20 km. These characteristics are consistent with the occurrence of rocks of acidic composition and low to high (granulite facies) metamorphic grade²³. P-wave velocities of 6.8–7.4 km.s⁻¹ and density ~3.0 g cm⁻³ are usually inferred for the lower continental crust. Some geophysicists²⁴ believe that higher seismic velocities and densities of the deep continental crust are due to a progressive increase of metamorphic grade. However, P-wave velocities higher than 6.8 km.s⁻¹ have been measured in granulitic facies rocks with at least 40% pyroxene plus hornblende at pressures of 4–10 kbar²⁵. Although the available seismic data indicate a very complex structure for the continental lower crust²⁵, the increase of metamorphic grade must be accompanied by a change of chemical composition toward intermediate or mafic in order to fit the seismic velocities²³.

Curie temperatures of acidic rocks are unlikely to be lower than 500 °C. It is well known that plutonic rocks and lavas of acidic composition contain almost pure magnetite as the main ferrimagnetic phase²⁶. Curie temperatures above 500 °C have also been measured in acidic rocks which are stable in the lower continental crust. Saturation magnetisation versus temperature curves for two charnockites outcropping along the coast of São Paulo State, Brazil, are reported in Fig. 2. One curve has a clear Curie temperature at 580 °C, indicating that pure magnetite is the main ferrimagnetic phase. The other sample is characterised by a more complex curve, with a tail to about 700 °C. This is probably a consequence of low temperature oxidation of the rock with resulting production of maghemite (Curie temperature 545 °C), which, being unstable at temperatures between 250

and 550 °C, tends to transform into hematite (Curie temperature 860 °C). High grade metamorphism of acidic rocks is hence unlikely to give rise to low Curie temperature ferrimagnetic phases.

Ferrimagnetic phases contained in alkali-basaltic and andesitic rocks are intermediate members of the ulvöspinel-magnetite solid solution series, with Curie temperatures in the range 100–300 °C^{26,27}. No experimental data are available on Curie temperatures of high grade metamorphic rocks of intermediate to mafic composition. However, in this case magnetisation of the intermediate members of the ulvöspinel-magnetite solid solution series would produce Curie temperatures in the range 300–450 °C⁷. Curie temperatures of rocks of intermediate (andesitic) or mafic composition are in the range of possible crustal temperatures of Precambrian areas. The Curie temperature at depth can therefore correspond to the surface where a change of chemical composition of crustal rocks occurs.

Figure 3 shows this correspondence with reference to the crustal model of southeastern Minas Gerais, Brazil. The Curie temperature is assumed to be 580 °C in the upper crust and about 200 °C in the lower crust. The bottom of the magnetised layer (dashed area) is slightly below the top of the lower crustal layer and corresponds to a compositional change of the crust towards more mafic terms.

If this interpretation is correct, the depth of Curie temperature is not sensitive to small variations in the thermal state of the crust, but only to the composition of rocks at that depth. The depth of Curie temperature can be used therefore as a marker of vertical changes in chemical composition of the continental crust in normal heat flow areas.

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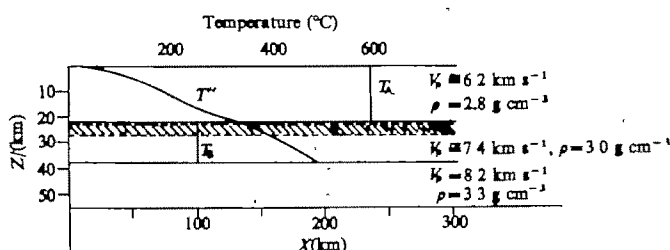


Fig. 3 Geophysical model of the continental crust in southeastern Minas Gerais from gravity¹¹, seismic¹², heat flow¹³ and aeromagnetic data. Curie temperature of crustal rocks at depth 0–22 km (T_A); at depth 22–38 km (T_B); T_m , maximum allowable temperature in shield areas; hatched area is the bottom of the magnetised layer.

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Palaeo-oceanography of the post-glacial Eastern Mediterranean

DURING the late Pleistocene the hydrography of the Eastern Mediterranean underwent severe changes in response to climatic fluctuations. Among the most striking of these changes were periods of deep water stagnation. Episodes of oxygen depletion are indicated by the presence of layers of dark sapropelic muds enriched in organic carbon, devoid of the remains of benthic organisms¹⁻⁴. While stagnant conditions prevailed in the deep basin, the water column above 1,000–800 m remained ventilated, and cores raised above this depth do not contain sapropels⁴⁻⁶. Several theories have been proposed to explain the past stagnations. They all use a mechanism which stabilises the water column by forming a steep density gradient in the upper waters. In this way the vertical mixing, and hence the ventilation, are retarded, oxygen is depleted in the deep water and stagnation commences. Olauson's theory⁷, whereby influx of freshwater leads to reduced surface density and the interruption of normal vertical circulation, is the most widely accepted at present. This theory is supported by recent studies⁸⁻¹⁰, which use measurements of oxygen isotope composition in shells of planktonic foraminifera. These studies demonstrate that many of the sapropels correspond to $\delta^{18}\text{O}$ minima, suggesting freshwater influx, and thus a reduced upper water salinity at the time of the sapropel formation. Moreover, the epipelagic species record greater depletion of ^{18}O than the mesopelagic species, indicative of the establishment of salinity and density gradient in the upper waters¹⁰. Using a similar approach we have attempted to investigate in detail the hydrographic changes associated with the deposition of the most recent sapropel, which took place about 7,000–9,000 yr BP⁶.

Core GA32 from the continental slope off Israel—an area of very rapid accumulation of Nile derived sediments¹¹⁻¹³—was sampled every 10 cm, which represent intervals of about 300 yr. The epipelagic planktonic species *Globigerinoides ruber* and the benthonic species *Uvigerina mediterranea* were chosen for analyses of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$. Being a shallow core, GA32 does not contain any sapropels. However, determination of the core interval corresponding to the time of the last sapropel deposition in the deep cores is straightforward when the $\delta^{18}\text{O}$ minimum of *G. ruber* (230–320 cm, Fig. 1) is used to correlate other core records such as RC9–181 and TR172–22^{8,9}. A time scale based on this correlation and on an assumption of constant accumulation rate is used in Fig. 1. Note that this scale should be used only as a rough guide because the accumulation rate of the core might not be uniform¹².

In general the benthonic species is enriched in ^{18}O relative to the planktonic species. At present this difference is primarily due to two effects. The oxygen isotopic fractionation between the calcitic shells and the seawater is temperature dependent, therefore at 590 m ($\sim 14^\circ\text{C}$) ^{18}O becomes more enriched in the shells than at the surface ($18\text{--}28^\circ\text{C}$). In addition, nonequilibrium isotopic fractionation due to vital effects¹⁴⁻¹⁶ would tend to increase the difference between *G. ruber* and *U. mediterranea*.

Assuming that *G. ruber* has not undergone significant environmental adjustments such as a change in its depth habitat or a change in the season of optimal shell production, and that the isotopic fractionation between the shells of both species and the seawater has remained nearly constant during the past 12,000 yr, hydrographic conditions can be inferred from the isotopic variations.

Rapid ^{18}O depletion in both the planktonic and the benthonic species is registered in the lower part of GA32. This depletion corresponds to a transition from cool to warmer faunal composition in the area which was attributed to the ending of the last Glacial^{12,17}. Nevertheless, it seems that temperature alone would not account for all the isotopic change, as the faunal change over the same period is small, and temperature estimates

based on this change suggest that the temperature rise did not exceed $2\text{--}3^\circ\text{C}$ ⁹. Thus the major part of the isotopic change ($\sim 1.5\%$) has to be accounted for by an influx of freshwater. It has been suggested that glacier melt-water was the major source of this influx¹⁰, but increased precipitation at about the same time cannot be neglected⁹. In either case, the freshwater input would result in a considerable reduction of the surface salinity ($2\text{--}3\%$)¹⁰.

It is significant that the salinity changes on the bottom lag behind the surface changes. This lag is explained by a very slow mixing of the low salinity surface layer. For $\sim 1,500$ yr there was enough freshwater input to cope with the vertical mixing, and throughout this period stable stratification of the upper water column was maintained, as is evident from the difference between the isotopic composition of the surface dwelling and bottom dwelling foraminifera (Fig. 1). Following a brief reduction in the steepness of the density gradient ($\sim 7,500$ yr BP), stable water-mass stratification was re-established for several hundred years. It seems then that the supply of freshwater was not steady throughout the deposition of the last sapropel. Further indication of this is found in cores from the Adriatic Sea, and also in some deep cores from the Levantine basin, where the last sapropel is interrupted by a short period of ventilated bottom water^{4,8}. However, in many deep cores from the Eastern Mediterranean there is no such interruption. This might indicate that only part of the basin was ventilated in this short period, or that the rate of accumulation of many cores is too slow and a short event cannot be recorded.

Following the sapropel time, the $\delta^{18}\text{O}$ curves register only small fluctuations, and thus indicate relatively stable climatic and hydrographic conditions. However, in the period 4,000–3,000 yr BP the planktonic species was enriched in ^{18}O , and the difference between the lower and upper waters is reduced. This might indicate a more arid climate during this period.

Unlike the $\delta^{18}\text{O}$ in the foraminiferal shells, the $\delta^{13}\text{C}$ is not directly influenced by changes in the evaporation-precipitation budget, and its temperature dependence is small. Rather, it depends mainly on the isotopic composition of the dissolved CO_2 in the seawater, and on some biogenic effects which influence the isotopic fractionation^{15,16,18,19}.

The $\delta^{13}\text{C}$ in *G. ruber* from GA32 is 1.4% on the average and

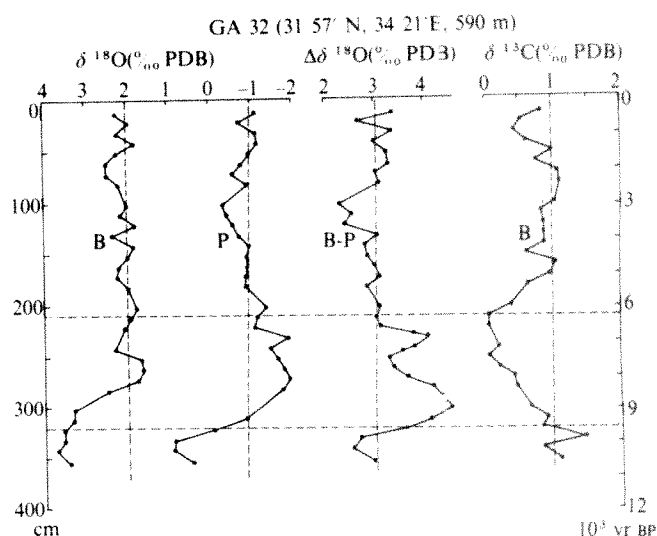


Fig. 1 $\delta^{18}\text{O}$ curves of the benthonic (B) species *Uvigerina mediterranea*, the planktonic (P) species *Globigerinoides ruber*, the difference ($\Delta\delta^{18}\text{O}$) between the planktonic and the benthonic species, and $\delta^{13}\text{C}$ curve of the benthonic species. Standard procedures were followed in the isotopic analyses²³, and 20 specimens of the planktonic species and five specimens of the benthonic were analysed on a Micromass 602C mass-spectrometer. Conversion to PDB was done through the CalTech standard PDB IV.

varies by about $\pm 0.5\%$. However, its changing values do not show an understandable trend. In this respect the record of GA32 is no exception to other such records of planktonic foraminifera from the Eastern Mediterranean^{9,20}. The $\delta^{13}\text{C}$ curve obtained for the benthonic species in turn shows a distinct minimum centred around 7,000 yr BP. This minimum probably resulted from increased proportion of dissolved carbon dioxide which originated from oxidation of organic matter. A similar process leads today to the formation of the $\delta^{13}\text{C}$ minimum which is closely associated with the oceanic oxygen minimum layer^{20,22}. Thus during periods of oxygen depletion in the deep parts of the Eastern Mediterranean basin, the $\delta^{13}\text{C}$ of the dissolved CO_2 decreased. The layer of the low oxygen content and low $\delta^{13}\text{C}$ propagated slowly upwards, and thus the minimum values reached the depth of a few hundred metres only 1,500 yr after the initiation of increased freshwater influx. Once this influx decreased and the ventilation resumed, the carbon isotope composition returned back to the pre-sapropel values. It is also possible that the ^{13}C -depleted carbon dioxide was not derived from direct oxidation of organic matter in the water column, but from oxidation of organic remains in the sediment. The latter process is slower, and might explain the slow response of the $\delta^{13}\text{C}$ to the changes in surface conditions.

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Evidence against Fisher's theory of dominance

FISHER'S theory of the evolution of dominance is based on the fact that heterozygotes for rare deleterious alleles, maintained by recurrent mutation from the wild-type allele at the locus, greatly outnumber homozygotes in a random-mating population at equilibrium¹. The fitness of a heterozygote carrying a mutant and the wild-type allele can be written as $1 - hs$ ($1 \gg h \gg 0$), compared with fitnesses of 1 and $1 - s$ for wild-type and mutant homozygotes respectively. Fisher postulated that mutant alleles were originally semidominant, so that $h > 0$. He explained the fact that most mutations are observed to be

recessive or nearly recessive to wild type as the result of selection for modifier genes which increase the fitness of the heterozygotes alone. He pointed out that selection to improve the fitness of the mutant homozygotes is likely to be relatively ineffective because of their rarity compared with the heterozygotes, and also because the presence of the wild-type allele in heterozygotes renders them more amenable to selection towards the wild type. Using this theory, the level of dominance of the mutant alleles, h , is gradually reduced towards zero, while s remains constant. Wright² criticised this because the heterozygotes for mutant alleles are so rare that the intensity of selection on a gene which modifies h is of the same order as the rate of mutation, u , to deleterious alleles at the locus undergoing dominance modification. He suggested that such a weak selection pressure is unlikely to overcome the effects of random genetic drift, or the selective consequences of pleiotropic effects of the dominance modifier. Later theoretical work^{3,4} has confirmed Wright's conclusion about the intensity of selection on dominance modifiers, so that many geneticists have come to doubt the validity of Fisher's theory⁵. The principle of dominance modification when applied to balanced polymorphisms (for example, Batesian mimicry⁶) is not, of course, subject to this objection and is generally accepted. Fisher¹, however, believed that the recessivity of mutant genes demonstrated the ability of even a minute selection pressure to cause significant evolutionary change. The issue cannot be settled by purely theoretical considerations, and it is evident that some empirical evidence capable of providing a critical test is required. (Demonstrations that dominance can be modified by artificial selection^{6,7}, although of great interest, are not relevant to the question of the efficacy of selection pressures as small as those postulated in Fisher's theory.) This paper points out recent experimental evidence on the heterozygous effects of mutations affecting viability in *Drosophila*⁸ that seems to be inconsistent with Fisher's theory, and also looks at two alternative theories proposed by Haldane⁹.

Fisher's theory implies that the intensity of selection on a modifier of dominance depends only on the mutation rate and the extent to which the level of dominance is affected by the modifier, and is independent of the homozygous effect of the mutant alleles^{2,3}. The reason for this can be seen as follows. The increment in fitness to the heterozygote after reducing h by a quantity Δh is $s\Delta h$. But the frequency of the heterozygotes is approximately $2u/hs$ ^{1,2}, so that the net intensity of selection on a modifier with effect Δh is $2u\Delta h/h$, which is independent of s . This argument tacitly assumes that linkage disequilibrium between the modifier and the primary locus can be neglected. Exact numerical computations of population trajectories suggest that this assumption gives an excellent approximation^{3,10}, except when dominance modification has proceeded so far that the mutant alleles have been rendered almost recessive. A similar conclusion about the lack of dependence of the strength of selection for dominance modification on s is true for the two models suggested by Haldane⁹. The first of these involves the selection of wild-type isalleles which are more dominant than the original wild type over the mutant alleles at a locus. The second involves selection for modifiers that strengthen the dominance of a favourable gene which is in the process of replacing an alternative allele. All three models predict that dominance should be reduced at about the same rate for genes with drastic effects on fitness as for genes with minor effects, so that no relationship between h and s should be observable.

The extensive literature on the heterozygous effects of deleterious genes affecting egg-to-adult viability in *Drosophila* was recently reviewed by Simmons and Crow⁸. It seems clear that newly-arisen spontaneous lethal mutations lower viability by 2–4% when heterozygous, giving an average h value of about 0.03 (ref. 11). The evidence is less clear for lethals derived from natural populations. Some studies have suggested heterotic effects of lethals^{12–14}, although most experiments seem to show small, deleterious effects of heterozygous lethals⁸. There is no indication, however, of anything but a small h value, of the order

of a few per cent, so lethals are usually nearly recessive in their effects on viability. This contrasts with findings on mildly deleterious genes. The evidence suggests an average h value of about 0.35 for newly-arisen, spontaneous mutations of small homozygous effect^{15,16}, and about 0.2–0.3 for mildly deleterious mutations carried on chromosomes extracted from natural populations^{8,16}. Although there are some technical problems associated with these estimates^{8,17,18}, the biases are in the direction of underestimating h for mildly deleterious genes. The experimental evidence therefore seems to contradict the predictions of the above models for the evolution of dominance, as h is found to be negatively correlated with s .

In contrast, Wright's² physiological theory of dominance predicts this type of relationship between h and s (ref. 19). This theory assumes that the function of most gene loci is to specify the structure of enzymes, and that most mutations cause a reduction in enzyme activity. If the wild-type enzyme is so active that the concentration of substrate rather than enzyme is rate-limiting for the enzyme-catalysed reaction in wild-type homozygotes, then the rate of the reaction is likely to show a law of diminishing returns as a function of the concentration or activity of the enzyme. This leads to the property that an allele that only slightly reduces enzyme activity is more nearly semi-dominant in combination with a wild-type allele conferring full activity, than an allele which reduces activity to near zero. If there is a relationship between fitness and the rate of production of the end product of the pathway in which the enzyme is involved, alleles with a high s will have a low h , and vice versa¹⁹. This theory requires that the wild-type enzyme has such a high activity that halving the concentration of enzyme produces only a small reduction in the reaction rate². Plunkett²⁰ and Muller²¹ have proposed models in which selection for buffering of the wild-type homozygotes provides an explanation for this apparently unnecessarily high efficiency of the wild-type enzyme.

In summary, the observation that h is negatively correlated with s for viability mutations of *Drosophila* suggests that either dominance of the wild-type allele is a purely biochemical phenomenon and has not been selected for, or that it has evolved by mechanisms other than those suggested by Fisher¹ and Haldane⁹.

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Delayed implantation is under environmental control in the badger (*Meles meles* L.)

IN the wild state, the European badger (*Meles meles* L.) becomes pregnant once a year during a post-partum oestrus in February or March¹. Fertilisation is followed, at the blastocyst stage of development, by a prolonged embryonic diapause which ends, when implantation occurs, in December or January. Implantation is thus delayed for 10 months² and it may be assumed, in accordance with Fries's hypothesis³, that the resumption of development in the winter is a genetically determined and adaptive phenomenon which results in birth during the spring when conditions for the young are most favourable. This pattern suggests that the time of implantation is related to some environmental factor such as photoperiod or temperature. To test this hypothesis, wild female badgers were captured in their set after fecundation and maintained during summer in conditions of 'artificial winter'. We report here that in such conditions implantation, gestation and birth can be brought forward 4 months.

Six females were kept at +5 °C in short photoperiod (10 h light: 14 h dark) from July to September. Lighting was provided by fluorescent lamps and the mean intensity of light was about 300 lx. Females were bled once a week and progesterone in plasma was determined, using a radioimmunoassay previously described⁴, to show the changing level of luteal secretion during the period of observation. In natural conditions of daylight and temperature, embryonic diapause in the badger is characterised by a low level of progesterone, the mean not exceeding 6.51 ± 1.60 ng ml⁻¹ (ref. 4). In contrast, just before implantation at the end of December or in January we have found that the level reaches the highest mean value achieved after fertilisation (16.9 ± 2.39 ng ml⁻¹) (ref. 4). When the photoperiod and temperature were decreased experimentally in July, there was a sharp increase in plasma progesterone to peak values (18 ± 0.91 ng ml⁻¹) similarly observed at implantation in January (20.16 ± 1.58 ng ml⁻¹). Experimental females whelped in early September, with plasma progesterone levels during gestation similar to those characterising pregnancy in animals kept in normal conditions of light and temperature (Fig. 1). The litters born in September 1978, that is during the summer, would have

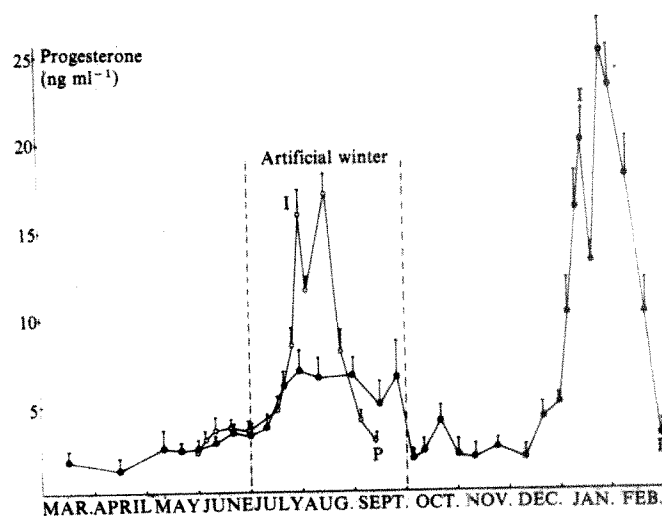


Fig. 1 ●, Progesterone profile during pregnancy in five control badgers (natural daylight and temperature conditions, mean $\pm$ s.e.m.). ○, Progesterone profile in six experimental females subjected to "artificial winter" (mean $\pm$ s.e.m.). With environmental parameters: daylight 10 h, temperature +5 °C unchanged from the beginning of experiment. I, implantation; P, parturition.

been dropped in the following spring had the animals not been exposed to our experimental conditions.

Many mammals undergo well defined annual cycles of sexual activity, and it has been demonstrated in several species that the cycle is influenced by photoperiod^{5,6}. In the mink⁷⁻⁹ and in the marten¹⁰ gestation becomes shorter when the photoperiod is increased artificially.

The experiment described here shows that full luteal function can be stimulated in the badger by manipulating the photoperiod and temperature, and it also suggests that in artificial conditions badgers could have two litters a year. The result conforms with our hypothesis that the hormonal changes which control implantation in the badger are activated by what we have called an 'impulsive factor'¹¹. Further experiments are underway to determine the relative significance of temperature and light in the operation of this factor.

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Pattern-selective adaptation in visual cortical neurones

PROLONGED viewing of a grating pattern produces striking 'after-effects', involving changes in the detectability, apparent size, orientation and contrast of subsequently viewed gratings¹⁻³. Studies of perceptual after-effects have been used to infer properties of neurones in the human visual cortex^{2,4,5} similar to those pattern-selective neurones whose sensitivities have been directly measured in the visual cortex of cats and monkeys^{6,7}. Such inferences are based on two assumptions: first, that perceptual changes result from changes in the distribution of activity within the responding population of neurones; second, that the effect of adaptation on each neurone of the population is to reduce its sensitivity uniformly to all stimuli. The experimental results reported here support the first but challenge the second assumption, as they show that after adaptation to a particular grating the sensitivity of a single neurone to that grating may be reduced more than its sensitivity to other gratings.

We recorded the activity of single neurones in the portion of the striate cortex representing the central visual field in young adult cats anaesthetised with nitrous oxide, using methods described in detail elsewhere⁸. The after-effects of prolonged stimulation by grating patterns were examined in 20 cells in five cats. Initially, we explored the receptive fields of each neurone with bars, edges and spots, and as a result classified them as simple, complex or hypercomplex^{6,7}. The face of a display

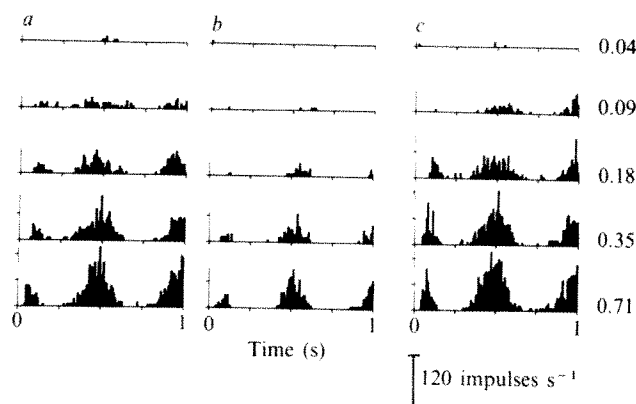


Fig. 1 Responses of a simple cell to gratings of different contrast, obtained before (a), during (b) and after (c) a period of adaptation. a Shows averaged responses to gratings of 0.75 c deg^{-1} , moving at 2 Hz in the optimal direction, presented at the five contrasts indicated on the right. Each grating was presented 20 times, each time for two stimulus cycles; each histogram thus shows the response to the passage of two bars of the grating across the receptive field. b Shows the responses to the test stimuli while the neurone was fully adapted to the same grating presented at high contrast (0.71). Each test stimulus was preceded by a presentation of the adapting stimulus. c Shows responses to the same test stimuli, measured in an experiment begun 12 min after the end of the adaptation experiment.

oscilloscope, which subtended a circle 10° in diameter at the cat's eye, was then centred on the receptive field (in the dominant eye); the other eye was covered.

The luminance of the oscilloscope screen (which was kept at a space average of 6 cd m^{-2} throughout) could be spatially modulated by signals from a computer to produce sinusoidal gratings. These are patterns of light and dark bars whose luminance profile perpendicular to the bars is a sine wave; their spatial frequency is the number of cycles that subtend one degree of visual angle, and their contrast is the difference between the maximum and minimum luminance divided by twice the mean luminance. All the gratings moved steadily in a direction orthogonal to their bars at 2 Hz (so that two cycles crossed any point on the screen in 1 s). The computer controlled the orientation, direction of movement, spatial frequency and contrast of the grating, and also, within the limits of the 10° field, its spatial extent. While presenting grating patterns, the computer counted action potentials and compiled them into average response histograms; these were saved on disk for later analysis.

For each neurone we first established the spatial frequency, orientation, extent and direction of movement of the optimal grating; then, using optimal stimuli, we measured the relationship between response and stimulus contrast. Figure 1a shows the results of a set of such measurements. In this and all other experiments, the stimuli were presented in blocks. Within a block, each stimulus was presented once for 1 s, during which two cycles of the grating crossed the receptive field. The order of presentation within a block was random and different within each block, of which there were normally 20 in an experiment.

The contrast-response relationship was then determined again, while the neurone was fully adapted to a high-contrast grating. Each block of this 'adaptation' experiment began with a 32- or 64-s presentation of the adapting grating; each test stimulus after the first was preceded by a 4- or 8-s topping-up presentation of the adapting stimulus². Figure 1b shows the results of an experiment in which the adapting stimulus was of optimum spatial frequency and orientation. A comparison of these responses with their counterparts in Fig. 1a shows clearly that prolonged stimulation by a high-contrast grating greatly reduced the strength of responses. In separate experiments we established that the initial period of adaptation used was

sufficient to produce the maximum reduction in sensitivity, and that the topping-up period was sufficient to maintain it.

Figure 1c shows the results of measurements begun 12 min after adaptation, by which time responses had fully recovered. In all cases, except three where the unit was lost, we verified that within 5–15 min after the end of adaptation, responses were similar to those observed before adaptation.

Figure 2A shows the full results of this experiment, which was carried out on a simple cell, and Fig. 2B shows the results of a similar experiment on a complex cell. For both neurones, Fig. 2 shows the contrast-response relationship measured before adaptation (a), while the cell was fully adapted (b) and after a period of recovery (c). For both neurones, adaptation raised the contrast required to produce a given response by a factor of about four, and recovery returned it to very nearly its former value. Although the changes brought about by adaptation are apparently very similar in form for the two units when expressed in this way, the extent to which the response to a grating of fixed contrast is reduced by adaptation may be a poor guide to the extent of this adaptation. Thus, in the case of the complex cell, adaptation produces a much smaller reduction in the response to high-contrast stimuli than it does to ones of lower contrast. Increase in the contrast required to produce a given response (that is, reduction in sensitivity) seems to be the appropriate measure of adaptation.

To ascertain how great a change in sensitivity is brought about by an adapting stimulus, one must compare responses to stimuli of different contrasts presented before, during and after adaptation. Adaptation brought about clear reductions in sensitivity in 19 of the 20 neurones we studied; the remaining unit, a

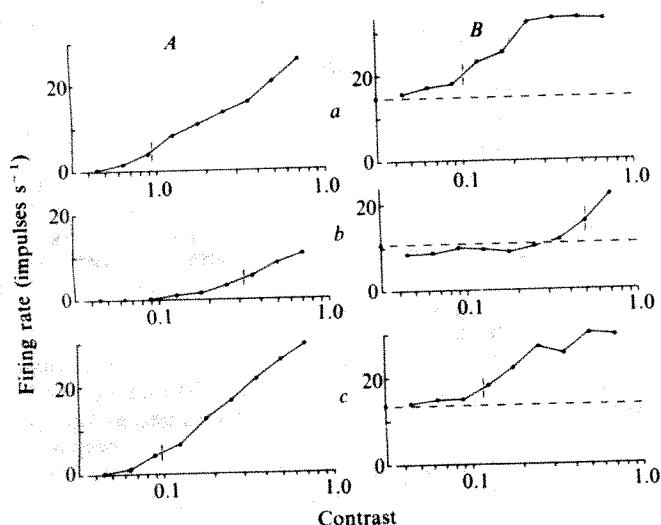


Fig. 2 The effect of adaptation on contrast-response relations. **A**, Results of the experiment partially illustrated in Fig. 1. **a** Shows the contrast-response relationship measured before adaptation, **b** shows the relationship while the cell was fully adapted, and **c** shows it after a 12-min recovery period. **B**, Results of a similar experiment on a complex cell; the spatial frequency of all gratings was 2.0 c deg^{-1} , and they moved at 2 Hz. Horizontal dashed lines indicate spontaneous activity. As in **A**, the three graphs show measurements made before (**a**), during (**b**) and after (**c**) adaptation. The vertical bar on each graph indicates the contrast necessary to elicit a criterion response of 5 impulses s^{-1} above spontaneous firing. Comparison of these criterion contrasts provides a useful index of adaptation; within limits, the choice of criterion does not strongly affect the index obtained. Here and in Fig. 3, the response plotted is the average discharge rate elicited by the stimulus. This is the natural measure of the response of complex cells, which do not give modulated responses to most gratings; simple cells do give modulated responses but as they generally lack maintained discharge, measures of modulated or unmodulated responses are very well correlated (see Fig. 1).

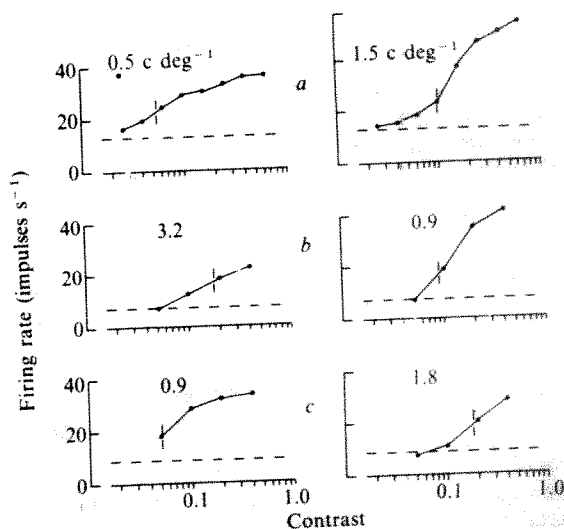


Fig. 3 The spatial frequency selective nature of adaptation. In these experiments, on a complex cell, test gratings of 0.5 and 1.5 c deg^{-1} were used. **a** Shows the contrast-response relationship for both spatial frequencies, measured before adaptation. **b** Shows the results of an adaptation experiment in which the adapting grating had a contrast of 0.71 and a spatial frequency of 0.5 c deg^{-1} . **c** Shows the results of a similar experiment in which the adapting grating had a contrast of 0.71 and a spatial frequency of 1.5 c deg^{-1} . The vertical bars on each graph, as in Fig. 2, indicate the contrast needed to elicit a criterion response (here, $10 \text{ impulses s}^{-1}$ above spontaneous level). Each adaptation experiment was followed by a period of recovery, during which we verified that responses had returned to pre-adaptation levels. The ratios of the post-adaptation and pre-adaptation contrasts needed to elicit the criterion response are shown on each of the four lower graphs; values greater than 1 indicate an elevation of this 'threshold', values less than 1 a reduction.

monocularly driven neurone with unusually broad orientation selectivity, showed no reliable effects. Spontaneous activity was not always depressed by adaptation.

We were especially interested to know whether the reduction in sensitivity that followed adaptation was confined to stimuli of the same spatial frequency as the adapting pattern; we examined this question in experiments where the spatial frequencies of the test and adapting patterns were varied. The graphs in Fig. 3a show contrast-response relationships for a complex cell, obtained with gratings of 0.5 and 1.5 c deg^{-1} ; vertical bars mark the contrast needed to produce a response of $10 \text{ impulses s}^{-1}$. The graphs in Fig. 3b show the same relationships obtained during adaptation to a grating of 0.5 c deg^{-1} . Responses to gratings of this spatial frequency were much reduced by adaptation (the contrast required to produce a response of $10 \text{ impulses s}^{-1}$ was elevated by a factor of more than three), whereas responses to gratings of 1.5 c deg^{-1} were hardly affected. The graphs in Fig. 3c show the results of a complementary experiment, in which the spatial frequency of the adapting grating was 1.5 c deg^{-1} . In this case, sensitivity for test gratings of 0.5 c deg^{-1} was unaffected, whereas sensitivity for gratings of 1.5 c deg^{-1} was much reduced. The effect of adaptation on this neurone cannot be explained by a uniform change in sensitivity; some spatial frequency selective mechanism must be involved. We observed behaviour of this kind in all seven complex neurones in which we studied the spatial frequency selectivity of adaptation; of three simple cells, one seemed to show spatial frequency selective adaptation, one definitely did not, and the third gave variable results.

Our experiments confirm two earlier reports that the behaviour of cortical neurones may be altered following a period of prolonged stimulation^{9,10}. However, it is necessary to

measure sensitivity in order to characterise the changes adequately; alterations in spontaneous activity, or in responses to high-contrast stimuli, provide an incomplete description of the effects of adaptation.

Our most surprising observation is that the loss of sensitivity in cortical neurones can be specific to the adapting stimulus. Our observations on complex cells might be explained by supposing that each complex cell is excited by a group of simple cells having different frequency selectivities. However, because the spatial frequency selectivity of complex cells is little different from that of simple cells¹¹, and because we have observed frequency-selective adaptation in a simple cell, this is not a convincing argument. Alternatively, adaptation in both simple and complex cells might result from changes in the afferent relay from the lateral geniculate nucleus. Again, this seems unlikely, as the spatial frequency selectivity of cortical neurones is far greater than that of geniculate neurones¹², and also as we have found in separate experiments that the responses of geniculate cells are unaffected by adaptation. We are left with the suggestion that some pattern-selective, perhaps inhibitory, cortical mechanism is involved.

Our observed frequency-selective loss of sensitivity is inconsistent with some models of perceptual after-effects^{2,5}. Although our experiments do not question the idea that human perceptual adaptation reflects adaptation in cortical neurones, they do limit the inferences about the properties of these neurones that may be based on adaptation experiments in man.

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Effects of calcium on the intensity-response curve of toad rods

THERE is evidence that Ca^{2+} is stored in the saccules in the outer segments of vertebrate rods, and released by light (for review, see ref. 1). Thus, light apparently increases $[\text{Ca}^{2+}]_i$, the cytoplasmic Ca^{2+} concentration. It now seems unlikely that this increase of $[\text{Ca}^{2+}]_i$ serves as an internal transmitter in generating the light response²⁻⁴. However, there is compelling evidence in the ventral photoreceptors of *Limulus*^{5,6} that it reduces photoreceptor sensitivity during light adaptation. In vertebrate rods an alteration of $[\text{Ca}^{2+}]_o$, the external Ca^{2+} concentration, alters $[\text{Ca}^{2+}]_i$, and the primary effects of changing $[\text{Ca}^{2+}]_o$ seem to be exerted inside the cell^{1,7,8}. Increased $[\text{Ca}^{2+}]_o$ mimics certain aspects of light adaptation, including hyperpolarisation of the resting membrane potential and reduction of light responses⁹⁻¹¹. Light adaptation also shifts the entire

intensity-response function (the V -log I curve) towards higher intensities on the intensity axis, thus reducing the sensitivity of toad rods¹¹. We report here that increased $[\text{Ca}^{2+}]_o$ can mimic this important aspect of light adaptation, whereas lowered $[\text{Ca}^{2+}]_o$ mimics dark adaptation. Control experiments indicate why altered $[\text{Ca}^{2+}]_o$ did not shift the V -log I curve in the recent work of Lipton, Ostroy and Dowling¹¹, who thus concluded that Ca^{2+} does not control this major aspect of photoreceptor sensitivity.

Experiments were carried out in the isolated, inverted and superfused retina of the toad, *Bufo marinus*, as described previously¹⁰. The receptor surface was viewed with a high-resolution infrared imaging system, and only retinal areas were used where the receptors were normal in appearance over a large area. Contact of the electrode tip with the distal end of an outer segment was readily visualised, and most recordings were from outer segments¹⁰. Deeper recordings, some of which were probably from inner segments, gave results similar to those obtained in outer segments. All recordings were from red rods, as determined by maximum sensitivity at about 500 nm. Ultra-fine micropipette electrodes were formed and advanced by previously described techniques¹²; intracellular recordings were readily obtained showing relatively large membrane potentials and light responses that could be maintained stably for up to 3 h. Light stimuli were introduced from below and focused by the condenser system of the microscope. Our control perfusate contained: NaCl (94 mM), KCl (2 mM), MgCl_2 (1 mM), NaHCO_3 (15 mM), glucose (10 mM) and CaCl_2 (1.8 mM). After bubbling with 98.5% O_2 and 1.5% CO_2 , it had a pH of 7.7-7.8; the temperature was maintained at 21-23°C. In this solution both the concentration of bicarbonate and the pH at our maintained temperature were based on an analysis of blood plasma in *Bufo marinus*¹³.

Figure 1 shows fully dark adapted V -log I curves of a single cell at three values of $[\text{Ca}^{2+}]_o$. It is critical to test ionic effects on photoreceptor sensitivity in dark adapted conditions, as ionic alteration of sensitivity to a steady background tends to cancel the measured alteration of sensitivity to the test stimulus. The reasons for this, and our methods for ensuring complete dark adaptation, have been described elsewhere¹⁴. Each V -log I curve required about 15 min, because of the stimulus spacing necessary to maintain dark adaptation. Following each change of solution, turnover in the perfusion chamber required less than 1 min. At least 5 min were always allowed before taking records, at which time the resting membrane potential and light responses had stabilised in the new solution. In Fig. 1 the value of $[\text{Ca}^{2+}]_o$ was at the control level of 1.8 mM for curve 1, then elevated to 6.0 mM for curve 2, returned to 1.8 mM for curve 3, lowered to 0.3 mM for curve 4, and again returned to 1.8 mM for curve 5. Each smooth curve in Fig. 1 is the computer-generated best fit to the data points, using the method of least squares and the Naka-Rushton equation, $V/V_{\max} = I^n / (I^n + \sigma^n)$. In this equation, V is the peak response amplitude elicited by a given stimulus intensity, I , and $V_{\max}$ is the maximum (saturated) response amplitude. The constant, σ , is the stimulus intensity eliciting a response of one-half the maximum amplitude, and in normalised curves the constant, n , is proportional to the slope at σ .

As previously demonstrated¹¹, and confirmed by us, the effects of light adaptation on the V -log I curve of toad rods include a reduction of $V_{\max}$, a reduced slope of the rapidly rising central portion of the curve, and a shift of σ to the right (towards higher intensities). Figure 1 shows that all these effects were mimicked by increasing $[\text{Ca}^{2+}]_o$ from 1.8 to 6.0 mM, whereas lowering $[\text{Ca}^{2+}]_o$ from 1.8 to 0.3 mM had the opposite effects. After the supra-normal $[\text{Ca}^{2+}]_o$, curve 3 shows almost complete recovery on return to the control solution. Following the sub-normal $[\text{Ca}^{2+}]_o$, curve 5 shows a more distinct loss of response amplitude on return to the control solution. In another experiment, however, when we waited 30 min after returning from 0.3 mM to 1.8 mM of $[\text{Ca}^{2+}]_o$, the final curve closely matched the initial control curve. As the effects of elevated and lowered

$[Ca^{2+}]_o$ were reversible, they apparently did not damage the photoreceptors. Effects of both elevated and lowered $[Ca^{2+}]_o$ have been tested in 10 different cells, and the results have been highly consistent. When $[Ca^{2+}]_o$ was increased from 1.8 to 6.0 mM the σ shift averaged +0.26 log units, and when $[Ca^{2+}]_o$ was lowered from 1.8 to 0.3 mM the σ shift averaged -0.49 log units. In both cases the s.e.m. was 0.04 log units. These σ shifts seem highly significant, as they show that altered $[Ca^{2+}]_o$ can mimic this important aspect of adaptation, with σ serving as a conventional index of the position of the V -log I curve on the intensity axis.

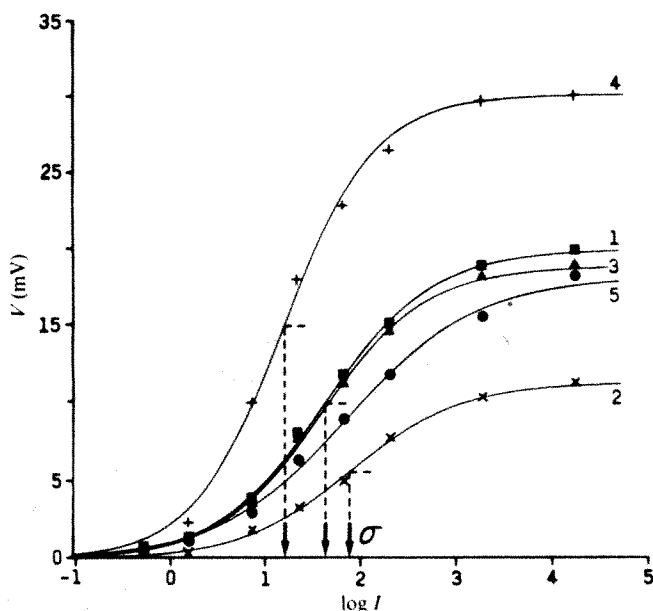


Fig. 1 Effects of $[Ca^{2+}]_o$ on the V -log I curve recorded intracellularly in the outer segment of a dark adapted red rod. Each point represents peak response amplitude. For each curve, responses were recorded in the order of increasing intensity. The sequence of curves is indicated by the numbers at the right. $[Ca^{2+}]_o$ was 0.3 mM for curve 4, 1.8 mM for curves 1, 3 and 5, and 6.0 mM for curve 2. A circular stimulus spot was centred on the electrode tip and focused on the outer segments, using direct viewing with infrared light in the stimulus beam. The stimulus spot was 1.0 mm in diameter at the outer segments, which more than covered the rod's receptive field¹⁷. Flashes of 500 nm light were 100 ms in duration, with a sufficient interval after each flash to maintain dark adaptation¹⁴. Stimulus intensities are expressed in photons absorbed per rod-flash, as described elsewhere¹⁴. The recording bandpass was 0–30 Hz, which did not affect response time course or amplitude. The resting membrane potential in control solution was -38 mV and showed no significant change during the experiment. In elevated $[Ca^{2+}]_o$ the membrane hyperpolarised by about 1.0 mV, and in lowered $[Ca^{2+}]_o$ depolarised by about 6.0 mV. With raised $[Ca^{2+}]_o$ the value of n remained constant at the control value of 0.75, so in high $[Ca^{2+}]_o$ the reduced slope of the V -log I curve resulted only from the decrease of V_{max} . In lowered $[Ca^{2+}]_o$ the value of n increased to 0.86, which contributed part of the observed increase in slope of the V -log I curve.

In their work on toad rods, Lipton *et al.* observed effects of $[Ca^{2+}]_o$ on V_{max} and the slope of the V -log I curve that were similar to the effects reported here. We therefore examined why they did not observe effects of altered $[Ca^{2+}]_o$ on the value of σ , even when the preparation was reported to be dark adapted. First, our perfusate contained 15 mM of bicarbonate, which was used for buffering. The perfusate of Lipton *et al.* contained only 0.13 mM of bicarbonate, and buffering was done with HEPES.

Our higher bicarbonate concentration seems preferable, as it is based on a plasma analysis in *Bufo marinus*¹³. Also, there is evidence that a bicarbonate concentration of about 25 mM is required in some other vertebrates for maintaining normal function of either photoreceptors¹⁵ or the adjacent pigment epithelium¹⁶. When both solutions were tested on the same cells, our control perfusate yielded V -log I curves with significantly higher values of V_{max} than obtained in the control perfusate of Lipton *et al.* When $[Ca^{2+}]_o$ was raised to 6.0 mM or lowered to 0.3 mM, we found σ shifts in their perfusate, but these were smaller and less consistent than those obtained in our own perfusate. Second, Lipton *et al.* increased $[Ca^{2+}]_o$ to only 3.2 mM, which we found to have little effect on σ , whereas our higher value of 6.0 mM was significantly more effective. Third, Lipton *et al.* used 1.0 mM of EGTA to produce a very low $[Ca^{2+}]_o$ of about 10^{-9} M. As demonstrated by them, and confirmed by us, this results in a continuous loss of sensitivity until the cell is almost completely unresponsive. As this occurred, they showed that the value of σ in the V -log I curves moved rapidly to the right. Their earliest curve was taken between 4 and 6 min after starting superfusion with the low $[Ca^{2+}]_o$; this was the curve chosen for comparison with the control solution and which showed no σ shift from control conditions. By extrapolation, curves taken at earlier times would be expected to show a σ shift to the left, but such curves were not shown. It is not clear why a given curve was chosen, from a rapidly changing set of curves, for comparison with the curve taken in normal $[Ca^{2+}]_o$. Fourth, our results indicate that a V -log I curve cannot be obtained within 2 min without significantly light adapting the cell by the test stimuli. Hence, if the low $[Ca^{2+}]_o$ of Lipton *et al.* shifted σ to the left, this may have been masked by a light adaptive shift of σ to the right. Because of these problems in very low $[Ca^{2+}]_o$, our approach was to use a subnormal value of $[Ca^{2+}]_o$ that was sufficiently low to yield clear effects but not low enough to cause continuous loss of sensitivity. The value of 0.3 mM proved adequate for this purpose, as it provided a steady-state condition during which a fully dark adapted V -log I curve could be obtained.

Thus, our results indicate that alteration of $[Ca^{2+}]_o$ can control the value of σ in the V -log I curve of toad rods. These results are consistent with a role for $[Ca^{2+}]_o$ in the control of sensitivity in vertebrate photoreceptors. Hence, changes of $[Ca^{2+}]_o$ may partially control the changes of sensitivity that occur during light and dark adaptation.

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Irazepine, a noncompetitive, irreversible inhibitor of ^3H -diazepam binding to benzodiazepine receptors

THE identification and characterisation of stereospecific, high-affinity binding sites for benzodiazepines in the mammalian CNS has been described both *in vivo*¹⁻³ and *in vitro*⁴⁻⁶. The correlations obtained between the *in vitro* affinities of a series of benzodiazepines for these binding sites and their clinical potencies as muscle relaxants², anxiolytics⁶ and anticonvulsants⁶ strongly suggest that these sites are pharmacological receptors mediating the therapeutic actions of the benzodiazepines. Benzodiazepines such as clonazepam, oxazepam and flurazepam are rapidly reversible, competitive inhibitors of ^3H -diazepam binding to synaptosomal membranes^{7,8}. Competitive inhibition of ^3H -diazepam binding has also been observed with the triazolopyridazines, which possess both anticonvulsant and anxiolytic activity⁹, and with the purines inosine and hypoxanthine^{10,11}, which may be endogenous ligands to the benzodiazepine receptor¹⁰⁻¹⁴. The development of noncompetitive, irreversible ligands has been invaluable in the characterisation, isolation and purification of both peripheral and central neurotransmitter receptors¹⁵, and may also prove useful for studying the benzodiazepine receptor. We now report the synthesis of a novel benzodiazepine, irazepine, which acts as a noncompetitive, irreversible inhibitor of ^3H -diazepam binding to synaptosomal membranes.

Irazepine [1-(2-isothiocyanatoethyl)-7-chloro-1,3-dihydro-5-(2-fluorophenyl)-2H-1,4-benzodiazepine-2-one] (Fig. 1a) was prepared as follows. A mixture of 203 mg (0.5 mmol) of Ro7-1986/1 (Fig. 1b), 10 ml of H_2O , 25 ml of CHCl_3 and 252 mg (3 mmol) of NaHCO_3 was stirred vigorously for 10 min, and then treated with 80.5 mg (0.7 mmol) of redistilled thiophosgene. Following vigorous stirring for 60 min, the CHCl_3 layer was separated, washed with 10 ml H_2O , dried with MgSO_4 and evaporated to give 182 mg of irazepine as a yellow foam. Thin layer chromatography of this material (silica gel GF, ether, ultraviolet visualisation) revealed essentially only one spot. The yellow foam was dissolved in 3 ml of warm isopropanol, rendered acidic with a slight excess of HCl gas and cooled to give 164 mg (80% yield) of crystalline irazepine·HCl. This material was recrystallised from methanol-diethyl ether to give analytically pure material: melting point (m.p.), 217–220 °C; mass spectrum, m/e 373/375 (M^+), infrared ν_{max} 2,110 cm^{-1} (NCS). Analysis: calculated for $\text{C}_{18}\text{H}_{14}\text{Cl}_2\text{FN}_3\text{OS}$: C, 52.69; H, 3.44; N, 10.24. Found: C, 52.42; H, 3.65; N, 10.14. In another preparation, the foamy residue from evaporation of the CHCl_3 extract was crystallised from isopropanol to give 151 mg (80% yield) of irazepine as the crystalline base, m.p. 142–144 °C. This material was readily recrystallised from absolute ethanol to give pure irazepine, m.p. 144.5–146 °C.

Specific binding of ^3H -diazepam to crude synaptosomal fractions of rat cerebral cortex was determined as described previously¹⁶, with the following modifications. Synaptosomal

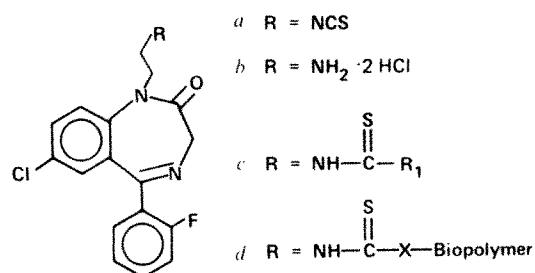


Fig. 1 Structure of irazepine (a), Ro7-1986/1 (b) and presumed reaction products (c, d) with bionucleophiles.

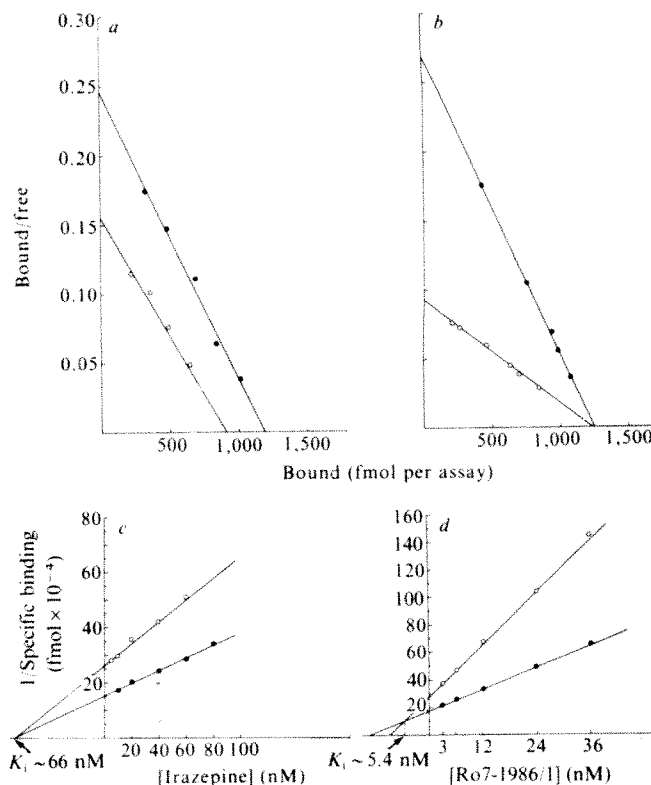


Fig. 2 Scatchard analysis of ^3H -diazepam binding to rat synaptosomal membranes in the presence of: a, irazepine·HCl (20 nM) (○), control (●); b, Ro7-1986/1 (6 nM) (○), control (●). Dixon plot of ^3H -diazepam binding in the presence of variable concentrations of c, irazepine·HCl, 2.88 nM (○) and 6.77 nM (●); d, Ro7-1986/1: 3.33 nM (○) and 7.03 nM (●). Plots are representative experiments; individual data points were determined in triplicate for total binding and duplicate for nonspecific binding (see ref. 16).

membranes were incubated with irazepine·HCl, Ro7-1986/1, clonazepam or distilled water at 0–4 °C for 30 min before addition of ^3H -diazepam. The incubation was then continued for 20 min at 0–4 °C, and terminated by filtration as described previously. Clonazepam (final concentration 1 μM) was used to determine nonspecific binding.

The inhibition of ^3H -diazepam binding by Ro7-1986/1 and irazepine was characterised *in vitro* by Scatchard and Dixon analyses (Fig. 2). No significant change in the maximum number of binding sites (B_{max}) was observed when synaptosomal membranes were incubated with Ro7-1986/1, whereas there was a concentration-dependent decrease in the apparent affinity using between 6 and 18 nM Ro7-1986/1 (Fig. 2b and unpublished observations). In contrast, incubation of tissue with irazepine·HCl (20 nM) resulted in a significant reduction in B_{max} ($21.4 \pm 3.8\%$ reduction in five experiments; $P = 0.02$) and a consistent but not statistically significant increase in K_d (3.7 ± 0.3 nM compared with 4.6 ± 0.5 nM; $P = 0.1$) (Fig. 2a). These findings are consistent with a competitive inhibition of ^3H -diazepam binding by Ro7-1986/1 and a noncompetitive inhibition by irazepine HCl. These observations were confirmed by Dixon analyses of the inhibition of ^3H -diazepam binding by the two compounds (Fig. 2c,d). The K_i values for Ro7-1986/1 and irazepine·HCl were estimated to be 5.4 and 66 nM, respectively.

The noncompetitive inhibition of ^3H -diazepam binding by irazepine indicated that the binding of this ligand, in contrast to that of other benzodiazepines, may be irreversible. Thus, the binding of ^3H -diazepam was then studied in the presence of

fixed concentration of irazepine-HCl and a variable amount of receptor (tissue) (see ref. 17) in a manner analogous to that used for determination of enzyme inhibition by noncompetitive inhibitors. The effects of increasing receptor (tissue) concentrations on the inhibition of ^3H -diazepam binding by irazepine-HCl suggest an irreversible inhibitor (Fig. 3). These observations are further supported by the ready reaction of irazepine with certain model bionucleophiles.

To determine the reactivity of irazepine with model bionucleophiles ($-\text{NH}$, $-\text{SH}$ and $-\text{OH}$ groups) *in vitro*, irazepine base was treated in 70% aqueous ethanol (at 25 °C) with an excess of compounds containing these functional groups. The relative velocity of the reactions was monitored by observing the disappearance of irazepine by thin-layer chromatography. Irazepine reacted rapidly with both diethylamine and methyl mercaptoacetate. These reactions were virtually complete in less than 5 min, presumably yielding (Fig. 1) the corresponding thiourea (Fig. 1c, $\text{R}_1 = \text{N}(\text{C}_2\text{H}_5)_2$) and the dithiocarbamate (Fig. 1c, $\text{R}_1 = \text{S}-\text{CH}_2\text{CO}_2\text{CH}_3$) derivatives, respectively. In contrast, reduced glutathione reacted slowly with irazepine in these conditions; a substantial amount of irazepine remained after 24 h. Little, if any, reaction of irazepine with 70% ethanol was observed after 24 h. The reaction of the isothiocyanate function with primary and secondary amines as well as with mercaptans has been described (for review, see ref. 18), and with aliphatic amines the reaction is usually quite rapid¹⁹. These observations suggest that a covalent binding of irazepine to the benzodiazepine receptor could occur by reaction with a free amino or sulphhydryl group, for example, from a lysine or cysteine residue, yielding a thiourea or dithiocarbamate (as in Fig. 2d, $\text{X} = \text{NH}$ or S , respectively). These *in vitro* studies do not, however, exclude a covalent link between irazepine and other bionucleophiles possibly associated with the receptor (such as a hydroxy or guanidino moiety).

Our data suggest that in the conditions used, irazepine is a noncompetitive, irreversible inhibitor of ^3H -diazepam binding. This activity is unique, as the binding of other benzodiazepines,

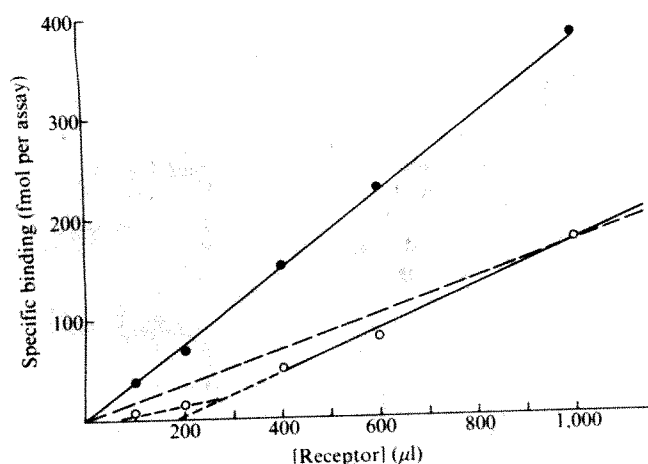


Fig. 3 Determination of irreversible nature of irazepine binding to benzodiazepine receptors *in vitro*. Ordinate: specific binding (fmol per mg protein); abscissa: μl of receptor (tissue) added. Incubations were performed as described in text. Upper line: observed points for control incubations (no irazepine); middle line: theoretical line for a noncompetitive reversible inhibitor, assuming the lines should pass through the origin and the 1,000 μl per incubation point. Bottom line: observed points in the presence of 100 nM irazepine-HCl. The line generated in the presence of irazepine indicates irreversible binding, analogous to determination of reversible/irreversible nature of enzyme inhibitors (see ref. 17) at low concentrations that is, of receptor, virtually no binding of ^3H -diazepam is observed. ○, Irazepine; ●, control; ---, reversible (expected).

triazolopyridazines and purines to the benzodiazepine receptor⁷⁻¹¹ is competitive. Irazepine is potentially useful in receptor isolation and purification, biochemical characterisation and histochemical localisation of the receptor, as well as estimation of receptor turnover *in vivo*. Further studies will be necessary to test the usefulness of this compound as a probe of the benzodiazepine receptor.

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Collagenous bone matrix is a local mitogen

THE importance of collagens in growth and differentiation^{1,2} and in cell adhesion³ is well documented. We have previously shown that cell-free, demineralised, collagenous bone matrix induces the formation of cartilage, bone and bone marrow⁴⁻⁶. One of the early responses to implanted collagenous matrix is the appearance of connective tissue mesenchymal cells adjacent to the implanted matrix before their differentiation into cartilage. We present here direct evidence that collagenous bone matrix is a local mitogen for connective tissue cells. We find that the collagenous bone matrix is a potent inducer of ornithine decarboxylase (EC 4.1.1.17, ODC), an enzyme involved in polyamine biosynthesis⁷ and considered as an early marker of cell proliferation⁸. These studies have been corroborated by incorporation of ^3H -thymidine into acid-precipitable material and by autoradiography. Our results reveal the local influence of insoluble collagenous bone matrix on proliferation of mesenchymal cells and imply a role for this extracellular matrix in anchorage-dependent⁹ events in cell growth and differentiation.

Demineralised bone matrix powder prepared from rat diaphyses was transplanted subcutaneously in the anterior thoracic region of 28-35-d-old, male rats of Long-Evans strain

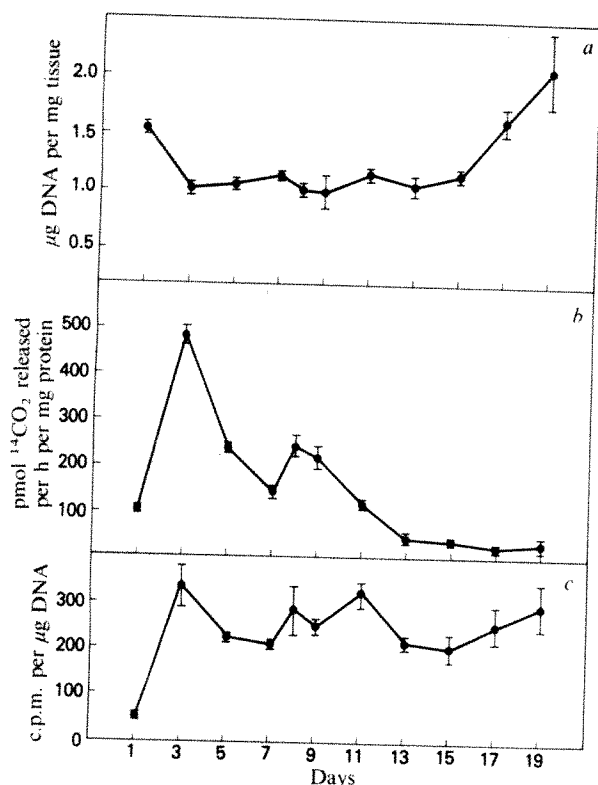


Fig. 1 Changes in the concentration of DNA (a), ornithine decarboxylase (b) and ³H-thymidine incorporation (c) during collagenous matrix-induced endochondral bone development. Plaques from individual rats were homogenised in ice-cold buffer containing 50 mM Tris-HCl, 10 mM dithiothreitol and 1 mM EDTA at pH 7.4, and centrifuged for 30 min at 30,000 g. ODC in the supernatant was determined by the method of Jänne and Williams-Ashman¹⁰; protein was determined according to Lowry *et al.*¹³. ³H-thymidine incorporation and DNA content were determined as described in the text. Day 1, polymorphonuclear leukocytes; day 3, fibroblasts; day 7, cartilage; day 11, bone; day 19, bone marrow.

as described previously⁴. The day of transplantation was designated as day 0. On different days after transplantation, the rats were injected intravenously with ³H-thymidine at a dose of 1 μCi per g body weight, 2 h before the end of the experiment. At autopsy the plaques were dissected free of adhering tissue; one portion from each plaque was used for determination of the activity of ODC^{10,11}, and another portion was processed for autoradiography by conventional methods with Kodak NTB-3 nuclear track emulsion. The rest of the plaque was homogenised in cold 10% trichloroacetic acid (TCA) containing 1 mM thymidine, and the incorporation of ³H-thymidine into acid soluble and insoluble portions was determined. Following acid hydrolysis, DNA in the TCA extract was measured. Calf thymus DNA served as the standard¹². The results were statistically evaluated by means of the Student's *t* test.

On day 3 collagenous bone matrix induced an increase in the ODC activity (Fig. 1b), which correlated with the incorporation of ³H-thymidine into the acid-precipitable fraction (Fig. 1c). Autoradiography at the light microscope level revealed cells labelled with ³H-thymidine in the vicinity of the implanted matrix (Fig. 2a). The high content of DNA on day 1, as determined by the diphenylamine method¹², is attributed to migration of polymorphonuclear leukocytes into the implant site, because there was no detectable incorporation of ³H-thymidine. There was a decline in both ODC activity and ³H-thymidine incorporation on days 5–7, when cartilage differentiation was in progress. On days 8–9 there was a second

peak of proliferation of cells (Fig. 2b) before impending osteogenesis and vascular invasion, as indicated by a peak of ODC and ³H-thymidine incorporation (Fig. 1). The ODC activity then steadily declined, but incorporation of ³H-thymidine continued and increased further during haematopoiesis on days 18–21. This increased incorporation was correlated with an increase in accumulated DNA as determined by a chemical procedure¹².

The proliferative response to collagenous bone matrix seems to be quite specific, because collagenous matrix from rat tail tendon is weak in this respect¹¹. It is worth recalling that tendon matrix is incapable of inducing endochondral bone¹.

The mechanism(s) underlying the local mitogenic action of the collagenous bone matrix is not known. Although the role of soluble growth factors in cell proliferation has been studied¹⁴, virtually nothing is known about the role of insoluble, extracellular, matrix macromolecules such as collagen and associated components. In many tissues collagenous matrix is the extracellular framework with which cells interact. The importance of collagenous matrix in development and differentiation and in the specification of positional information has been emphasised earlier¹. Recent investigations show that collagen is required for the proliferation of normal connective tissue cells but not for their transformed counterparts¹⁶. The study of skeletal and dental tissues, which abound with these constituents, may provide important clues to the possible function of the extracellular collagenous matrix and its associated components. The cellular

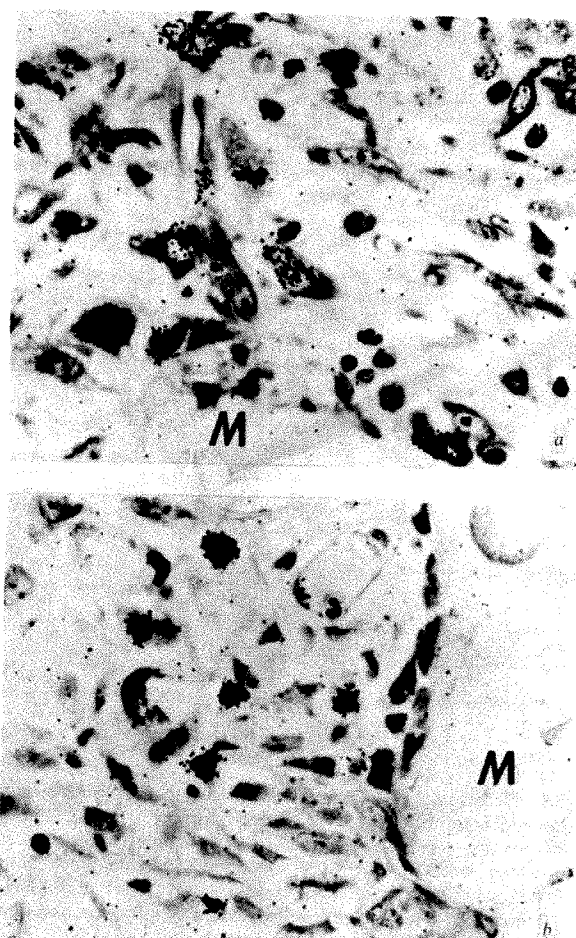
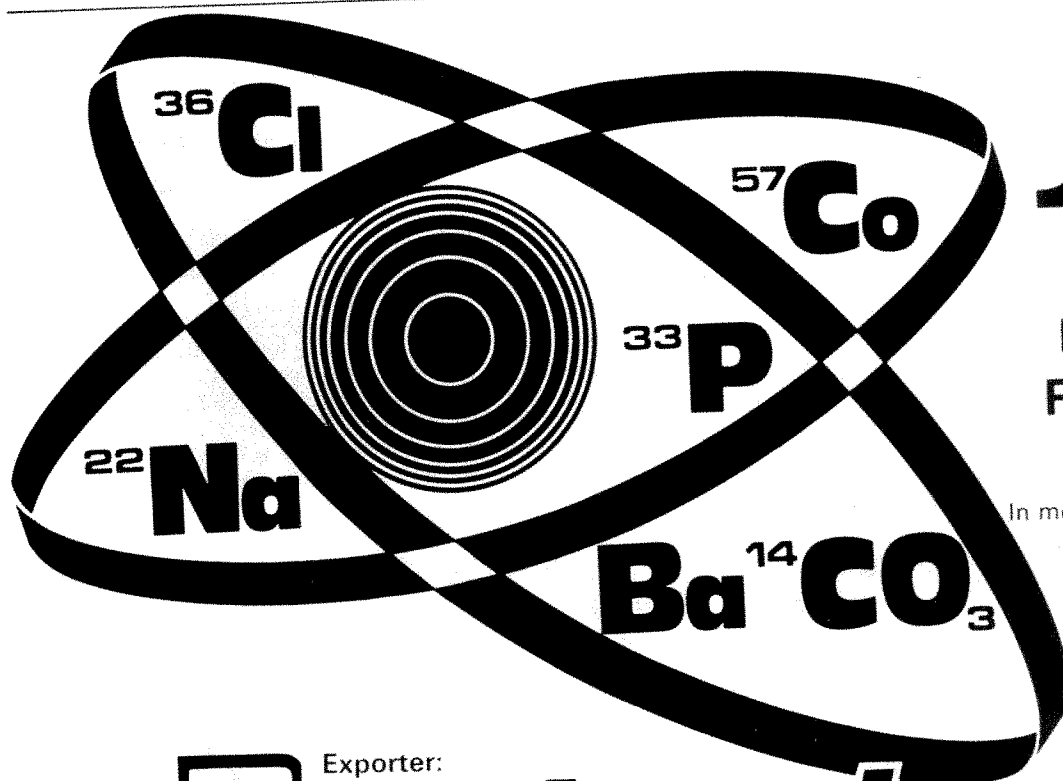


Fig. 2 Autoradiographic localisation of ³H-thymidine incorporation in the collagenous matrix-induced plaques on different days following implantation of matrix. a, Day 3, the label is mainly in the mesenchymal cells in the vicinity of the matrix particles (M). ×860. b, Day 8, label is localised in the mesenchymal cells ×860. M, matrix particle.



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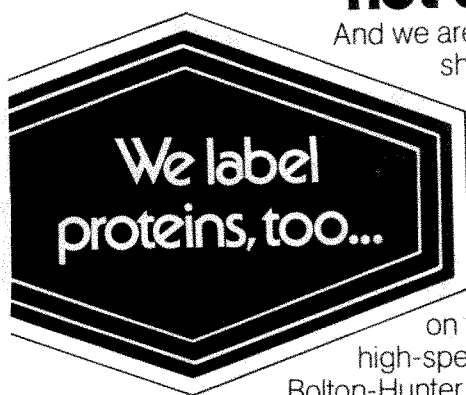


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cascade accompanying the collagenous bone matrix-induced formation of endochondral bone^{1,4-6} is remarkably similar to that seen during fracture healing in man. Collagens and their peptide constituents are chemotactic to fibroblasts¹⁵. The present study provides evidence that collagenous matrix from bone has a local mitogenic action. These observations may explain the local nature of fracture- and wound-healing responses and also provide a basis for further studies in clinical areas such as dental implants and periodontal diseases.

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Mutagenesis *in vitro* by DNA polymerase from an RNA tumour virus

THE fidelity of DNA synthesis has usually been determined from polynucleotide templates of restricted base composition¹⁻³. Mis-matched nucleotides were simply identified as those non-complementary to the template. Error rates estimated in this way are less than were predicted on the basis of Watson-Crick base pairing, 10^{-2} (refs 4-6), but greater than were inferred from spontaneous mutation frequencies, 10^{-8} - 10^{-11} (ref. 7). However, *in vitro* error rates measured for the same enzyme vary from one synthetic template to another^{3,8,9}. In addition, the repeating structure of homopolymers and copolymers may, by slippage and looping-out of mis-matched bases¹⁰, allow a higher frequency of mis-pairing than natural DNA of random sequence. To avoid these problems, we have recently developed a method for measuring the accuracy of *in vitro* DNA synthesis using a natural DNA template¹¹. We now report that a DNA polymerase which has a high frequency of mis-incorporation with synthetic polynucleotides¹ is also mutagenic when copying natural DNA *in vitro*.

Briefly, mutagenesis is measured *in vitro* with biologically active DNA in the following way. The template is the single-stranded circular DNA from bacteriophage Φ X174 and contains one nucleotide substitution mutation (*am3*) located in the overlapping genes *D* and *E* (refs 12, 13). Synthesis is initiated on a DNA restriction fragment (*Hae*III Z-5) hybridised to the template. The 3'-OH terminus of Z-5 is located 83 nucleotides from the *amber* mutation¹³. Purified DNA polymerase from any source may be used to copy the DNA in defined *in vitro* conditions. The accuracy with which the DNA polymerase has copied the *amber* mutation is then measured by

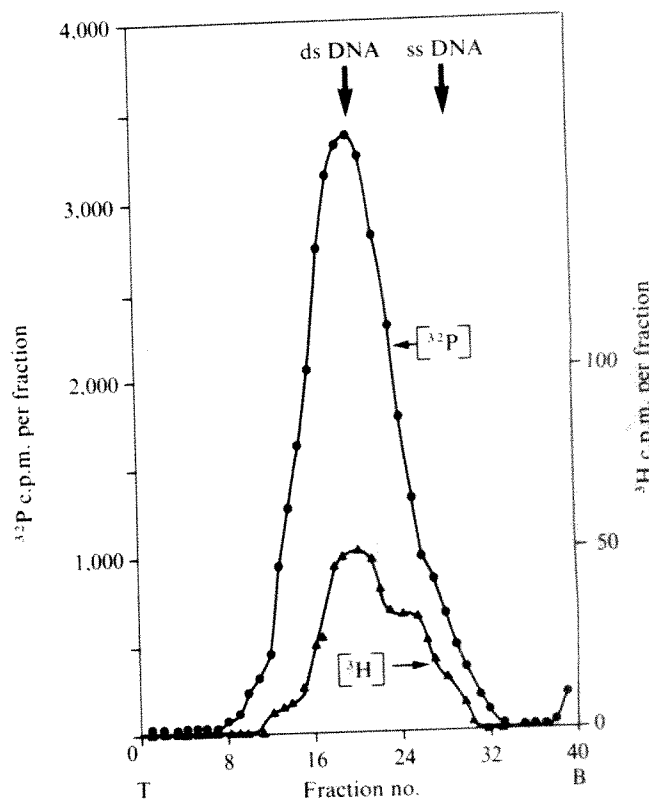


Fig. 1 Sedimentation of Φ X174 DNA after copying by AMV DNA polymerase. ^3H -labelled *am3* Φ X174 DNA was hybridised to the Z-5 restriction fragment and copied by AMV DNA polymerase as indicated in the legend to Table 1. A 20- μl sample of the reaction mixture after termination with EDTA (before top annealing) was applied to a 5-20% neutral sucrose gradient containing 10 mM Tris-HCl (pH 7.5), 10 mM EDTA, 1.0 M NaCl and 0.1% sarkosyl. Gradients were centrifuged at 45,000 r.p.m. at 20°C for 16 h in a Spinco SW 50 rotor. Fractionation of the gradients was from the top, and the total amount of radioactivity present in each fraction was determined after adding 50 μl calf thymus DNA (1 mg ml⁻¹), 1 ml 5% trichloroacetic acid and collecting the precipitates on glass fibre filters. ^3H -radioactivity was determined after the ^{32}P -radioactivity had decayed for 4 weeks. The ss DNA marker was single-stranded ^3H - Φ X DNA. The ds DNA marker was ^{14}C -labelled double-stranded Φ X DNA. In control experiments, the single-stranded Φ X174 DNA hybridised to the Z-5 fragment sedimented coincidentally with the single-stranded Φ X174 DNA marker.

transfection. The copied DNA is used to infect *Escherichia coli* spheroplasts. Accurately copied DNA will contain the *amber* codon in both parent and daughter strands, and give rise to mutant phage only. Inaccurately copied DNA will contain mis-matched bases, some of which will code for a revertant daughter strand at the *am3* codon. The fidelity of DNA synthesis *in vitro* is obtained from the reversion frequency of the phage produced from copied DNA.

Φ X *am3* DNA was copied by homogeneous avian myeloblastosis virus (AMV) DNA polymerase in the presence of 8 mM MgCl₂ and 100 μM of each of the deoxynucleoside triphosphates and then used to infect *E. coli* spheroplasts. The infected spheroplasts were immediately plated onto indicator bacteria either permissive or non-permissive for *am3*, to determine how many infective centres produced mutant *am3* phage and how many revertant phage (Table 1). *am3* DNA copied by AMV DNA polymerase produced phage with an average reversion frequency of 25.8×10^{-5} . This is about 4.5 times higher than the average background reversion frequency, 5.76×10^{-5} , which results from infection by the same *am3* DNA template incubated with reaction mixture in the absence of DNA polymerase. These results indicate that copying by AMV

Table 1 Reversion of Φ X174 *am3* DNA after copying by AMV DNA polymerase

Expt	Nucleotides incorporated per Φ X template	Total infective centres	Wild-type infective centres	Reversion frequency	Calculated error rate
1	0	115,200	5	4.34×10^{-5}	1/392
	1,410	122,800	46	3.75×10^{-4}	
2	0	819,000	37	4.52×10^{-5}	1/937
	1,080	130,700	24	1.84×10^{-4}	
3	0	30,000	1	3.33×10^{-5}	1/1,300
	520	15,000	2	1.33×10^{-4}	
4	0	830,000	20	2.41×10^{-5}	1/820
	497	10,900	2	1.83×10^{-4}	
5	0	3,890,000	455	1.17×10^{-4}	1/1,645
	420	428,000	84	1.96×10^{-4}	
6	0	11,525,000	950	8.24×10^{-5}	1/329
	767	460,000	220	4.78×10^{-4}	

am3 Φ X DNA was copied *in vitro* at 37°C for 45 min in a 50- μ l reaction mixture containing 40 mM Tris-HCl (pH 7.5), 5 mM dithiothreitol, 8 mM MgCl₂, 2×10^{11} molecules of Φ X174 *am3* single-stranded DNA hybridised to an *am3* *Hae*III Z-5 fragment, 100 μ M each of dATP, dGTP, dCTP and [α -³²P]dTTP (specific activity, 200 d.p.m. pmol⁻¹), and 10 pmol of purified AMV DNA polymerase. Synthesis was terminated by the addition of EDTA to 20 mM, and acid-precipitable counts were determined on aliquots of 2 μ l. The number of nucleotides incorporated per template was determined by dividing the number of nucleotides incorporated by the number of Φ X template molecules added to the reaction mixture (5,376 nucleotides per genome). The remainder of the copied DNA was hybridised to *Hae*III restriction fragments from *am3* Φ X DNA ('top annealing', ref. 11) and used to infect spheroplasts from *E. coli* KT-1 (su⁺, λ ⁻, Φ X^r) as described by L.A.W. and L.A.L.¹¹ In experiments 5 and 6, top annealing was unnecessary¹⁹. The infected spheroplasts were immediately plated onto indicator bacteria *E. coli* HF 4704 and *E. coli* 4714 (restrictive and permissive for the *amber* codon) to assay the number of wild-type and total infective centres. Uncopied DNA was subjected to exactly the same conditions except that DNA polymerase was omitted from the reaction mixture. The AMV DNA polymerase used in this study was purified to homogeneity from isolated virions by the method of Kacian *et al.*²⁰. The preparation used was free of detectable DNA, endonuclease, exodeoxyribonuclease and DNA ligase²¹.

DNA polymerase has led to an increased frequency of back mutations (revertants) on the Φ X DNA.

To calculate the frequency of revertants in the daughter DNA strand from the reversion frequency of the phage after infection, it is necessary to correct for penetrance, the frequency with which the *in vitro* synthesised DNA strand is expressed in the progeny phage. A value of 0.13 ± 0.06 was obtained from six experiments in which spheroplasts were infected with *am3* Φ X (+)strand DNA circles hybridised to a saturating concentration of wild-type restriction fragments (*Hae*III Z-7) which cover the *am3* codon¹¹. The calculated error rates of DNA synthesis past the *am3* mutation by AMV DNA polymerase therefore averaged 1 in 904 in the six determinations (Table 1).

Our calculations assume that every infecting Φ X DNA molecule contained a daughter DNA strand which extended at least past the *am3* codon. This assumption was tested by sedimentation analysis of the Φ X DNA product following *in vitro* synthesis by AMV DNA polymerase. The single-stranded Φ X DNA template was labelled with tritium and synthesis was monitored by incorporation of [α -³²P]dTTP. The DNA product, copied to the extent of an average of 1,050 nucleotides per added template, was sedimented in a neutral, high salt sucrose gradient. In these conditions, Φ X DNA circles migrate as double-stranded molecules when they are replicated 20% or more¹⁴. After synthesis, 70% or more of the templates and all of the product sediment coincidentally with double-stranded Φ X174 DNA (Fig. 1). (The Z-5 primer plus the 83 nucleotides to the end of the *am3* codon constitute 393 nucleotides, or only 7.3% of the 5,376 nucleotide-long genome¹³.) To the degree that some molecules of template remain uncopied, the error rate could be underestimated by as much as 30%.

These experiments represent the first estimate of the error rate with which AMV DNA polymerase copies a natural DNA template *in vitro*. The error rates are similar to those observed with homopolymer templates. This measurement with Φ X DNA does not take into account substitutions at the *amber* codon that are lethal, or the effects of substitutions at other positions on the genome. Studies with *E. coli* DNA polymerase

in mutagenic conditions indicate that distant incorrect substitutions invariably decrease the observed reversion frequency at the *am3* codon (T.A.K. and L.A.L., unpublished results). Thus, the error rates reported represent a minimum value.

The high error rate observed in copying natural DNA, one mismatch per 1,000 nucleotides polymerised, is nearly one order of magnitude greater than that observed in initial experiments with *E. coli* DNA polymerase I, 1 in 6,000 to 1 in 20,000 (ref. 11 and T.A.K. and L.A.L., unpublished results). However, we have observed certain preparations of AMV DNA polymerase which copy both poly (dA-dT) and Φ X DNA at lower error rates. The different error rates among different preparations of AMV DNA polymerase might be due to the degree of phosphorylation of the enzyme itself. A protein kinase has been purified from AMV which is able to phosphorylate AMV DNA polymerase, thereby increasing the rate of catalysis¹⁵. The *sarc* gene, the viral gene responsible for transformation in culture and the induction of sarcomas in animals, has been demonstrated to be a protein kinase¹⁶.

Because DNA polymerases from AMV and other RNA tumour viruses seem to be highly error prone *in vitro*, we may ask whether RNA tumour viruses have a correspondingly high mutation rate *in vivo*. This correspondence would be consistent with the hypothesis of Eigen and Schuster¹⁷ relating error rate of replicating enzymes to genome size. A spontaneous mutation rate in the host-range marker in the RNA tumour virus B77 has been measured to be about 1 per 100 pro-virus replications¹⁸ and a similarly high mutation rate has been observed in the *sarc* gene of other strains of Rous sarcoma virus (B. Mason and D. K. Dube, unpublished results). These high spontaneous *in vivo* mutation rates observed for RNA tumour viruses may in part be caused by the viral DNA polymerase which frequently incorporates mis-matched nucleotides during DNA synthesis. The ability of DNA polymerases to copy DNA templates from any source has led to the hypothesis that DNA polymerases from oncogenic viruses copy host DNA inaccurately, causing mutations and initiating tumorigenesis⁶.

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Ribose methylation and resistance to thiostrepton

THE antibiotic, thiostrepton, produced by *Streptomyces azureus*, inhibits bacterial protein synthesis by binding to 50S ribosomal subunits with 1:1 stoichiometry. Recently¹, we showed that *S. azureus* prevents its own death by methylating 23S ribosomal RNA (rRNA) so that its ribosomes do not bind the drug. Intact ribosomes are not substrates for the methylase and those experiments involved methylation of 'core particles' derived from the (thiostrepton-sensitive) ribosomes of *Streptomyces coelicolor*. Ribosomes can be reconstituted from core particles by adding back the missing 'split proteins'. Presumably, in *S. azureus*, methylation of 23S rRNA occurs before assembly of the 50S ribosomal subunit is completed. Here we report that the 'thiostrepton-resistance' methylase of *S. azureus* is an RNA-ribose methylase which introduces a single methyl group into adenosine in 23S rRNA. This is a novel antibiotic-resistance mechanism.

Crude methylase was prepared from *S. azureus* as previously described¹ and was used to methylate 3 M LiCl core particles from *S. coelicolor* ribosomes together with *S*-adenosyl[methyl-³H]methionine cofactor. As shown in Fig. 1, ³H-methyl groups were incorporated into at least three different residues in rRNA one of which predominated greatly over the others. Acid hydrolysates of rRNA prepared as in Fig. 1 contain mainly purine

bases, pyrimidine nucleoside monophosphates and ribose phosphate. When the major peak of radioactivity was eluted from chromatograms such as that shown in Fig. 1 and incubated with acid phosphatase, the resultant material was not adsorbed by activated charcoal (thus, it did not contain a purine or pyrimidine base) and did not migrate on high voltage paper electrophoresis (HVPE) at any pH between 2.5 and 10.5. We therefore concluded that the major methylated product visualised in Fig. 1 was 2'-*O*-methylribose-5'-phosphate and that it had probably been derived from a purine nucleotide in rRNA. The minor methylated products in Fig. 1 were purine bases and we have identified one as *N*⁶-methyladenine (data not given).

The stoichiometry of methylation of *S. coelicolor* ribosomal core particles by *S. azureus* crude methylase corresponded at saturation to 1.1–1.2 methyl groups incorporated per core (Fig. 2). Because in Fig. 1 ~85% of the ³H-methyl groups incorporated were recovered in the major product (2'-*O*-methylribose phosphate), we concluded that this material had been produced to the extent of one residue per core particle. In contrast, the more abundant of the minor products (*N*⁶-methyladenine) occurred on average only once per 7.5–8.0 core particles. As core particles methylated in these conditions completely lost the ability to support thiostrepton binding (after reconstitution with split proteins), we concluded that a causal connection exists between ribose methylation and resistance to the drug. This conclusion was supported by the observation (Fig. 2) that the time courses of methylation and acquisition of resistance to thiostrepton were superimposable. In other experiments (data not given) we confirmed that methylribose phosphate was produced exclusively in 23S rRNA.

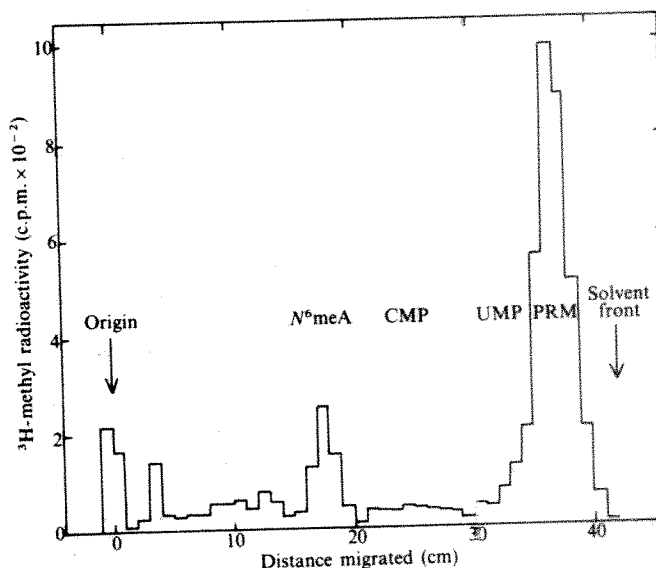


Fig. 1 Chromatography of acid hydrolysates of methylated rRNA from *S. coelicolor*. 500 pmol *S. coelicolor* 3 M LiCl cores (prepared as described previously¹) were incubated at 30 °C for 3 h with crude *S. azureus* methylase¹ together with 25 μ Ci *S*-adenosyl[methyl-³H]methionine (500 mCi mmol⁻¹). This was carried out in 1 ml of buffer containing 50 mM HEPES-KOH, pH 7.5 at 20 °C, 7.5 mM MgCl₂, 37.5 mM NH₄Cl, 3 mM mercaptoethanol. Cores were then extracted twice with water-saturated phenol and rRNA was precipitated from the aqueous phase with 2.5 volumes of ethanol at -20 °C for at least 2 h. Precipitation with ethanol was repeated after re-dissolving the RNA in 0.25 M sodium acetate. Hydrolysis of RNA in 1 M HCl was carried out for 60 min in a boiling water-bath and the hydrolysate was applied to Whatman 3 MM paper. Descending chromatography was carried out in solvent containing isopropanol-water-concorder UV light and radioactivity in sample tracks was estimated by liquid-scintillation spectrometry after cutting the tracks into strips 1 cm wide. '*N*⁶meA' represents *N*⁶-methyladenine; 'PRM', 2'-*O*-methylribose-5'-phosphate.

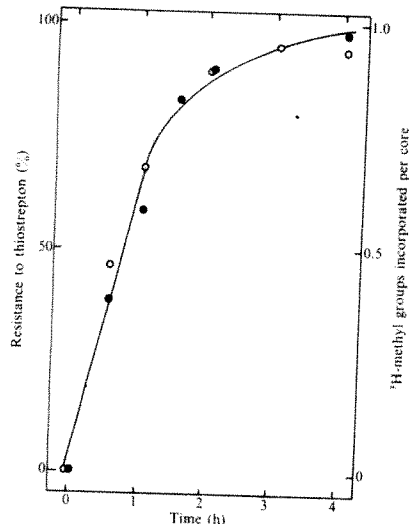


Fig. 2 Time courses of methylation and acquisition of thiostrepton resistance. **A**, Methylation: *S. coelicolor* cores were methylated as in Fig. 1 except that the incubation volume was reduced to 200 μ l. At the times indicated samples (20 μ l) were removed into 2 ml ice-cold 10% (w/v) trichloroacetic acid (TCA) and after 30 min at 0°C precipitates were collected on glass fibre disks (Whatman GFC). These were washed with 5 $\times$ 4 ml of 5% (w/v) TCA and dried before estimation of their radioactivity by liquid-scintillation spectrometry. Counting efficiency was approximately 26% so that 1 pmol of ^3H -methyl radioactivity gave 285 c.p.m. **B**, Thiostrepton resistance ($\circ$): *S. coelicolor* cores were methylated as in **A** above except that non-radioactive *S*-adenosylmethionine (0.5 mM final concentration) was used. Samples (20 μ l) containing 16 pmol of cores were removed as indicated and were incubated with a twofold excess of *S. coelicolor* 3 M LiCl split proteins (prepared as previously described¹) and then with ^{35}S -thiostrepton (35 pmol) as described earlier¹. Binding of ^{35}S -thiostrepton to reconstituted particles was assayed by gel filtration¹. The specific radioactivity of ^{35}S -thiostrepton was 1,053 c.p.m. pmol⁻¹. Results have been normalised. Cores do not bind ^{35}S -thiostrepton and reconstituted non-methylated particles do so with approximately 1:1 stoichiometry (this latter level of drug binding was taken as 100%).

The major methylated nucleoside produced in 23S rRNA by the action of *S. azureus* methylase was identified by a combination of paper chromatography and HVPE following digestion of the RNA with snake venom phosphodiesterase and bacterial alkaline phosphatase². Thus, the methylated nucleoside migrated as a single peak of radioactivity during paper chromatography in butanol-ammonia solvent (Fig. 3a). This procedure also served to deproteinise the nucleoside preparation so that, following elution from the paper and lyophilisation, it could be further analysed by HVPE. As shown in Fig. 3b, the methylated nucleoside migrated with adenosine and cytidine at pH 2.5 and was well separated from guanosine and uridine, which are uncharged at this pH. At pH 3.5 (data not given) cytidine migrated well ahead of adenosine and the methylated nucleoside co-migrated with the latter. Also, the methylated nucleoside remained at the origin during HVPE at pH 9.3 in borate buffer (data not given). In these latter conditions nucleosides containing non-methylated ribose migrate towards the anode as borate complexes. Further evidence that the methylated nucleoside contained 2'-*O*-methylribose was obtained by HVPE at pH 2.5 after heating in a boiling water bath for 20 min with 1M HCl (Fig. 3c). All the radioactivity which had previously migrated as a cation with adenosine (Fig. 3b) was now found to remain near the origin with uncharged markers (for example, ribose and deoxyribose). Accordingly, and because there was no reason to suppose that the adenine ring was modified in addition to ribose, we concluded that the methylated nucleoside under investigation was 2'-*O*-methyladenosine. (For the chromatographic properties of various base-modified adenosines see ref. 4.)

Ribose-methylation is a novel mechanism of resistance to an antibiotic. There are only two other examples of antibiotic resistance known to involve changes in the pattern of post-transcriptional modification of rRNA, and both of these involve base methylation. Thus, kasugamycin-resistant mutants (*ksgA* mutants) of *Escherichia coli* possess 16S rRNA which is under-methylated in comparison with wild type and lacks geminal residues of *N*⁶,*N*⁶-dimethyladenine normally located near the 3' terminus⁵. Conversely, certain strains of *Staphylococcus* and *Streptococcus*, which possess the so-called MLS phenotype

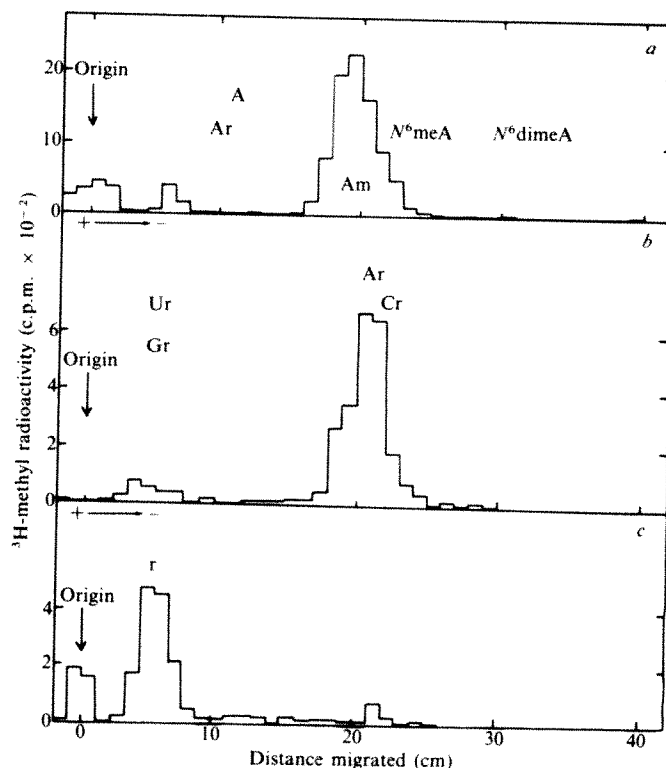


Fig. 3 Identification of methylated nucleoside. *S. coelicolor* 3 M LiCl cores (500 pmol) were methylated with the crude *S. azureus* methylase and *S*-adenosyl[methyl- ^3H]methionine and then RNA extracted as described in Fig. 1. RNA was dissolved in 25 ml 10 mM Tris-HCl, pH 8.0, and incubated for 18 h at 37°C with 2 mg crude snake venom phosphodiesterase (from *Crotalus atrox*) and at the same time 1.3 mg of bacterial alkaline phosphatase². Finally, the reaction mixture was lyophilised. **a**, The lyophilised digest, still containing protein, was dissolved in a minimum volume of water and applied to Whatman 3 MM paper for descending chromatography in solvent containing butanol:water:concentrated ammonia solution in the proportions 86:14:5. Routinely, such chromatograms ran 50 cm in 15–18 h (the full length of the paper) and were removed for drying after 20–24 h. Markers were detected under UV light and radioactivity in the sample tracks was estimated by liquid-scintillation spectrometry after cutting the tracks into strips 1 cm wide. Material corresponding to the single peak of radioactivity running on these chromatograms was eluted, lyophilised and taken up in water. This preparation of methylated nucleoside was further analysed by HVPE as described below and in the text. **b**, Methylated nucleoside was applied to Whatman 3 MM paper and subjected to HVPE using 50 mM glycine-HCl buffer, pH 2.5. Electrophoresis was for 45 min at 4,000 V in a flat-bed water-cooled apparatus very similar to one described elsewhere³. Detection of markers and of radioactivity in the sample track was carried out as above. **c**, Methylated nucleoside was hydrolysed by addition of an equal volume of 2 M HCl followed by immersion in a boiling water bath for 20 min. The hydrolysate was then subjected to HVPE at pH 2.5 as in **b**. Ribose and deoxyribose markers were detected by standard staining techniques. Following electrophoresis, uncharged markers such as ribose, deoxyribose and (at pH 2.5) uridine were located a short distance from the origin (**b**, **c**) due to endosmotic flow. Abbreviations: Ar and similar = nucleosides; Am = 2'-*O*-methyladenosine; *N*⁶meA = *N*⁶-methyladenine; *N*⁶-dimeA = *N*⁶,*N*⁶-dimethyladenine; r = ribose.

resistance to macrolide, lincosamide and streptogramin B-type antibiotics), possess 23S rRNA which is over-methylated. In this case, sub-lethal concentrations of erythromycin (a macrolide) induce the synthesis of a plasmid-encoded enzyme which produces approximately two residues of N^6,N^6 -dimethyladenine in 23S rRNA (ref. 6). Recently, there has been evidence to suggest the existence of a similar enzyme in *Streptomyces erythreus*, the organism which produces erythromycin⁷. The only other resistance phenotype known to involve some property of rRNA concerns resistance to viomycin in *Mycobacterium smegmatis*. Mutants of this organism possess lesions in either 16S or 23S rRNA, but the nature of such lesions is unknown⁸.

In conclusion, we note that whereas ribose methylation occurs only rarely in the rRNA of prokaryotes⁹ it occurs much more frequently in the rRNA of eukaryotes¹⁰. It is possible that the different sensitivities of prokaryotic and eukaryotic ribosomes to thiostrepton might ultimately be determined by patterns of ribose methylation in rRNA.

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Lobster shell carotenoprotein organisation *in situ* studied by photoacoustic spectroscopy

A SINGLE carotenoid pigment, astaxanthin, bound to specific proteins in a complex quaternary structure, provides shell coloration in the lobster *Homarus americanus*¹⁻⁸. The absorption of light by these carotenoproteins is uniform over a remarkable spectral range, as evidenced by the reflectance of a blue shell fragment which is low (5-6%) and essentially constant from 400 to 700 nm (ref. 9). This uniform absorption cannot be assigned to any combination of the carotenoprotein complexes which have been isolated. To assess the significance of these chemical species and to make specific inferences about carotenoprotein function *in vivo*, it is essential to measure the absorption spectrum *in situ*¹⁰. Several astaxanthin-protein complexes which absorb in the visible region have been isolated and spectroscopically characterised^{1-9,11}. These include α - and γ -crustacyanin with absorption maxima at 632 and 625 nm, respectively, and a second complex called the yellow protein^{7,11} which absorbs maximally at 410 nm. An early *in situ* absorption measurement of a carapace sample taken immediately after ecdysis and before calcification has been reported by Cheesman *et al.*⁴. However, the absence of structure in the reflectance measurements⁹ from a mature, opaque carapace suggests that conventional spectroscopy can yield little information about absorption maxima *in situ*. By using a new spectroscopic technique, photoacoustic spectroscopy (PAS), we have obtained spectra of a native shell fragment and here report the existence of a near continuum of astaxanthin absorption energies which span the visible spectral region. Furthermore, these pigments are in an anisotropic distribution within the endocuticle, that is, those absorbing in the blue are near the surface whereas those absorbing in the red are in the interior.

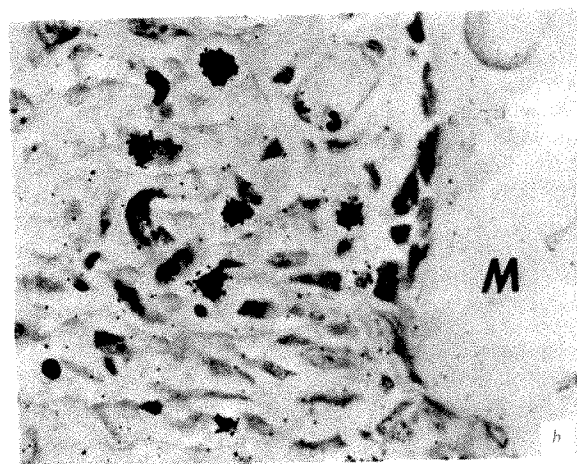
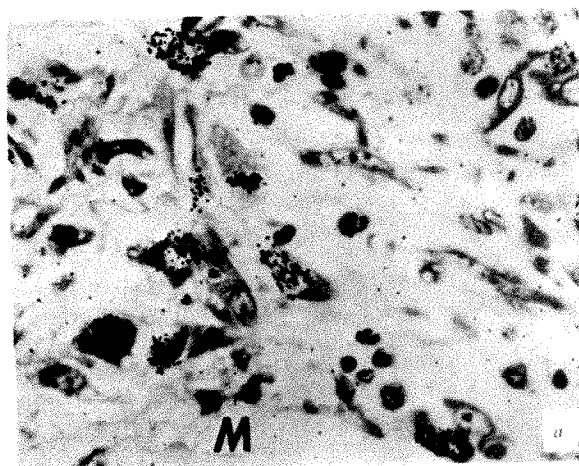


Fig. 1 Photoacoustic absorption spectra of native (a) and denatured (b) lobster shell as a function of modulation frequency ω (indicated with each spectrum) and phase ϕ . The interior spectra (—) were recorded with ϕ selected for maximum discrimination against the surface signal. In this case, decreasing ω allows response from deeper within the sample. The surface region spectra (---) were recorded with ϕ selected for maximum response from the surface. This selection of phase angle restricts the response to the surface region and results in a spectrum which is essentially independent of ω . Square wave modulation used in our spectrometer results in a triangular waveform from a carbon black (soot) sample and a concomitant 90° phase shift from the reference. Thus, the surface spectrum is optimised at $\phi = 90^\circ$ and discriminated against at $\phi = 0^\circ$. These spectra were recorded with a 10 nm spectral bandpass and a 0.03 Hz bandwidth (4-s time constant) at the lock-in output. The signal intensities are only qualitative.

PAS is ideally suited for measuring absorption maxima of opaque materials, and as it depends on thermal as well as optical properties of the sample, provides different information from reflectance measurements. It involves the measurement of heat produced as an excited species relaxes by a nonradiative path¹²⁻¹⁸. The exciting light is chopped at a suitable frequency and the resulting modulated heat flow is detected as pressure fluctuations using a microphone and a lock-in amplifier. As the exciting light is scanned, a spectrum similar to the absorption spectrum is obtained in which the response is proportional to both the absorption cross section and the thermal diffusivity of the sample. It is convenient to specify a thermal diffusion length¹³, $\mu = (2\alpha/\omega)^{1/2}$, where α is the thermal diffusivity and ω the chopping frequency. The definition of μ is such that light which penetrates the sample deeper than μ before absorption does not contribute to the response, as the periodicity is damped out

before the heat diffuses to the surface. Additionally, the phase (ϕ) of the signal at the phase-sensitive detector is critically dependent on the thermal diffusion time, as phase shifts are induced by the time required for thermal diffusion to the sample surface¹⁵⁻¹⁷. Spectral band shapes are influenced by several factors including wavelength-dependent relaxation pathways, saturation effects¹⁴ and, in layered samples, the filter effect of pigments located near the surface. Nevertheless, band maxima from PAS agree very well with measurements by conventional absorption techniques, and the spectrum is independent of ω and ϕ for an isotropic sample, provided saturation is avoided¹⁴. A key feature of PAS is that both ϕ and μ are experimental parameters that can be chosen to optimise the response from different levels of the sample^{16,17}. In certain cases the pigment locations in anisotropic (layered) samples can be determined¹⁵.

The spectra presented here are of a small sample ($\sim 4 \text{ mm}^2$) of uniform deep blue-green colour which was removed from the carapace of a live, mature lobster and used without further preparation. Samples removed from regions of carapace of similar appearance yield indistinguishable results, and samples from regions of obviously different coloration, such as the yellow underside of the claw, yield distinctively different spectra.

Figure 1a presents a series of spectra obtained at different modulation frequencies and phases. It is immediately apparent that the pigment distribution in the carapace is anisotropic so that by changing ω and ϕ one gains access to the different classes (layers) of pigments. Beginning with the surface region, absorption in the near UV by the epicuticle (which consists of wax, polyphenols and various lipoproteins^{4,19}) is evident, together with a band at $\sim 425 \text{ nm}$ which is assigned to the outermost carotenoprotein in the endocuticle. The spectra from the interior of the endocuticle demonstrate a remarkable bathochromic shift in the carotenoprotein absorption, from ~ 450 to $\sim 625 \text{ nm}$, as the modulation frequency is lowered from 326 to 26 Hz. The exception to this trend is the band at 390 nm which is clearly evident in the 26 Hz spectrum and not present in the spectrum from the surface region.

Figure 1b presents the results of a similar study on the shell fragment after denaturation of the carotenoproteins by boiling. The surface region spectrum shows that although the absorption of the epicuticle remains unchanged, the carotenoprotein band at $\sim 425 \text{ nm}$ is shifted to $\sim 450 \text{ nm}$. Only a small change in the interior spectrum is observed as ω is changed. The absorption in the red has disappeared and there is a general appearance of absorption strength in the region 450–560 nm.

We propose that the progressive bathochromic shift of the pigment absorption as a function of depth in the native endocuticle is due to highly specific protein-protein, pigment-protein and pigment-pigment interactions. If this is correct, then it is expected that denaturation would tend to convert all the chromophores to a common form. Indeed, this is the case, and thus the observation of the expected spectra from the denatured sample strongly supports the assignment of an anisotropic distribution of bathochromically shifted pigments in the native endocuticle.

The assignment of bands observed *in situ* to crustacyanins which have been isolated and purified is not straightforward. The spectra presented here, as well as the reflectance spectrum, are characteristic of an essentially continuous shift in the absorption maximum of the crustacyanins. This shift is produced by the interactions described above, which are expected to be a part of the ultrastructure of the endocuticle. Thus, it may not be possible to isolate discrete chemical species corresponding to each absorption. In the case of the denatured shell, the range of absorption maxima between 450 and 560 nm is characteristic of β -crustacyanin and other products of the denaturation of α -crustacyanin^{2,7,8}.

Astaxanthin aggregates, which have been described in terms of exciton states^{11,20}, absorb maximally at 390 nm. This corresponds exactly to the 390 nm band in the native shell and suggests the assignment of this band to the yellow protein *in situ* in which exciton interactions have also been found, although

after isolation and purification, absorption shifts to 410 nm (refs 2,7,9). Assuming a simple exciton model^{21,22} for this species, a monomer absorption maximum at 490 nm and the allowed exciton state at 390 nm, one calculates the corresponding bathochromically shifted and less allowed exciton state to be at 660 nm. Indeed, the absorption spectrum of purified yellow protein² clearly shows absorption by the less allowed transition in this region. Thus, the anisotropic distribution of bathochromically shifted excited states is such that the lowest energy level, the bathochromically shifted exciton state, is in the interior of the endocuticle. This assignment of an interior location to the yellow protein is in contrast to the recent suggestion based on resonance Raman scattering that it is at the shell surface⁹.

A quantitative description of the location of the various carotenoproteins may be obtained from measurements of the phase shift as a function of ω , provided α is known^{16,17}. In the present study, a qualitative estimation of depth is given by μ which ranges from 10 μm at 326 Hz to 35 μm at 26 Hz, if α is taken as 0.001 cm^2s^{-1} . This value of α is typical of biological materials²³ similar to chitin and yields values of μ in excellent agreement with the observed thickness of the epicuticle and depth of the pigmented zone¹⁹.

Although the work described here is structural in that it reports only the detection of a variety of carotenoprotein species and their organisation in the lobster shell, several interpretations are possible. The anisotropic arrangement of the pigments may not serve any photobiological role and may simply be a consequence of the ordered synthesis of the pigmented zone during moulting. Functions such as cryptic coloration may or may not be a property of the distribution of the pigments in layers. However, two possible functions which are dependent on pigment arrangement may be suggested. First, with different receptors serving specific layers of pigment a form of colour discrimination is obtained which could be important in a variety of photobiological processes. Second, pigments arranged with the lowest excited state in the interior could function as an antenna which absorbs light throughout the visible range and transfers excitation energy vectorially to the interior of the endocuticle where receptor sites may be located.

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Session 2 : **Immunoglobulin genes and their expression**

Speakers : Ph. LEDER (Bethesda), T.H. RABBITS (Cambridge), F. ROUGEON (Paris), I. SCHECHTER (Rei

Session 3 : **Immunoglobulin receptors and secreted antibody products**

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521(A)

UNIVERSITY OF PAPUA
NEW GUINEA
(PORT MORESBY)

Applications are invited for the position of

LECTURER/
SENIOR LECTURER
IN AGRONOMY

in the DEPARTMENT OF AGRICULTURE. Applicants should have a good first degree in Agriculture with post-graduate experience in Agronomy, preferably at the Ph.D. level. Preference will be given to applicants with university teaching and research experience in tropical agriculture. The appointee will be responsible for courses in the agronomy of plantation crops and pasture, up to honours degree level and may be required to assist in other courses according to experience and interests. He/she will be expected to supervise postgraduate students and to carry out independent research. The appointee may also be asked to assist with agronomic projects on the departmental farm and undertake other relevant duties as may be assigned from time to time. Currently the department is split between Port Moresby and Lae, but from February 1981 will be unified in Port Moresby. The appointee may be asked to teach one or two semesters in Lae initially but in the long term will teach full time in Port Moresby. Salary scales: Senior Lecturer K15,150 p.a. Lecturer K13,300 p.a. (21 sterling=K1.49). Family passages, 3 year contract, gratuity of 24% of salary, of which 5% is taxable, research allowance, baggage allowance, overseas leave. Detailed applications (2 copies) with recent small photograph with curriculum vitae and naming 3 referees to be sent direct to Secretary, Box 4820, University PO, Papua New Guinea by May 25, 1979. Applicants resident in the UK should also send one copy to Inter University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address 530(A)

AMGUEDDFA
GENEDLAETHOL CYMRU
NATIONAL MUSEUM
OF WALES
APPOINTMENT OF
PRINCIPAL OFFICER,
Oriol Eryri,
The Environmental Centre,
Llanberis, Gwynedd

Applications are invited for the post of Principal Officer at Oriol Eryri, the environmental gallery at Llanberis, Gwynedd.

The gallery, which is to be the first major outstation of the National Museum in North Wales is currently being built and will be opened to the public in 1982.

It will, in essence, be an outstation of the Departments of the earth and biological sciences at the Museum and will complement the work currently being carried out at the North Wales Quarrying Museum and at the Roman Fort Museum at Segontium, which are respectively outstations of the Welsh Industrial and Maritime Museum and the Archaeology Department.

The centre will contain an interpretative gallery illustrating the relationship of man and his environment in North West Wales, an exhibition gallery with facilities for displaying works of art, laboratories, lecture rooms and a general purpose hall which will be available for use in the evenings by local people.

Candidates must have a degree either in the earth or biological sciences and preferably have some experience in Museum work including administration. They should have the ability to make contact with both the general public and scholars.

A knowledge of Welsh is essential. Contributory pension rights. Salary scale £5,717 to £8,856 (under review).

Further particulars may be obtained from the Secretary, National Museum of Wales, Cardiff CF1 3NP, to whom applications should be submitted not later than Tuesday, May 31, 1979. 559(A)

UNIVERSITY OF MAINE

With the establishment of the Center for Marine Studies, the University of Maine is placing priority emphasis on the development of a strong, competitive research and graduate education program in marine resources. Nominations and applications are invited for the following positions.

CHAIRPERSON
DEPARTMENT OF OCEANOGRAPHY

The Oceanography Department, a graduate department offering M.S. and Ph.D. degrees, is primarily housed at the University's marine laboratory at Walpole, Maine. It currently has a faculty of 8. Present research activities include ecology of sub-boreal estuarine organisms, environmental biology and geochemistry, aquaculture and geological oceanography.

Responsibilities:

to serve as chief administrative officer; and develop a coordinated research program in physico-chemical estuarine processes.

Qualifications:

- (1) innovative leadership and administrative capability,
- (2) continuing scholarly achievements,
- (3) evidence of consistent success in funded research,
- (4) earned doctorate.

Academic appointment as Associate or Professor, tenure track. Salary open within University constraints.

FISHERIES POPULATION DYNAMACIST
DEPARTMENT OF ZOOLOGY

The Zoology Department, with a current faculty of 18 is primarily housed on the Orono campus, and includes the Cooperative Fisheries Unit. Ten of the faculty have research interests in marine or freshwater biology.

Responsibilities:

to develop a graduate education and research program on population dynamics of fishes.

Qualifications:

- (1) earned doctorate,
- (2) additional professional experience in marine fisheries biology desirable.

Academic appointment as Assistant Professor, initially one year with probability of additional 2-4 year University commitment.

MIGRATORY FISH BIOLOGIST
MIGRATORY FISH RESEARCH INSTITUTE

The Migratory Fish Research Institute is an interdisciplinary group of primarily Zoology faculty whose research interests focus on the biology of migratory fishes.

Responsibilities:

to collaborate in research on oceanic and estuarine migrations of larval and juvenile seas, and to assist in research proposal development.

Qualifications:

- (1) earned doctorate,
- (2) training and experience in fish migration and orientation preferred, and,
- (3) marine background or orientation.

Research appointment, initially one year with probability of second year continuation and possibility of third year depending on extramural program funding.

MARINE STATION
ADMINISTRATIVE OFFICER

The Ira C. Daring Center is the marine laboratory of the University of Maine. It is located on a 152 acre site on the Damariscotta estuary and includes Lowe's Cove. The facility comprises 10,000 square feet of laboratory and teaching space, including an Aquaculture Center, a developing conference center, a library, dockside facilities, and a fleet of small vessels.

Responsibilities:

to serve as the Chief Administration officer of a semi-isolated marine facility in support of educational and research programs, to be responsible for budget development and control, and for an expanding promotional/developmental activity.

Qualifications:

- (1) extensive administrative experience,
- (2) evidence of ability to communicate effectively with the public; and,
- (3) experience in sea-oriented organization including logistics, desirable.

Salary \$16,000 to \$18,000

Applicants should send complete resumes, samples of publications, and names of three referees, by May 15, 1979, to

Secretary, Search Committee
Center for Marine Studies
Room 14, Coburn Hall
University of Maine at Orono
Orono, ME 04468 U.S.A.

The University of Maine is an Equal Opportunity/Affirmative Action Employer. W70(A)

FACULTY OPENINGS exist at the Higher Institute of Technology (Brack) Socialist Peoples Libyan Arab Jamahlriya

In

ENVIRONMENTAL TECHNOLOGY DEPARTMENT

Applied Statistician/Biometrician: M Sc, Ph D, to teach basic statistics applied to biology, ecology and medicine

Analytical Chemist: M S., Ph D, Teaching and practical experience in general analytical and instrumental methods, or experience in water or environmental analysis.

Environmentalist: M.S., Ph.D., in environmental studies and geographical aspects Teaching and practical experience required

Ecologist: M S, Ph D, Experience in warm climates preferred Interest in field work is essential

Environmental Engineer: M Sc., Ph D., or Engineering Degree. To teach environmental engineering relevant to a developing country. Teaching and practical experience in "warm" climates is an advantage

Technologists, Technicians/Demonstrators:

- 1 With degree or extensive experience in preparing and teaching ecology laboratory practicals Knowledge of electronics is helpful
- 2 Experience in analytical chemistry and instrumental methods of analysis Environmental Microbiologist with Water, Soil and Air analysis experience.

Medical Technology Department

Hematologist - Serologist: M.S, Ph D or Certified Medical Technologist To teach Hematology and Serology. Teaching and practical experience desired.

Demonstrators, Technologists, Technicians: For teaching laboratory in Biochemistry, Clinical Chemistry, Hematology and Medical Microbiology

Food Technology Department Lecturers

Food Analytical Chemist: M Sc, or Ph D with teaching and practical works experience

Food Nutritionist: B Sc, or M Sc., with teaching and laboratory experience

Technologist/Demonstrator and 2 Technicians: B Sc /HND or equivalent qualifications for laboratory demonstration

All applicants must be fluent in spoken English, the language of instruction. Appointments depend on qualifications of appointees. No age limit exists on applicants. Fringe benefits include free air conditioned housing, medical services, transportation and terminal gratuity

Applications should be addressed to
The Dean
Higher Institute of Technology
Lyster Campus, Hal-Far, Malta.

LIFE SCIENTISTS A support Role in Agricultural Research

The successful candidates will join teams concerned with investigational and development work, and advising farmers.

Vacancies will probably be in the following locations. ANALYTICAL CHEMISTS—Newcastle and Wye; ENTOMOLOGISTS—Cambridge, Evesham, Leeds, Reading and Wye; MICROBIOLOGISTS—Newcastle, Reading and Wye; PLANT PATHOLOGISTS—Evesham, Newcastle or Wye; SOIL SCIENTISTS—Newcastle, Starcross and Wolverhampton.

Candidates (normally aged under 27) must have (or expect to obtain in 1979) a degree, HND, HNC or an equivalent qualification in an appropriate scientific subject. Relevant experience an advantage.

Salary. £2,555 to £4,415 Starting salary may be above minimum Non-contributory pension scheme Promotion prospects.

For further details and an application form (to be returned by 21st May, 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB; or telephone Basingstoke (0256) 68551 (answering service operates outside office hours) Please quote ref. S/5130.

MAFF — Agricultural Development and Advisory Service

55

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF GENETICS LECTURESHIP

Applications are invited for the post of Lecturer in Genetics. Candidates with qualifications in any field of Genetics will be considered, but preference will be given to candidates with experience in both pre- and post-doctoral levels in the field of molecular genetics.

Salary will be on the scale £3,883 to £7,754 (under review), plus superannuation.

Further particulars are available from the Assistant Registrar (Science and Engineering), University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (three copies) including curriculum vitae and naming three referees, should be sent by Monday May 14, 1979.

Please quote ref NL6 541(A)

UNIVERSITY OF EDINBURGH DEPARTMENT OF PHYSICS

Applications are invited for the post of University Demonstrator in the above Department. Teaching duties (laboratory and tutorial work) will not normally exceed 10 hours per week. The successful candidate will be expected to take part in the research programme of the Department and to register for a higher degree, if he/she does not already hold one. Details of research can be obtained on application to the Department.

Salary in accordance with experience and qualifications, between £3,384 and £4,882 per annum without a Ph D degree and between £3,883 and £5,600 with a Ph D degree. The appointment is for three years, and will commence on October 1st, 1979.

Applications by letter, including a curriculum vitae and the names of two referees, should be sent to the Secretary to the University, Old College, South Bridge, Edinburgh, EH8 9YL, not later than July 22nd. Please quote reference 1040.

PUBLIC HEALTH LABORATORY SERVICE BOARD

Centre for Applied Microbiology and Research, Porton Down
Genetic Manipulation Laboratory

Applications are invited for scientists with enthusiasm and ability to join the Genetic Manipulation Laboratory, within the Centre for Applied Microbiology and Research, Porton Down.

- 1 Senior/Basic Grade Microbiologist to join a small research team working on the development and practical uses of genetic manipulation techniques. The successful applicant will be expected to take part in the on-going research programme and to initiate independent research in related areas. Some work will take place under conditions of containment and appropriate training will be given where necessary. Applicants should have a Ph D acceptable equivalent and practical knowledge of genetic manipulation.
- 2 Medical Laboratory Scientist Officer to assist in the work of the genetic manipulation laboratory. Applicants should have a BSc HNC and familiarity with techniques employed in molecular biology would be highly desirable. NHS Terms and Conditions of Service. Annual salaries will be according to age and experience. The following scales: Senior Microbiologist, £5,451 to £6,837; Grade Microbiologist, £2,991 to £3,261 to £4,680.

Further details of the above can be obtained from Mrs M I at PHLS CAMR, Porton Down, Wiltshire, to whom applications with full career details, qualifications and names and addresses of referees should be submitted by 11 May 11, 1979. The director of the laboratory, Dr P Greenaway, will be pleased to discuss the posts with interested candidates. SC

Birds Mammals Agriculture

These posts are in the Land Pests and Bird Research Department which undertakes ecological, biological and pathological studies with a view to controlling harmful birds and mammals. There are excellent library and experimental facilities.

Vertebrate Ecologist

... to develop and test the potential of new methods of rabbit control, e.g. poison baiting. This will involve selecting test sites and studying the social behaviour and feeding habits of rabbits. In addition, the ecologist will be responsible for evaluating the extent of crop damage caused by rabbits and assessing the effectiveness of new control methods.

Candidates, normally aged under 30, should have a good honours degree or equivalent and at least 2 years' relevant postgraduate experience, ideally in vertebrate ecology or animal behaviour. Appointment as Higher Scientific Officer.

Ethologist

... to lead a small team studying the foraging strategies and food preferences of birds, particularly bullfinches, under natural conditions with a view to developing repellants for use in crop protection. This will involve planning and carrying out aviary and field experiments and publishing results.



Candidates, normally aged under 30, should have a good honours degree or equivalent in an appropriate discipline. At least 2 years' postgraduate experience in the study of animal behaviour and a sound knowledge of statistical methods is essential. An interest in optimal foraging theories and experience of working with birds is highly desirable. Appointment as Higher Scientific Officer.

Ornithologist/Ecologist

... to initiate a study of the relationship between the roosting and feeding habits of starlings in agricultural habitats. The work will also involve analysing the factors that may influence the selection of feeding grounds, assessing the profitability of producing cheap alternative food for starlings in an attempt to reduce damage to economically important crops, investigating breeding control methods and preparing results for publication.

Candidates, normally aged under 27, should have a good honours degree or equivalent in an appropriate discipline. *Final year students may apply.* Appointment as Scientific Officer.

Ornithologist

... to join a small team studying the behaviour of birds in airfield environments and advising on the potential danger to aircraft. The work will involve collecting and analysing data on populations and movements of lapwings whose affinity for airfields by both day and night is well established but little understood. In addition, the successful candidate will improve methods to alleviate bird hazards, advise civil and military aviation authorities on ornithological problems and instruct airfield personnel on bird control techniques.

Candidates, normally aged under 27, should have an honours degree or equivalent in an appropriate discipline. *Final year students may apply.* Appointment as Scientific Officer.

Salary for Higher Scientific Officer ranges between £4,100 and £5,450 and for Scientific Officer between £2,840 and £4,410. *Salaries under review.* Promotion prospects. Non-contributory pension scheme.

Men and women may apply. For further details and an application form (to be returned by 21 May, 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote ref. SB/40/AF.* Agricultural Science Service Worplesdon Laboratory, Surrey 589(A)

**Science
Group**
CIVIL SERVICE

THE UNIVERSITY OF SHEFFIELD RESEARCH IN INFORMATION SCIENCE Information Science Studentships and Postdoctoral Fellowships

Applications are invited from well-qualified graduates for consideration for the award of research studentships and postdoctoral fellowships for research in information science.

Candidates for studentships should normally have, or expect to obtain by October 1979, at least an upper (or equivalent undivided) second class honours degree in science or technology. Candidates for fellowships should hold, or expect to be awarded by October 1979, a Ph.D. in science or technology. Conditions of award will be similar to those applied by the S.R.C. including the usual British citizenship and residence requirements.

Current research in the School includes studies of computer-based information storage and retrieval systems, application of information theory in information systems, user studies in all disciplines, national information system design, information services in local authority social services departments as well as work on computer-based chemical information storage and retrieval systems.

Applications should be submitted to Professor M. F. Lynch, Chairman, Postgraduate School of Librarianship and Information Science, by May 25, 1979. Quote ref: R 288/G. 556(A)

MEDICAL RESEARCH COUNCIL LABORATORY ANIMALS CENTRE RESEARCH OFFICER

The Director's research programme is primarily concerned with an evaluation of the advantages which accrue from the use in research of specific pathogen free animals, in particular the cat. A person in the Research Officer category is required to assist the Director in this programme. Opportunities will occur for collaborative work with other departments in the Centre, which cover the whole spectrum of Laboratory animal science.

The Successful applicant will have a degree or equivalent in a biological science, and at least two years relevant research experience. Salary will be related to age, qualification and experience and is supplemented by £354 London Weighting. Contributory pension scheme.

Applications, together with a copy of curriculum vitae and the names of two professional referees, should be addressed to The Director, MRC Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey, SM5 4EF. 561(A)

MRC

Medical Research Council

Leeds POLYTECHNIC

School of Health and Applied Sciences LECTURER II IN NUTRITION AND DIETETICS (NEW POST)

Applications are invited from State Registered Dietitians having some community and/or clinical experience, to join a young and enthusiastic team concerned with the teaching of Nutrition and Dietetics to Degree and Diploma students in a well established school.

Salary Scale: £4,101 to £6,558.

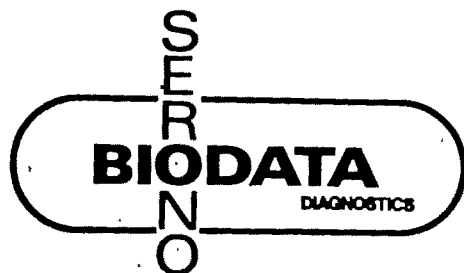
Details from:

The Services Officer (ND/30), Leeds Polytechnic, Calverley Street, Leeds LS1 3HE. Tel: 0532 462355.

Informal enquiries may be made to Dr K. R. Fell, Head of School. Tel: 0532 462941.

Closing Date: May 21, 1979. Please enclose s.a.c.

577(A)



International Co-Ordinator Diagnostics

World wide expansion of Biodata Diagnostics has resulted in the need for a scientist who will co-ordinate and direct the activities of Biodata at international level.

The person appointed will be expected to assume overall accountability for R & D, quality control, production and world wide strategy for the marketing and profitability of Biodata Diagnostics.

Applicants should possess a postdoctoral degree together with wide experience in radio-immunoassay, enzyme immunoassay based diagnostics, together with the ability to lead a multi-national team of scientists.

Though based in the U.K. (Hertfordshire), the position will involve frequent visits to Companies and laboratories within the Group in Germany, Israel, Italy, Switzerland, North and South America.

Reporting directly to the Group's Chairman in Geneva this appointment presents a unique opportunity in developing the existing range of diagnostics and opening up new areas through internal R & D, and/or acquisition of products, techniques or Companies. An ability to communicate and negotiate at the very highest level with scientists and commercial groups throughout the world is essential.

Remuneration and fringe benefits are commensurate with this important international appointment.

Candidates should write, enclosing a comprehensive C.V., and marking the envelope "STRICTLY CONFIDENTIAL" to:

Dr L. Margarito,
Sero Laboratories (U.K.) Ltd.,
Beesmer Road,
Welwyn Garden City,
Hertfordshire, AL7 1HH.

568(A)

SCIENTISTS

required for the
Institute of Meteorology and Arid Land Studies
King Abdul Aziz University, Saudi Arabia

Scientists at all ranks are needed in the following departments:

- 1) Meteorology Department
- 2) Arid Land Studies Department
- 3) Hydrology and Water Shed Management Department

Our pay scales are attractive and furnished housing is provided. Two months leave for the candidate and family is given with round trip air travel tickets (4 maximum) between Jeddah and his home town.

Send your applications as soon as possible by air mail and have two letters of reference sent directly to:

Dr. Fawaz Alamy, Dean, Institute of Meteorology and Arid Lands Studies,
King Abdul Aziz University, P.O. Box 1540, Jeddah, Saudi Arabia. 581(A)

UNIVERSITY OF BRISTOL

Applications are invited for post of

LECTURER IN ANATOMY

A vacancy exists for a lecturer with a primary responsibility to the teaching of medical students. As a being responsible for the teaching gross anatomy to medical, veterinary and science student Department offers courses in anatomy, endocrinology, reproductive embryology, dental anatomy, physical anthropology, with special teaching in each of these topics Honours degree level.

The Department has excellent facilities for research and is pre-organised into a number of research groups working in the following: biochemistry and physiology of secreting neurones, neuroanatomical dopamine pathways, electrophysiology of the CNS, control of parturition, bone mechanics, tooth eruption physical anthropology.

Salary will be on the scale £3, £7,754 (under review) and letter application including the name addresses of three referees should be sent to the Registrar and Secretary, University of Bristol, Senate House, Bristol BS8 1TD, before May 1979 (quoting ref TLJ). Late persons are welcome to visit Department by arrangement with Head of the Department's secretary (Tel: 0272-24161, Ext 367) 55.

UNIVERSITY OF MELBOURNE

LECTURESHP/

SENIOR LECTURESHP

in the

DEPARTMENT OF ANATOMY

With the development of new research and teaching initiatives in the Department of Anatomy, applications are invited for a position as Lecturer or Senior Lecturer in Anatomy. Applicants should have considerable research experience in one of the following fields: cell biology/histology, neurosciences, or topographical anatomy. Teaching experience would be a advantage.

The Department is well provided with transmission and scanning electron microscopes and ancillary equipment. Technical assistance is available. Participation in interdisciplinary research is actively encouraged.

Salary: Lecturer — \$A15,782 to \$24,687 p.a. In addition, a special loading of \$A2,500 p.a. is payable to a medically qualified appointee.

Additional details may be obtained from Professor G.B. Ryan in the University.

Further information regarding application procedure and conditions of appointment is available from Staff Officer, University of Melbourne, Parkville, Victoria, Australia 3010, from the Association of Commonwealth Universities (Appts), 36 G Square, London WC1H 0PP. Applications close on June 8, 1979.

527

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL (University of London) POSTDOCTORAL RESEARCH SCIENTIST

An experienced postdoctoral research scientist is required to join a Group funded by the Cancer Research Campaign and based at the Cancer Institute of Biochemistry in The Middlesex Hospital Medical School.

This Group is concerned with competing pathways of carcinogenesis, activation, inactivation and control of gene expression with macromolecular interactions. Particular attention is focused on: thione and interactions with branched and proteins both cytoplasmic and nuclear.

The possibility of tenure for post is under consideration.

Applicants should write to Dr Ketterer, Courtauld Institute of Chemistry, The Middlesex Hospital Medical School, London W1P 7P 522

Biological Assessment of Medical Products

c. £55000/£60000

The Radiochemical Centre Limited is a world leader in the development and manufacture of a range of radioactive products. Group sales are £32m. per annum and the company has been growing at more than 20% each year. Expansion has occurred in each part of the business and particularly in the development of medical products.

This is a new post for an additional professional scientist to assist with the development of new radiopharmaceuticals for in vivo diagnostic applications. This work will involve close co-operation with project leaders, often chemists, in setting up and supervising screening protocols for speculative products, more detailed testing procedures for identified products and collation of biological data for presentation to drug regulatory authorities. The job holder will play a part in identifying new areas for work in the

Department and eventually take over leadership of certain projects.

Applicants should hold a good honours degree in a biological science and have undertaken post-graduate research to the level of Ph.D. in a paramedical or biological subject (Physiology, Pharmacology or Biochemistry would be good examples). Practical experience of in vivo biological experimentation is also necessary, as is the ability to communicate easily with other staff.

Starting salary will be around £5500/£6000 p.a. according to qualifications and experience. Conditions of employment are excellent and include assistance with re-location if appropriate.

Please write with comprehensive career details to: Dr. J. S. Allinson, Personnel Officer.



The Radiochemical Centre
Amersham Bucks



1974

582(A)

HARVARD UNIVERSITY FULL/ASSOCIATE PROFESSOR IN OBSERVATIONAL OPTICAL ASTRONOMY

The Department of Astronomy of Harvard University is seeking to fill a position in Observational Optical Astronomy either at the level of Full Professor with tenure or at the level of Associate Professor to be considered or tenure within three years. The appointment can commence on or after July 1980. In association with the Harvard College Observatory and the Smithsonian Astrophysical Observatory, the Department of Astronomy has access to optical observing facilities at the Agassiz Station in Massachusetts and at Mount Hopkins in Arizona. At Mount Hopkins the facilities include the Mautimirror Telescope which is jointly operated by the Smithsonian Astrophysical Observatory and the University of Arizona. Other active observational programs in progress in the Department include X-ray observations using the Einstein Observatory, VLBI observations using the Harvard Radio Astronomy Station in Fort Davis, Texas and balloon-borne infrared observations.

Expressions of interest, inquiries and applications are invited. Please write to the Chairman, Search Committee, Department of Astronomy, 60 Garden Street, Cambridge, Massachusetts 02138. Candidates must have substantial observing experience using either ground-based or space-borne instrumentation. We explicitly encourage applications from women and minority group candidates.

W82(A)

MEDICAL RESEARCH COUNCIL LABORATORY ANIMALS CENTRE RESEARCH OFFICER

The Centre's Accreditation Department manages a national scheme concerned with monitoring the quality of laboratory animals of many species produced by commercial breeders and suppliers. The scheme aims to ensure that adequate standards of housing and husbandry are maintained; it acts as an information exchange on sources of supply; and it provides veterinary advice to the centre.

A vacancy in the Research Officer category exists in the department. The post holder will be expected to travel widely in Great Britain. Applications are invited from persons with a degree or equivalent qualification in a biological science, and at least two years research experience. Salary will be related to age, qualification and experience and is supplemented by £354 London Weighting. Contributory pension scheme.

Applications, together with a copy of curriculum vitae and the names of two professional referees, should be addressed to The Director, MRC Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey, SM5 4EF.

562(A)

MRC

Medical Research Council

ORGANIC ANALYTICAL CHEMIST

Applications are invited from young chemists for a position in the research and development laboratories of Weddel Pharmaceuticals Ltd., a subsidiary of the Union International Company Ltd.

This position would interest B.Sc. graduates wishing to extend their knowledge of development and application of physical methods of analysis. This would be applied to organic compounds and pharmaceutical preparations, using modern instrumentation. Some experience of gas or high performance liquid chromatography would be an advantage.

The laboratories are situated at St Albans in pleasant surroundings and within easy reach of the city centre. A staff restaurant is available and the Company operates a contributory pension scheme.

Please write, giving full personal and career details to:

**The Staff Manager (AD 6085),
The Union International Company Ltd.,
14 West Smithfield,
London EC1A 9JN.**

535(A)

The Research Division of Synthelabo located in
PARIS
requires a
SENIOR CARDIOVASCULAR PHARMACOLOGIST
to join its Department of Biology

The high success rate and rapid growth of this pharmaceutical group is related to the quality of people they employ and their continued commitment to research and development.

For this key position in the Cardiovascular Group we require a professional and highly motivated scientist who will be responsible for various projects within the group and should also be able to conduct independent research programmes.

Applicants can be either male or female and should have relevant postdoctoral experience (a maximum of five years). It is essential that the candidate should have experience of the pharmaceutical industry.

A realistic salary can be expected and relocation assistance will be available with other financial benefits.

All applications will be treated in the strictest confidence and should be sent directly to:

"NATURE"
c/o Box No. W67(A) 3 DYERS BUILDINGS
HOLBORN, LONDON EC1N 2NR

W67(A)



ELSEVIER'S SCIENCE DIVISION

BIOCHEMICA ET BIOPHYSICA ACTA

Applications are invited for a post in the BBA EDITORIAL SECRETARIAT.

The BBA Editorial Secretariat, located in Amsterdam, is a small team of biochemists involved in the selection of manuscripts for publication in the world's largest biochemical journal. The work involves the choosing of reviewers for submitted articles, and subsequent correspondence with authors and editors, leading to eventual acceptance or rejection of the manuscripts. The Secretariat also maintains close contact with the journal sub-editing team, and advises on matters such as nomenclature and terminology. They thus play a central role in the quality control of the journal.

Candidates should meet the following requirements:

- a wide knowledge of the biochemical sciences
- 2-4 years of postgraduate research experience
- initiative, decisiveness, tact and an eye for detail
- an excellent command of the English language
- age under 30 and resident in Europe.

The job offers modern working conditions including excellent social services and a good salary and bonus system. Holidays amount to 24 working days per year, plus public holidays.

Applications, including curriculum vitae, should be addressed to the Personnel Department Elsevier's Science Division, P.O. Box 2400, 1000 CK Amsterdam, The Netherlands. W69(A)

UNIVERSITY COLLEGE OF NORTH WALES, BANGOR

SCHOOL OF PHYSICAL AND MOLECULAR SCIENCES

Studies of
unstable molecules by
Infrared and Microwave
Spectroscopy.

Applications are invited for the S.R.C. supported post of Postdoctoral Research Officer to work in collaboration with Drs S. W. Charles, J. N. Macdonald and N. L. Owen on spectroscopic investigations of small acetylenic and ethylenic derivatives. Applicants should preferably have experience in either infrared or microwave spectroscopy of small molecules.

The appointment, which will be for one year in the first instance, will normally be renewable for a second year, and the preferred commencing date is October 1979. The starting salary will depend on age and experience, and will be in the range £3,883 to £4,382 per annum (under review).

Applications (two copies), giving details of qualifications and research experience, together with names and addresses of two referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG. 502(A)

UNIVERSITY OF MANCHESTER LECTURER IN PHARMACOLOGY

Applications are invited from science and medical graduates for this post, tenable from October 1. Science graduates will normally be expected to have obtained a Ph.D. and to have some postdoctoral research experience as well as teaching experience in pharmacology. Salary range: £3,883 to £7,754 p.a. Superannuation. Further particulars and application forms (returnable by May 10 from the Registrar, The University, Manchester M13 9PL. Quote ref: 85/79/N. 337(A)

PHYSIOLOGIST/ PHYSIOLOGICAL ECOLOGIST

Applications and nominations are invited for a tenured professorship in the Department of Biology at Harvard University (although candidates at the non-tenured levels of assistant and associate professor will be considered). The position involves teaching and research in the field of whole plant physiology and/or physiological ecology. A specialist in photosynthesis or water relations is preferred, who will join an active and growing group of ecologists and population biologists in an academic environment.

Letters of application, with résumé and three references, should be sent by August 1, 1979, to:

Professor Peter S. Ashton,
Harvard University Herbaria
Building,
22 Divinity Avenue,
Cambridge, Massachusetts 02138.

An affirmative action/equal opportunity employer W79(A)

Manchester Area Health Authority (T) Central District SENIOR MEDICAL LABORATORY SCIENTIFIC OFFICER (or Senior Scientific Officer N.H.S.)

Required for the cellular immunology section of the Department of Medical Genetics. The work involves cellular matching in renal transplantation and research in the immunogenetics of human leukaemia. A desire to participate in research and a knowledge of immunology would be an advantage.

Send applications and the names of two referees to Dr R. Harris, Department of Medical Genetics, St. Mary's Hospital, Hatheridge Road, Manchester M13 0JH. 542(A)

UNIVERSITY COLLEGE
OF NORTH WALES,
BANGOR

RESEARCH ASSISTANT:
WOOD DEGRADATION

Applications are invited for the above post, in the College's Department of Forestry and Wood Science, to assist with the examination of water-soluble and their performance when added to wood. Applicants should be holding, or have, a good Honours degree in Chemistry, Physics, Wood Science/Wood Technology, or Materials Science, and have an interest in surface phenomena or in the use of wood as an industrial material.

The appointment is grant-funded, and will be for one year in the first instance, commencing October 1, 1979, with the possibility of extension for a further two years.

The appointment will be within the salary range: £3,384 to £3,883 per annum (under review).

Applications (two copies), giving details of age and qualifications, together with the names and addresses of two referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, from whom further particulars may be obtained. Closing date for applications: May 1, 1979. 501(A)

LA TROBE UNIVERSITY
Melbourne, Australia
VISITING
FACULTY POSITION
IN
ORGANIC CHEMISTRY

The Department of Organic Chemistry wishes to appoint a visiting faculty member for a period of 6 months commencing about mid-February 1980. The successful applicant will undertake some undergraduate teaching and give lectures in his specialty at the post-graduate level.

A major objective of the appointment is to encourage close contact between the present staff and research workers in the Department and an organic chemist of wide University research experience.

The total remuneration, for the 6-month period, will be \$A9,500. There is some possibility that the position could be extended beyond 6 months but this will not be known until later in 1979. The appointment may be held concurrently with study or sabbatical leave from the appointee's own University.

Further information and application forms are available from the Association of Commonwealth Universities (Apts.), 36 Gordon Square, London WC1H 0PF. Applications close on June 8, 1979. 529(A)

OVERSEAS DEVELOPMENT

KNOW-HOW: vital to developing countries

Tropical Hardwoods Research Project — A Forester/Plant Physiologist/Biometrician

To join a team working on the propagation and improvement of West African timber trees, particularly *Triplochiton Scleroxylon*. The appointee will be located in the Forest Research Institute and will work in liaison with the staff there and with others at the Institute of Terrestrial Ecology, Edinburgh.

Applicants should have a good honours degree in forestry and/or botany; at least 5 years post-graduate experience, with evidence of involvement in designing, managing and analysing field and laboratory experiments; an appreciation of the physiological processes affecting tree form. Special knowledge of biometrics/statistics will be an advantage.

Appointment 3 years. (2 tours of 18 months each). Salary (U.K. taxable) in range £8,100—£9,350 p.a., plus a variable tax-free overseas allowance currently in range £4,095—£8,685 p.a. (Ref. 329 X).

The post is wholly financed by the British Government under Britain's programme of aid to the developing countries. In addition to basic salary and overseas allowances other benefits normally include paid leave, free family passages, children's education allowances and holiday visits, free accommodation and medical attention. Applicants should be citizens of the United Kingdom.

For full details and application form please apply, quoting reference stating post concerned, and giving details of age, qualifications and experience to:—



Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London SW1E 5DH

680(A)

HELPING NATIONS HELP THEMSELVES

The Queen's University of Belfast

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH FELLOW/
RESEARCH ASSISTANT

Applications are invited for two positions for work with Dr J. J. Rooney and Professor K. J. Ivin on the mechanism of olefin metathesis, especially in relation to the ring-opening polymerisation of cycloalkenes:

1 Postdoctoral Research Fellow: £3,885 to £5,130 (under review) with F.S.S.U./U.S.S.

1 Postdoctoral Research Assistant: £3,885 to £5,130 (under review) with F.S.S.U./U.S.S.

The research assistant post will be tenable for one year from October 1, 1979. The other post will be tenable for one year initially and may be renewed annually for further periods. Commencing salary will be at point appropriate to age, qualifications and experience.

Applications, giving a curriculum vitae and the names and addresses of two referees, should be sent to The Personnel Officer, The Queen's University of Belfast BT7 1NN, as soon as possible. Please quote Ref. 79/N. 547(A)

Queen
Charlotte's
Maternity
Hospital



TECHNICIAN OR BASIC GRADE BIOCHEMIST

There is a vacancy for a technician or basic grade biochemist to assist in the Routine Clinical Biochemistry Laboratory. The work is varied and may provide an opportunity to gain experience in some special techniques for estimating biogenic amine metabolites, for which this laboratory is a reference centre. Applications with the names of referees to Professor M. Sandler, Consultant Chemical Pathologist, Bernhard Baron Memorial Research Laboratories, Queen Charlotte's Maternity Hospital, Goldhawk Road, London W6 0XG. 572(A)

ROYAL GREENWICH OBSERVATORY

HEAD OF ASTROPHYSICS AND ASTROMETRY DIVISION

SENIOR PRINCIPAL SCIENTIFIC OFFICER

There is a vacancy for a senior research astronomer of high ability and wide interests to head the newly-created Astrophysics and Astrometry Division.

The Post

The person appointed will be required to manage and co-ordinate the research activities of scientific staff working in the fields of astrophysics and astrometry, and to develop a coherent research strategy appropriate to the primary role of the Observatory as a provider and operator of telescopes, instruments and measuring machines for use by all UK scientists engaged in ground-based optical astronomy. He/she will be expected to ensure that the Division contributes (in collaboration with University users) to the definition, design, operation and management of such national facilities. The main project now being undertaken is the procurement and subsequent operation of the proposed Northern Hemisphere Observatory in the Canary Islands. Initially, the Division will contain about 30 permanent staff, including at least 7 post-doctoral research astronomers, and 5 or 6 Research Fellows on short-term contracts. Observing is carried out on UK and overseas telescopes.

Qualifications/Experience

The person appointed is likely to be an energetic astronomer with an international reputation for collaborative research in ground-based optical astrophysics or astrometry. He or she should have wide interests and a thorough understanding of contemporary astrophysics. He/she should also have demonstrated ability for leadership of groups with diverse interests.

Salary

On a scale £10,043 to £10,671 to £11,300, subject to review. Non-contributory pension scheme.

Application forms and further information available from Mr T. A. Treglown, Personnel Section, Royal Greenwich Observatory, Herstmonceux Castle, Hailsham, East Sussex BN27 1RP. Closing date for applications June 1, 1979.

534(A)



Anglian Water Authority

Assistant Chemist

Cambridge

Post Ref. R2S15 Grade 4 £4,179—£4,476 p.a.
(including supplements)

Application forms for the above post may be obtained from the Divisional Scientist. The successful applicant will work in the Divisional Laboratory, which is well equipped with modern instrumental and automatic methods of analysis and handles some 10,000 samples per annum from all parts of the water cycle. A degree or equivalent in Chemistry is essential and practical experience in instrumental, classical and automatic monitoring methods of water analysis is desirable.

NJC Conditions of Employment for the Water Service are applicable and the closing date for receipt of applications is Monday, May 14, 1979.

GREAT OUSE RIVER DIVISION

Great Ouse House, Clarendon Road, Cambridge, CB2 2BL
Tel.: (0223) 61561 (Miss Hobbs)

MRC

Medical Research Council

National Institute for Medical Research Computing Laboratory

The Medical Research Council invites applications from suitably qualified candidates with wide experience of computer techniques for the appointment of Head of the Computing Laboratory. The Computing Laboratory, which will be moving into a new building at the end of this year, manages two time shared systems (DEC 2040 and HP 3000) for the Institute and is concerned with developing systems for real time and batch processing.

The Laboratory at present serves the Institute (staff about 600) and some smaller institutions in the London area; the off-site work is likely to increase. Provision has also been made for coupling into a Universities Computer network.

The salary will be within the range for the Council's Senior or Special Appointments grades, which is equivalent to the universities' range for Reader or Professor, plus £502 p.a. London Allowance and superannuation provision.

Applications, giving the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA, from whom further details may be obtained. Closing date for applications 15th June 1979.

506(A)

The Research Division of Synthelabo, an internationally recognised pharmaceutical industry located in Paris requires a

CARDIOVASCULAR PHARMACOLOGIST

to join its Department of Biology. For this position we require a candidate for performing work on conscious animals.

The successful candidate should have relevant practical experience and know basic surgical procedures to instrument animals for cardiovascular parameter measurements.

A realistic salary can be expected and relocation assistance will be available with other financial benefits.

All applications will be treated in the strictest confidence and should be sent direct to 'Nature' who will forward.

W78(A)

UNIVERSITY OF SYDNEY CHAIR OF PHARMACEUTICS

Applications are invited for the Chair of Pharmaceutics within the Department of Pharmacy.

Salary will be at the rate of \$A33,061 p.a.

A statement of Conditions of Appointment and Information for Candidates may be obtained from the Registrar, University of Sydney, NSW 2006, Australia, with whom applications close on June 30, 1979. Information also available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

523(A)

NMR SPECTROSCOPIST OCTOBER 1, 1979

Ph.D. Physical Science or Graduate degree in engineering. NMR background essential, experience in electronics or chemistry desired. Duties include design, construction, testing of NMR equipment, some instrument operation. \$18,500 or more depending upon qualifications. Closing date: August 1, 1979. Send curriculum vitae and three letters of recommendation to: Dr Stephen E. Ulrich, School of Chemical Sciences, University of Illinois, Urbana, Illinois 61801. An equal opportunity/affirmative action employer.

W83(A)

CHELSEA COLLEGE University of London LECTURER IN CHEMISTRY

Applications are invited for a post of LECTURER in CHEMISTRY which has arisen from a resignation in the Department of Chemistry.

Candidates must have good academic qualifications and a special interest in some aspects of ANALYTICAL CHEMISTRY. Industrial and/or teaching experience at the tertiary level would be an advantage.

Salary scale: £4,385 to £8,256 p.a. (under review) inclusive of £50 London Allowance.

The appointment will commence in September 1979 or as soon after as possible. Application forms and further particulars from the Personnel Officer, Chelsea College, Friese Greene House, Chelsea Manor Street, London SW3 3TW.

Closing date: May 18, 1979.

524(A)

MEDICAL RESEARCH COUNCIL Radiobiology Unit, Harwell TECHNICIAN FOR CELL MUTATION GROUP

The Cell Mutation Group at Harwell is studying the biochemistry and genetics of mutants of cultured mammalian cells induced by ionising radiation. A Technician, preferably with experience in cell culture techniques and/or biochemistry is required for this work.

Qualifications for a technician are H.N.C. or equivalent and salary will be in the scale £3,261 to £4,680. Assisted transport and modern hostel accommodation are available. Applications, giving full personal details including the names of two referees, should be addressed to the Administrator, M.R.C. Radiobiology Unit, Harwell, Didcot, OXON OX11 0RD.

507(A)

THE QUEEN'S
UNIVERSITY
OF BELFAST
DEPARTMENT OF
BIOCHEMISTRY
POSTDOCTORAL

RESEARCH ASSISTANTS

Research Assistant (preferably 4th Ph.D.) required in the Biochemistry Department to investigate the immunoglobins and viral antibodies in sera from psychiatric patients. Experience in virology and/or enzyme immunoassay techniques would be an advantage. Salary scale £3,384 to £3,885 with 1.3.3 Curriculum vitae with the names and addresses of two referees should be sent to the Personnel Officer, The Queen's University of Belfast, Belfast, BT7 1NN, Northern Ireland.

RESEARCH TECHNICIAN

Required to assist with a project concerned with the investigation of immunoglobins and viral antibodies in psychiatric patients. Experience in the use of tissue culture for growth of viruses would be an advantage although not essential. Salary scale (Grade 5) £3,474 to £4,056. Application forms obtainable from the Personnel Office, address as above.

The appointments will be from August 1, 1979 (or as soon thereafter) and will be for one year in the first instance, with a possible extension of a further one to two years. 544(A)

THAMES POLYTECHNIC
SCHOOL OF MATERIALS
SCIENCE AND PHYSICS
X-RAY AND NUCLEAR
LABORATORY TECHNICIAN
(Grade 5)

Applications are invited for a Technician for the X-ray and nuclear laboratories who will be required to operate X-ray equipment, handle radioactive sources and be responsible for keeping exposure records. Candidates should have a recognised apprenticeship or H.N.D./H.N.C. qualification and at least eight years' relevant experience.

Salary scale: £3,474 to £4,011 inclusive.

Further particulars and form of application may be obtained from the Staffing Officer, Thames Polytechnic, Wellington Street, London SE18 6PF, to whom completed applications should be returned by May 15, 1979.

555(A)

THE QUEEN'S
UNIVERSITY
OF BELFAST
RESEARCH ASSISTANTS
DEPARTMENTS OF
INDUSTRIAL CHEMISTRY
AND ZOOLOGY

Applications are invited for two research assistantships (Chemist and Zoologist) related to the utilisation of mollusc resources. The designated areas of research are structure and properties of mussel flesh, and the ecology of mussel growth in particular geographic areas respectively. The appointments are supported by a Wolfson Foundation Grant. Each is tenable for three years and the successful applicants may register for research degrees. Initial salaries are in the range £2,640 to £3,400. Applicants should possess (or expect to attain in 1979) a good honours degree in the appropriate field.

Applications with curriculum vitae and the names and addresses of two referees to the Personnel Officer, Queen's University, Belfast from whom further information can be obtained. Closing date: June 30, 1979. 545(A)

CARDIOVASCULAR RESEARCH

Pfizer Research in the U.K., which is part of an integrated international organisation, is located at Sandwich on the Kent Coast. Here in modern, well-equipped facilities, about 600 scientists and supporting staff are engaged in the discovery and development of novel compounds for use in human and veterinary medicine.

We now require an additional innovative pharmacologist to join the department of Developmental Biology, and are looking for a person with post-doctoral experience in cardiovascular research, and preferably in conscious animal work.

The appointment will involve the testing, detailed evaluation and development of compounds as potential antihypertensive agents. The emphasis will be on work with potential candidates for development as drugs and will involve close collaboration with biologists in our discovery biology department.

The man or woman appointed will carry considerable responsibility for planning and supervising the work of a team of some four people. The starting salary will reflect qualifications and experience and there is a good bonus potential. Pension, salary continuance and death benefit schemes are in operation and there is generous assistance with relocation expenses. Flexible hours are worked and the social/recreational facilities are first class.

Applications, which will be treated in confidence, giving details of qualifications, experience, age etc., should be sent to:- D. W. Sells, Pfizer Central Research, Sandwich, Kent.

565(A)

THE UNIVERSITY OF
THE WEST INDIES
TRINIDAD

Applications are invited for a post of
PROFESSOR/
SENIOR LECTURER

in the DEPARTMENT OF AGRICULTURAL ECONOMICS AND FARM MANAGEMENT IN THE FACULTY OF AGRICULTURE, preferably with interest in Quantitative Analysis and/or Agricultural Development. Interest in other areas of Agricultural Economics will be considered. Salary Scale 1977/78 (Under Review): Professor: TT\$36,729 to TT\$45,480 p.a.; Senior Lecturer: TT\$25,827 to TT\$35,901 p.a. (£1 sterling=TT\$4.96). F.S.S.U. Unfurnished accommodation if available at 10% or furnished at 12½% or housing allowance of 20% of pensionable salary. Family passages. Study and Travel Grant. Detailed application (2 copies) together with curriculum vitae and naming three referees to Secretary, U.W.I., St Augustine, Trinidad, as soon as possible. Applicants resident in the UK should also send one copy to Inter University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 517(A)

UNIVERSITY OF LONDON
KING'S COLLEGE
DEPARTMENT OF CHEMISTRY
LECTURESHIPS
IN CHEMISTRY

Applications are invited for two Lectureships, tenable from October 1, 1979 (or from a date to be agreed). Applicants should have an interest in either synthetic organic chemistry or in experimental physical organic chemistry/solution kinetics.

Current salary scale £3,883 to £7,754 p.a. (under review) plus £502 p.a. London Allowance. Universities Superannuation Scheme.

Further information and application forms from the Assistant Registrar, King's College, Strand, London WC2R 2LS, to whom applications (in triplicate) naming two referees should be sent, to arrive by June 11, 1979. 532(A)

UNIVERSITY OF BENIN Nigeria

Vacancies exist in the underlisted departments. Posts range from the level of Assistant Lecturer, for which the minimum qualification is a Second Class Honours (Upper Division) with adequate teaching or research experience, to the level (in some departments only) of Professor, for which higher academic qualifications and relevant University experience will be sought.

Departments of Biological Sciences; Biochemistry; Chemistry; Geology; Mathematics; Physics and Optometry; Civil Engineering; Chemical and Petroleum Engineering; Electrical and Electronic Engineering; Mechanical Engineering; Production Technology and Industrial Engineering; Obstetrics and Gynaecology; Medicine; Radiology; Surgery; Mental Health; Child Health; Community Health; Pathology; Physiology; Pharmaceutics and Pharmaceutical Technology; Pharmaceutical Chemistry and Pharmacognosy; Pharmaceutical Microbiology; Pharmacology; Periodontics; Restorative Dentistry; Preventive Dentistry; Oral Surgery

Salaries

Professor/Director	N 11,288 — N 12,428
Associate Professor	N 9,996 — N 11,028
Senior Lecturer	N 7,784 — N 8,724
Senior Research Fellow	N 7,104 — N 7,752
Lecturer Grade I	N 5,480 — N 6,432
Lecturer Grade II	N 4,368 — N 5,340
Research Fellow	
Assistant Lecturer	

N.B. N1 = approx. 0.78p, subject to exchange rates

Applications. For further details of the posts, specific qualification requirements and the method of application, please write to: Principal Assistant Secretary, Nigerian Universities Office, 180 Tottenham Court Road, London W1 9LE.

Closing Date: 11 May 1979.

583(A)

SOUTH OF SCOTLAND ELECTRICITY BOARD

HUNTERSTON POWER STATION

HEALTH
PHYSICS
ASSISTANT

Applications are invited for the post of Health Physics Assistant at Hunterston 'A' and 'B' Nuclear Power Stations. The Health Physics Department provides a comprehensive radiological protection service and Health Physics Assistants are concerned with the supervision of a personnel dosimetry section, environmental surveys and technical investigations on both 'A' and 'B' Stations.

The successful applicant will be appointed at Third Engineer Grade and given the opportunity to gain a full knowledge of other Health Physics activities with a view to promotion within the department.

Applicants should have a degree or equivalent in Physics or an associated subject; health physics experience is not essential and training will be given.

Salary, which is currently under review and includes responsibility and productivity payments, will be within the range £4,231 to £6,215 per annum (NJB Third Engineer Grade).



Applications (quoting reference number 43/G83/79) should be submitted on the standard form obtainable from and returnable to the Manager, Hunterston Power Station, West Kilbride, Ayrshire, KA23 9QJ, not later than 17th May, 1979. 574(A)

A position is available in the Department of Biochemistry, University of Alberta, Edmonton, T6G 2H7, beginning in the fall of 1979. Applicants should have a good background in DNA metabolism and DNA-drug interactions, both at the graduate and postdoctoral level. A strong background in physicochemical techniques is required for current studies on multistranded nucleic acids and their possible biological roles. Only Canadian Citizens may apply for this position. Please apply to Dr A. R. Morgan at the above address. W73(A)

RESEARCH CHEMIST

BREWING

SALARY RANGE £4,000 TO £5,500

A vacancy exists for an Experimental Officer in the Chemistry Section of the Brewing Research Foundation. The successful applicant will assist in the determination of better ways to utilise the raw materials employed in brewing. This will involve the isolation and characterisation of compounds which are of importance in beer flavour. The position would suit a chemist with a sound theoretical background in organic chemistry together with knowledge of modern instrumental techniques.

Candidates expecting to graduate this summer are invited to apply as also are graduates with relevant industrial experience.

Appointments will be made within the salary range £4,041 to £4,964 to £5,584. Conditions of employment are in keeping with a progressive organisation and include pension/life assurance schemes, social facilities and four weeks annual holiday.

Applications, including a curriculum vitae, should be sent to The Administrative Manager, Brewing Research Foundation, Lyttel Hall, Nutfield, Redhill, Surrey RH1 4HY, to arrive not later than June 1, 1979. 575(A)

LOUGHBOROUGH
UNIVERSITY
OF TECHNOLOGY
LECTURER IN PHYSICS

The Department of Physics has a temporary lecturer post available for one academic year from September 1, 1979 to July 31, 1980. It provides an opportunity for a younger physicist to obtain experience in a university. The person appointed would be expected to lecture to honours degree students and join in one of the research groups.

Salary is within scale £3,883 to £7,754 (under review).

Application forms and further particulars from Paul Johnson, Establishment Officer, Reference 79/15PH, Loughborough, Leicestershire. 511(A)

GLOBAL TECTONICS

The California Institute of Technology is seeking to create a professional position in the general area of global tectonics. Applications are invited for either a junior appointment, at the Assistant Professor level, or a more senior tenure appointment. The areas of specialisation preferred include mantle dynamics, theory and driving mechanism of plate tectonics, global geophysics, and internal fluid dynamics of the earth and planets.

Please send résumé, publications, and names of three references, to Barclay Kamb, Chairman, Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, California, 91125. An equal opportunity employer. W84(A)

ST THOMAS'S HOSPITAL
MEDICAL SCHOOL

(University of London)

London SE1 7EH

DEPARTMENT OF CHEMICAL
PATHOLOGY AND
METABOLIC DISORDERS

has vacancy for

MEDICAL LABORATORY
SCIENTIFIC OFFICER

Appointment for one year in the first instance, with possibility of renewal. The successful applicant will assist in the Department's lipid research programme, involving isolation and quantification of human plasma lipoproteins, previous similar experience not essential. Applications with reference to Professor B. Lewis, above address. 594(A)

POSTDOCTORAL RESEARCH
ASSOCIATE POSITION

starting July, 1979, available for Ph.D. with an interest in studying the immunology of pulmonary fibrosis. Applicants must have a strong background in cellular immunology and experience in B and T cell biological assays and enumeration techniques. The Associate will function as part of an ongoing interdisciplinary programme in pulmonary research associated with the Vermont Pulmonary Specialised Centre of Research (SCOR). The individual will have an opportunity to interact with biochemists, pharmacologists, physiologists, and cell biologists at the University of Vermont, as well as physicians in pulmonary medicine at the Medical Centre Hospital of Vermont. Applicants should forward a current curriculum vitae and three letters of reference to Dr Beth Hart, Department of Biochemistry, Given Medical Building, University of Vermont, Burlington, Vermont, 05405, by June 1, 1979. W77(A)

JUNIOR
MEDICAL LABORATORY
SCIENTIFIC OFFICER

required in Host Defence Unit to assist in the investigation of resistance to infection in animals and man. Ideal post for school leaver wishing to make a career in Medical Laboratory Sciences. Applicants should have five 'O' Levels including two relevant sciences. Day Release granted for O.N.C./H.N.C. studies. Apply giving age and qualifications to The Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP and quoting ref. 4DU2. 560(A)

UNIVERSITY OF LONDON
WELLCOME CHAIR OF
PHARMACOLOGY AT THE
SCHOOL OF PHARMACY

Applications are invited by Senate for the Wellcome Chair of Pharmacology tenable at The School of Pharmacy. The successful candidate will be Head of the Pharmacology Department in the School and take up his position as soon as possible after September 1, 1979, when the becomes vacant.

Applications (10 copies) should be received by the Academic Registrar (N), University of London, M Street, London WC1E 7HU, from whom further particulars should be obtained. Closing date May 1979. 520(A)

UNIVERSITY OF TORONTO
DEPARTMENT OF
MEDICAL BIOPHYSICS
POSTDOCTORAL POSITION
IN CHEMICAL
CARCINOGENESIS

is available immediately for research on mechanism of action of nitrosamines. Experience in enzymology, drug metabolism desirable. Salary \$12,050 to \$13,350 per annum. See curriculum vitae and references to: Michael C. Archer, Department of Medical Biophysics, University of Toronto, 500 Sherbourne Street, Toronto, Canada M4X 1K9. W75(A)

BIRZEIT UNIVERSITY

An independent Arab University, the Israeli-occupied West Bank, Jordan has the following faculty openings for the academic year 1979/1980: **Biology-Biochemistry, Chemist (Physical, Inorganic, Organic), Mathematics (Algebra, Analysis, Mathematical Statistics), Physics (Experimental and Theoretical).**

English is the basic language of instruction and candidates should have a Ph.D. preferably or a master degree with experience.

Starting annual salary for Ph.D. 2,700 Jordanian Dinars (current exchange rate 1 J.D. equals about £1.7). Optional summer teaching extra. Fringe benefits include: Relocation allowance (15% of salary). Transportation also provided.

All applications should be addressed to: Dean of the Faculty of Science, Birzeit University, Birzeit, West Bank, via-Israel. W71(A)

THE HOSPITAL FOR
SICK CHILDREN
AND
UNIVERSITY OF TORONTO
RESEARCH
PHARMACOLOGIST

The Hospital for Sick Children Research Institute invites applications for the position of Scientist in the Division of Clinical Pharmacology. Ph.D. or equivalent in pharmacology or related field with at least 2-5 years postdoctoral experience required. Emphasis on developmental biochemical pharmacology preferred. University rank and salary negotiable. Send letter of application, curriculum vitae and three reference letters to Dr S. M. MacLeod, Division of Clinical Pharmacology, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8. W74(A)

UNIVERSITY OF
LONDON
RESEARCH ASSISTANT

Graduate research assistant preferably with research experience in physiological psychology or psychopharmacology required as soon as possible for a project on brain control of body weight and motivation in rats, funded for one year in the first instance. Salary and superannuation on University scale. Apply with curriculum vitae and names of referees to Dr. L. J. Herberg, Experimental Psychology Laboratory, Institute of Neurology, National Hospital, Queen Square, London WC1. 525(A)

Liverpool Area Health Authority (T)

Regional Cardiac Centre, Sefton General Hospital,
Smithdown Road, Liverpool L15 2HE

BIOCHEMIST (Basic Grade)

Applications are invited for the post of Biochemist (Basic Grade) in the Centre Laboratory, which is concerned with biochemical aspects of heart disease.

Whitley Council conditions of pay and service apply. Newly qualified graduates will be considered.

Application forms obtainable from the Sector Administrator,
Sefton General Hospital, Smithdown Road, Liverpool L15 2HE.
Closing Date: 15th June 1979. 590(A)

RESEARCH OFFICER

Required at our Mill Hill Laboratories NW7 to assist with research into some aspects of the control of gene expression during development. This involves the utilisation of recombinant molecules containing segments of eukaryotic DNA to investigate the structure and transcription of developmentally regulated genes.

H.N.C. degree plus two years research experience essential. Some experience of biochemical techniques useful.

Salary £3,936 to £5,256. For further information and application form write or telephone Miss P. Maddox at the Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2, on 242 0200 ext. 305. 585(A)

UNIVERSITY OF EDINBURGH POSTDOCTORAL RESEARCH FELLOW

Applications are invited for this S.R.C. financed post to work on theoretical aspects of inelastic collisions between atoms and molecules. Close liaison is maintained with the Molecular Beams and the Spectroscopy Groups in the Physics and Chemistry Departments, and a background in either of these fields, or in theoretical chemistry is desirable. The work will be under the supervision of Dr K. P. Lawley.

Applications with a curriculum vitae and the names of two referees should be sent to Dr Lawley at the Department of Chemistry, West Mains Road, Edinburgh. Please quote Reference 5019.

549(A)

THE UNIVERSITY OF LANCASTER DEPARTMENT OF PHYSICS Postdoctoral Research Associate

Applications are invited for the position of Research Associate to work with Drs D. J. Meredith and J. K. Wigmore on a study of phonon echoes and non-linear acoustic interactions in solids. Candidates should have completed a Ph.D. in solid state physics, and particular experience with low temperatures, microwaves, or lattice dynamics would be advantageous but not essential. The appointment would be for two years beginning October 1, 1979 at a salary in the range £3,883 to £5,604 (depending on experience).

Further particulars may be obtained (quoting reference L129/B) from the Establishment Officer, University House, Bailrigg, Lancaster LA1 4YW, to whom applications (6 copies) naming three referees, should be sent not later than May 14, 1979. 591(A)

THE UNIVERSITY COLLEGE OF WALES ABERYSTWYTH LECTURER IN ANIMAL SCIENCE

Required—Lecturer in Animal Science in the Department of Agriculture tenable from October 1, 1979.

The successful applicant will participate in the teaching of undergraduate and postgraduate courses in Animal Science and Animal Health. Postgraduate qualifications or veterinary experience are highly desirable. Salary on the Scale for Lecturers £3,883 to £7,754 per annum (under review).

Application forms and further particulars from The Registrar, The University College of Wales, Aberystwyth SY23 2AX. Closing date for applications May 31, 1979. 504(A)

THE UNIVERSITY OF LANCASTER DEPARTMENT OF PHYSICS POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for the position of Research Associate to work with Drs D. J. Meredith and J. K. Wigmore on a study of phonon echoes and non-linear acoustic interactions in solids. Candidates should have completed a Ph.D. in solid state physics, and particular experience with low temperatures, microwaves, or lattice dynamics would be advantageous but not essential. The appointment would be for two years beginning October 1, 1979 at a salary in the range £3,883 to £5,604 (depending on experience).

Further particulars may be obtained (quoting reference L129/B) from the Establishment Officer, University House, Bailrigg, Lancaster LA1 4YW, to whom applications (6 copies) naming three referees, should be sent not later than May 14, 1979. 505(A)

UNIVERSITY OF MANCHESTER LECTURESHIP IN PHARMACY

Applications are invited from honours graduates in Pharmacy, who should preferably have a higher degree, for a lectureship in the Pharmaceutics area. Applicants should have an interest in physical pharmacy or pharmaceutical technology. Excellent facilities are available for research.

Salary range: £3,883 to £7,754 per annum. Superannuation.

Particulars and application forms (returnable by May 31) from the Registrar, The University, Manchester M13 9PL. Quote Ref. 84/79/N. 509(A)

Scientific Officer/ Higher Scientific Officer £2,839 – £5,448

Applications are invited for a pensionable post in the Field Botany Division of the Department of Agriculture situated at Newforge Lane, Belfast.

The successful candidate will carry out research in the field and under controlled environmental conditions aimed at increasing productivity and utilisation of pasture in hill land in Northern Ireland. The successful candidate may also be required to undertake lecturing duties in the Faculty of Agriculture and Food Science, Queen's University, Belfast.

Higher Scientific Officer

Under 30 years of age at 31 December 1979 and possess a first or second class Honours Degree in Agricultural Botany, Agriculture or Botany and at least 2 years' relevant post-graduate experience.

Scientific Officer

Under 27 years of age at 31 December 1979 with an Honours Degree as above.

A clean driving licence is essential.

Exceptionally applications may be considered from candidates over the age limits who have specialised experience.

The Civil Service Commissioners may decide to interview only those applicants who appear from the information available (including the level of academic attainment and relevant experience) to be best qualified.

Salary Scales

Higher Scientific Officer — £4,101-£5,448
Scientific Officer — £2,839-£4,415

(the salary scales will be subject to review from 1 April 1979).

Grading and starting salary will be related to qualifications and experience.

There are prospects of promotion to Senior Scientific Officer (£5,514-£6,898) and to Principal Scientific Officer (£6,609-£8,461).

Please write or telephone for an application form quoting job reference SB 198/79/NN to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585 ext 256). Completed forms must be returned to arrive not later than 22 May 1979. 510(A)



**NORTHERN IRELAND
CIVIL SERVICE**

Liverpool Area Health Authority (Teaching)

ROYAL LIVERPOOL HOSPITAL
PRESCOT STREET, LIVERPOOL L7 8XW

Clinical Biochemist (Basic Grade)

Applications are invited for the above post in the Department of Chemical Pathology and Metabolic Medicine at the Royal Liverpool Hospital.

The Department carries out the full range of routine, specialised and advanced assays for this new teaching hospital and is recognised for the M.R.C. Path and M.C.B. There are excellent opportunities of working for a higher degree.

Application forms are available from the Area Personnel Department, Liverpool Area Health Authority (T), Orleans House, Edmund Street, Liverpool L3 9NG.

Closing date: 25th May, 1979.

543(A)

UNIVERSITY OF ALBERTA

Applications are invited from graduates with a good background in biochemistry or chemistry to undertake fundamental research on proteoglycans or glycoproteins leading to an M.Sc. or Ph.D. degree. Well equipped laboratories include automated amino acid sequencer and amino acid and carbohydrate analysers. Stipends are available for suitably qualified persons and travel allowances may be provided.

Enquiries should be sent to Dr C. H. Pearson, Department of Oral Biology, Dentistry-Pharmacy Centre, University of Alberta, Edmonton, Alberta T6G 2N8, Canada. W76(A)

UNIVERSITY OF GLASGOW
ADMINISTRATIVEASSISTANT
INSTITUTE OF VIROLOGY

Applications are invited from university graduates for the post of Administrative Assistant in the Institute of Virology. Salary will be within the Range £3,384 to £5,604 (interim) with placing according to qualifications and experience. A degree in science, preferably biological, is desirable but not essential.

Further particulars may be had from the Secretary of the University Court (Room 18), University of Glasgow Glasgow G12 8QQ, with whom applications (eight copies) giving the names and addresses of three referees should be lodged on or before May 18, 1979.

In reply please quote Ref. No. 4434 M. 548(A)

STUDENTSHIPS

UNIVERSITY OF LONDON
MEDICAL
RESEARCH COUNCIL

Applications are invited for a postgraduate studentship, associated jointly with the MRC Cell Biophysics Unit, King's College, and the Department of Clinical Haematology, University College Hospital Medical School, under the MRC Partnership Award scheme. The research will be concerned with the mechanisms by which the cytoskeletal proteins of the red cell control its shape and the properties of the membrane, and in particular with the nature of the anomalies in functionally abnormal cells from subjects with haemolytic and other inherited red cell disorders. Applications, giving the names and addresses of two referees, should be sent to Professor E. R. Huehns, Department of Clinical Haematology, University College Hospital Medical School, University Street, London WC1. 570(F)

MINISTRY OF
AGRICULTURE,
FISHERIES AND FOOD
POSTGRADUATE
RESEARCH STUDENTSHIPS

The Ministry of Agriculture, Fisheries and Food are offering to suitable candidates two Studentships tenable at the School of Agriculture, Aberdeen.

The areas of research are:

1. Evaluation of practices aimed at reducing levels of pig mortality. (Dr P. R. English).
2. Nutrient requirements of bulls and castrates treated with anabolic compounds. (Dr H. Galbraith).

Candidates should possess at least an upper second class Honours degree in Agriculture or allied subjects, or be completing their Honours degree in June 1979. They must be normally resident in England or Wales and be studying at a University in the United Kingdom.

The value of the M.A.F.F. award in 1979 will normally be £1,610.

Interested applicants should write immediately to Professor G. A. Lodge, School of Agriculture, University of Aberdeen, stating for which research topic they wish to be nominated and giving the names of two persons to whom reference can be made.

586(F)

MEAT
RESEARCH INSTITUTE
SCIENTIFIC OFFICER

Applications are invited for a Scientific Officer post in the Division of Meat Structure involving work on proteins of the contractile apparatus of muscle.

Experience in protein chemistry and/or electron microscopy would be an advantage.

Qualifications: Honours degree or equivalent or an ordinary degree with relevant experience.

Salary: Scientific Officer Grade £2,839 to £4,415 (starting salary depending on qualifications and experience).

Non Contributory Pension Scheme. Closing date for return of application forms May 11, 1979.

Application forms from: The Personnel Officer, Meat Research Institute, Langford, Bristol BS18 7DY. 531(A)

UNIVERSITY OF BRISTOL
DEPARTMENT OF GEOLOGY
ROCK-SECTION
TECHNICIAN WANTED

Applications are invited for a person to take charge of the rock-cutting workshop. The appointed person will have had several years experience in this type of work together with some educational qualifications.

The position carries a salary of £3,474 to £4,056 per annum (Grade 5) (scale review). Applications, including the names of two referees, to: The Superintendent, Department of Geology, Queens Building, University Walk, Bristol BS8 1TR. To be received within two weeks of this advertisement appearing. 584(A)

APPLIED ECOLOGY
RESEARCH GROUP
REDLAND PURLE
RESEARCH
STUDENTSHIP

Applications are invited from candidates with a good Honours degree in Zoology or Biology for a research studentship concerned with a study of invertebrate communities in freshwater habitats adjacent to a landfill site.

The project which will involve close collaboration with Redland Purle Ltd, the industrial sponsors, will be based within the Applied Ecology Research Group of the Polytechnic of Central London, and will be at standard research council rates. The successful candidate will be registered for a higher degree with the C.N.A.A. A full current driving licence is essential.

Salary: £3,087 to £3,261 inclusive of London Allowance.

Application form and further details from the Establishment Officer, P.C.L., 309 Regent Street, London W1R 8AL (Tel 01-580 2020, Ext 212) 550(F)

THE ANIMAL VIRUS
RESEARCH INSTITUTE,
PIRBRIGHT
A.R.C. RESEARCH
STUDENTSHIP 1979

Applications are invited for an Agricultural Research Council Studentship to join a group working on the structure of foot-and-mouth disease virus RNA and the biochemistry of virus mutants as they relate to the function of the RNA. The Studentship will be tenable for up to 3 years from October 1979 and the successful candidate will be expected to read for a higher degree. Applicants should possess, or expect to obtain, a good honours degree in a Molecular Biological subject.

Application forms and further particulars may be obtained from the Secretary, The Animal Virus Research Institute, Pirbright, Woking Surrey, GU24 0NF, applications should be received by May 14, 1979. 571(F)

THE OPEN
UNIVERSITY
Physics
Department
Research
Studentships

Applications are invited from graduates interested in pursuing research towards the degrees of B.Phil, M.Phil, or Ph.D. in the following areas: biomagnetism (SQUID magnetometry); nanosecond molecular spectroscopy; optical properties of semiconductors; solar energy (interseasonal storage); triboluminescence. In each area the student would work under one or more members of the permanent academic staff. A small number of both the normal SRC grants and internal OU grants may be available to qualified candidates. Further particulars may be obtained from Dr. S. J. Swinbenby, Faculty of Science, The Open University, Walton Hall, Milton Keynes, MK7 6AA.

Please quote reference PY 3 Closing date for applications 10th May 519(F)

AGRICULTURAL
RESEARCH COUNCIL
STUDENTSHIP

Applications are invited for an A.R.C. postgraduate studentship tenable at the Meat Research Institute for research into the viscoelastic properties of semi-solid foodstuffs.

The work will involve a study of the transmission of mechanical pulses through foods and computer analysis of transient stresses generated with an Instron materials tester.

The project is expected to form the basis for a Ph.D. thesis in the University of Bristol and the appointment will commence in October 1979.

Candidates should possess a First or Upper Second Class degree in Physics or a related subject.

Further particulars of the award and allowances together with Application Forms may be obtained from the Personnel Officer, Agricultural Research Council, Meat Research Institute, Langford, Bristol BS18 7DY. 579(F)

UNIVERSITY OF
ABERDEEN
DEPARTMENT OF THERAPEUTICS
AND CLINICAL PHARMACOLOGY
AND DEPARTMENT OF
PHARMACOLOGY

Applications are invited for a Ph.D. studentship tenable in the above departments from October 1979.

The project will involve a comparison of the pharmacokinetic and pharmacodynamic effects of drugs used in the treatment of hypertension and/or cancer chemotherapy.

Applicants should have or expect to gain a good honours degree in Pharmacology or Biochemistry. A brief curriculum vitae and the names of two academic referees should be submitted within two weeks of the appearance of this advertisement to: Dr H. E. Barber, Department of Pharmacology, University Medical Buildings, Foresterhill, Aberdeen AB9 2ZD. 587(F)

UNIVERSITY OF LONDON
KING'S COLLEGE
DEPARTMENT OF CHEMISTRY
RESEARCH
STUDENTSHIPS

Applications are invited for College Tutorial and SRC (including CASE) postgraduate studentships in all areas of Chemistry. Applicants should hold or expect to obtain a First or Upper Second Class Honours degree from UK universities (or CNAAs) or Grad RIC of equivalent standing.

Further information and application forms from Prof V. Gold, FRSc, King's College, Strand, London WC2R 2LS. 533(F)

AGRICULTURAL
RESEARCH COUNCIL
POULTRY RESEARCH
CENTRE,
KING'S BUILDINGS,
WEST MAINS ROAD,
Edinburgh EH9 3JS, Scotland
NUTRITION
DEPARTMENT
RESEARCH
STUDENTSHIP

Applications are invited for three-year Agricultural Research Council Research Studentship study dietary factors affecting digestion and metabolism of or by poultry. A wide range of rational and physiological techniques are available. Supervision by J. M. McNab from whom full details of the proposed research can be obtained.

The person appointed must be a 1st or upper 2nd honours degree in agricultural science or nutrition and will be expected to apply for registration as a postgraduate student (Ph.D.) at the University of Edinburgh.

Application form and full details from the Assistant Secretary at the above address. 503(F)

UNIVERSITY
OF DUNDEEDepartment of Physiology
M.R.C. POSTGRADUATE
STUDENTSHIP

available from October 1. Applications, with the name of two referees, to Professor O. Peterson, Department of Physiology, The University of Dundee, DD1 4HN before May 10, 552(F)

UNIVERSITY OF LEICESTER
DEPARTMENT OF ZOOLOGY
SCHOOL OF
BIOLOGICAL SCIENCES
POSTGRADUATE
RESEARCH STUDENTS

Applications are invited for a graduate research studentship funded by the Medical Research Council for three years for the study of regulation and determinants of spatial patterns of cellular differentiation in arthropods.

The study would be of particular interest to those with some knowledge and background in Developmental Cell or Neurobiology, although candidates with other qualifications will be considered.

Applicants should send a curriculum vitae and names and addresses of referees to Dr P. M. J. Shelton, Department of Zoology, University of Leicester, Leicester LE1 7RH, as soon as possible. 51

THE ANIMAL VIRUS
RESEARCH INSTITUTE
Pirbright
A.R.C. RESEARCH
STUDENTSHIP 1979

Applications are invited for an Agricultural Research Council Studentship to join a group working on the structure of foot-and-mouth disease virus RNA and the biochemistry of virus mutants as they relate to the function of the RNA. The Studentship will be tenable for up to 3 years from October 1979 and the successful candidate will be expected to read for a higher degree. Applicants should possess, or expect to obtain, a good honours degree in a Molecular Biological subject.

Application forms and further particulars may be obtained from the Secretary, The Animal Virus Research Institute, Pirbright, Woking, Surrey, GU24 0NF, applications should be received by May 14, 1979. 55

UNIVERSITY OF NEWCASTLE UPON TYNE M.R.C. RESEARCH STUDENTSHIP

Applications are invited from good students with honours in a biological science and from students expecting to graduate in 1979 for an M.R.C. research studentship to study myofibrillar generation in muscular dystrophy in 1. The work will involve quantitative techniques of electron microscopy, light microscopy and, possibly, tissue culture, and will be carried out in the Muscular Dystrophy Research Laboratories at Newcastle General Hospital in association with Dr M. J. Lennox. The studentship (value £1,610 per annum) is tenable for three years and a successful candidate will be registered for the degree of Ph.D. Applicants should write to Dr M. J. Lennox, Muscular Dystrophy Research Laboratories, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, enclosing a curriculum vitae and names and addresses of two referees, by May 14, 1979. 460(F)

RESEARCH STUDENTSHIPS

Applications are invited from graduates with honours (or those about to graduate) in a biological science to undertake a 3 year project on the effect of hormones on mammalian heart muscle. (This is a joint project between the National Heart and Royal Free Hospitals and the Nuffield Laboratories of Comparative Medicine, Zoological Society of London.) Candidates should have an interest in pharmacology, electron microscopy and/or cardiac physiology and will be expected to register for a higher degree. Candidates of employment will follow those set out by the Research Councils for Studentships.

Applications accompanied by a full curriculum vitae and the names of two referees should be sent to Mrs J. French, Personnel Department, Royal Free Hospital, Pond Street, London, N.W.3. (Telephone 01-794 0500, Extension 4343) and from whom further particulars may be obtained. Candidates expecting to graduate this summer will be considered. Closing date May 14, 1979. 564(F)

FELLOWSHIPS

UNIVERSITY OF ABERDEEN GEORGINA McROBERT CANCER RESEARCH FELLOWSHIP

Applications are invited for a postdoctoral cancer research fellowship available in one of the departments of Anatomy, Bio-Medical Physics and Bio-Engineering, Therapeutics and Clinical Pharmacology, Pathology, Biochemistry, Bacteriology and Developmental Biology.

Salary within range of £3,883 to £6,555 per annum with appropriate housing. Initial appointment for three years, from October 1979, with possible extension to five.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by May 18, 1979. 558(E)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND CANADA DEPARTMENT OF GEOLOGY A POSTDOCTORAL FELLOWSHIP

is available for a condodont specialist with extensive documented knowledge of Middle Ordovician North Atlantic faunal province condodonts. The successful applicant will participate in an on-going sedimentological and paleoecological study of the Table Head Formation and related rocks in western Newfoundland.

The appointment will be effective September 1, 1979 at a salary of \$12,900 and will be for one year in the first instance.

Applications, including the names and addresses of two referees, should be sent by June 1, 1979 to: Head, Department of Geology, Memorial University of Newfoundland, St John's, Newfoundland, Canada A1B 3X5. 518(E)

UNIVERSITY OF SOUTHAMPTON DEPARTMENT OF CHEMISTRY

Several Postdoctoral Research Fellowships will be available from October 1, 1979 for development of new synthetic methods or synthesis of natural products under Professor R. Baker or Professor R. C. Cookson. A fellowship is also available in the Chemical Entomology Unit for a chemist with biological interests. Salary within range £4,133 to £4,382 (under review) plus superannuation.

Applications, including brief curriculum vitae and names of at least two referees, should be sent to Professor R. Baker or Professor R. C. Cookson, Chemistry Department, Southampton University, Southampton SO9 5NH. 566(E)

UNIVERSITY OF ABERDEEN DEPARTMENT OF CHEMISTRY POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a S.R.C. fellowship to work with Professor R. H. Thomson and Dr A. R. Forrester on the generation and reactions of atomic nitrogen in solution. Applicants with experience in synthetic and/or free radical chemistry will be preferred.

The appointment is for two years beginning July 1, 1979 or as soon as possible thereafter.

Commencing salary within Range 1A, £3,883 to £4,382 per annum (under review) with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by May 25, 1979. 569(E)

UNIVERSITY OF SOUTHAMPTON DEPARTMENT OF PHYSICS RESEARCH FELLOWSHIPS IN CRYOGENICS

Applications are invited for Postdoctoral Fellowships to work on the following projects:

1. Heat transfer in the reboiler/condensers of cryogenic plant, using model rigs and a full length test section.
2. Complex convective flow in stratified cryogenic vapours and liquids using Laser Doppler Anemometry.
3. Heat transfer and fluid behaviour of helium at 3,000 r.p.m. for the development of large superconducting machines.

Applicants should have experience in mechanical/chemical engineering or low temperature physics.

Salary in range up to £5,367 plus U.S.S. benefits.

Applications with names of two referees should be sent to Mrs E. C. P. Sears, Staffing Section, The University, Highfield, Southampton SO9 5NH. Please quote reference number 1607/R/N. 441(E)

QUEEN'S UNIVERSITY Kingston, Ontario

Applications are invited for a M.R.C. supported postdoctoral fellowship to work with Dr B. T. Eaton on the study of arbovirus persistence in mosquito cells. The appointment is available for two years and the initial salary is \$12,000 per annum. Prospective or recent Ph.D. graduates should send their curriculum vitae and the names of two referees to Dr Eaton, Department of Microbiology and Immunology, Queen's University, Kingston, Ontario K7L 3N6, Canada. W80(E)

The Queen's University of Belfast POSTDOCTORAL RESEARCH FELLOWSHIP

Pharmacokinetics and
Metabolism of Anticancer
Drugs

The Department of
Biochemistry

This work on drugs used for combination chemotherapy of breast cancer will be carried out in the Action Cancer Unit in collaboration with existing research staff. Techniques used include G.L.C. and radioimmunoassay. The post is tenable until September 30, 1982, on the scale £3,885 to £5,130 per annum, according to age and qualifications.

Further details are available from the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland, to whom applications, together with the names and addresses of two referees should be sent. 546(e)

UNIVERSITY OF SOUTHAMPTON DEPARTMENT OF CHEMISTRY

Applications are invited for a Science Research Council sponsored Postdoctoral Fellowship to work in the field of photoelectron spectroscopy. The work will involve studies of gaseous free radicals and molecular fragments generated by high temperature pyrolysis.

The post will be available from a suitable agreed date for one year in the first instance and is renewable for a second year by mutual agreement. This position should appeal to a chemist or physicist with related experience although this is not essential. Salary commensurate with age and experience, membership of U.S.S. available.

Applications, including a curriculum vitae and the names of two referees should be sent to: Professor N. B. H. Jonathan or Dr J. M. Dyke, Department of Chemistry, The University, Southampton SO9 5NH. Ref. No. N. 567(E)

UNIVERSITY OF ABERDEEN DEPARTMENT OF GEOLOGY AND MINERALOGY POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited from suitably qualified organic geochemists or clay mineralogists to work with Dr M. J. Pearson on the correlation of organic matter and clay mineral diagenesis in sediments from the North Sea Basin. The project involves the study of kerogen maturation and hydrocarbon generation during burial in relation to clay mineral transformations as a response to temperature increase and ion concentration in interstitial water.

The post is tenable for three years and is supported by the Natural Environment Research Council.

Salary £4,382 to £4,882 per annum on Research and Analogous Staff Range 1A Scale (under review), with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by May 18, 1979. 557(E)

CONFERENCES

Quekett Microscopical Club Lecture. Dr James Dyson, Sc.D., F.R.S., will speak on 'Interference and Image Shearing Microscopy' at 6.30 p.m. on Tuesday, May 8, at the British Museum (Natural History), Cromwell Road, South Kensington, London SW7 5BD. All Club members and visitors are welcome. 563(C)

ASSISTANTSHIPS

UNIVERSITY OF OXFORD DYSON PERRINS LABORATORY POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a postdoctoral research assistantship supported by the S.R.C. to develop new protecting groups for histidine in peptide synthesis. Previous experience in synthetic organic chemistry is essential, and practical knowledge of peptide, amino-acid or heterocyclic chemistry will be advantageous.

The appointment is for two years, commencing September 1, 1979, or as soon as possible thereafter. The salary will be on the Research Support Grade Scale 1A, initially not higher than £4,382 p.a. (under review), plus U.S.S. benefits.

Applications (one copy), with curriculum vitae and the names and addresses of two referees (overseas applicants should ask referees to write directly to the address below) should be sent as soon as possible and in any case not later than May 31, 1979, to Dr J. H. Jones, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY, England, from whom further details may be obtained. 588(P)

UNIVERSITY COLLEGE OF NORTH WALES, BANGOR RESEARCH POSTS IN ORGANIC CHEMISTRY

Applications are invited for postdoctoral research assistantships in the College's School of Physical and Molecular Sciences, to work in collaboration with Professor C. J. M. Stirling on measurement of the effect of ring strain on reactivity in organic reactions.

The appointments, to commence on October 1, 1979, or such earlier date as may be arranged, will be for one year in the first instance, with the possibility of extension for a further two years, and will be within the salary range:—£3,883 to £4,382 per annum (under review).

Applications (two copies), giving details of qualifications and research experience, together with the names and addresses of two referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd, as soon as possible. 538(P)

UNIVERSITY OF DURHAM DEPARTMENT OF PHYSICS

Applications are invited from physicists or astronomers, preferably with postdoctoral research experience, for a

SENIOR RESEARCH ASSISTANTSHIP

to be responsible, with an electronic engineer, for the design of a new experimental facility in high energy gamma-ray astronomy. The experiment will use the atmospheric optical Cerenkov radiation technique at present in use in a large cosmic ray detector array in Utah, U.S.A.

This appointment is tenable for two years from October 1979, and will involve working for a number of short periods overseas, most probably in the U.S.A. (Further details from either Dr K. E. Turver or Dr K. J. Orford. Tel. Durham 64971.)

Initial salary in the range £3,883 to £4,631 per annum (under review) plus superannuation.

Applications (three copies) naming three referees should be sent by May 31, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. 540(P)

ASSISTANTSHIPS—cont.

UNIVERSITY OF BRISTOL
DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL
RESEARCH ASSISTANTSHIP
IN PROTEIN
CRYSTALLOGRAPHY

Applications are invited for an S.R.C. funded postdoctoral research assistantship to study the high resolution crystal structure and the substrate and effector binding sites of Pyruvate Kinase.

The appointment is for a period of three years with an initial salary of up to £24,382 per annum (Point 3 on Range 1A of the University scales—scale under review). Applicants should have previous research experience in crystallography.

Applications, including a brief curriculum vitae, research experience and names and addresses of two referees, should be sent to Dr H. Muirhead, Department of Biochemistry, University of Bristol, Bristol BS8 1TD, as soon as possible. 513(P)

UNIVERSITY OF BRISTOL
DEPARTMENT OF BIOCHEMISTRY
RESEARCH ASSISTANT
IN BIOCHEMISTRY

Applications are invited for an S.R.C. funded postgraduate research assistantship. The successful applicant would help with the purification of enzymes, preparation of crystals and data collection in a protein crystallographic laboratory.

The appointment is for a period of three years with an initial salary of £3,384 per annum on Range 1B of the University scales (scale under review).

Preferred qualifications: Degree in Biochemistry or equivalent.

Applications, including a brief curriculum vitae, and names and addresses of two referees should be sent to Dr H. Muirhead, Department of Biochemistry, University of Bristol, Bristol BS8 1TD, as soon as possible. 512(P)

UNIVERSITY OF DURHAM
DEPARTMENT OF
BOTANY

Applications are invited from graduates in Botany, microbiology or bacteriology for a

**POSTDOCTORAL
SENIOR**

RESEARCH ASSISTANTSHIP

tenable for 2½ years from July 1, 1979 or as soon as possible thereafter, on a N.E.R.C. funded investigation into the physiological ecology of blue-green algae/cyanobacteria.

Salary at the appropriate point on Range 1A £3,883 to £6,555 plus superannuation.

Applications (three copies) naming three referees should be sent by May 8, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. 539(P)

COURSES

UNIVERSITY OF LEICESTER
DEPARTMENT OF CHEMISTRY
AND SCHOOL OF EDUCATION
Ph.D. IN CHEMISTRY WITH
GRADUATE CERTIFICATE
OF EDUCATION

Applications are invited from persons holding, or expecting to be awarded during 1979, a degree with First or Second Class Honours (Upper division), or equivalent qualifications, in Chemistry, Combined Sciences, Chemical Engineering, Physics, etc., who wish to pursue a 3-year course of research and teacher training.

The course may attract a limited number of Science Research Council quota awards, principally for new graduates, but candidates from industry or teaching may be eligible to apply for awards under other S.R.C. schemes.

For further information and application forms please write to Professor R. D. Peacock, Department of Chemistry, University of Leicester LE1 7RH.

The closing date for applications is May 31, 1979. 515(D)

GRANTS

**N.E.R.C. RESEARCH GRANTS IN GEOPHYSICS
AND PLANETARY PHYSICS**

Research studentships are available in the Department of Geophysics and Planetary Physics in the School of Physics at the University of Newcastle upon Tyne, for work starting in October 1979 and leading to a Ph.D. degree, in the following topics:

A Geophysical Study of Afar, a region of active plate boundaries (Dr R. W. Girdler)

Assessment of the contribution of altered silicates to the palaeomagnetic record (Dr W. O'Reilly)

Magnetic Fabric of Consolidated and Unconsolidated Sediments (Dr D. H. Tarling)

Laboratory Studies of flows in rotating, thermally-stratified fluids (Dr C. W. Titman and Dr P. A. Davies)

Experimental Fluid Dynamics related to topographically generated wind-storms (Dr D. J. Tritton)

Laboratory Experiments related to effect of Mantle Convection on Core Formation (Dr D. J. Tritton)

Laboratory Investigation of Mechanisms responsible for origin of planetary magnetic fields (Dr I. Wilkinson)

For further details, write to Mr J. M. Walmsley, School of Physics, The University, Newcastle upon Tyne NE1 7RU. 536(H)

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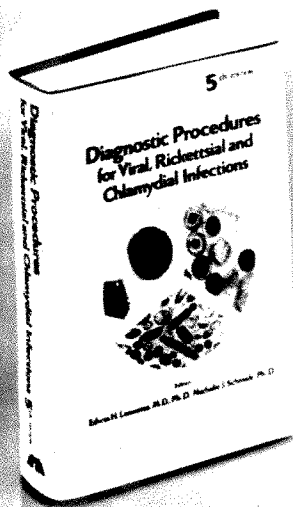
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